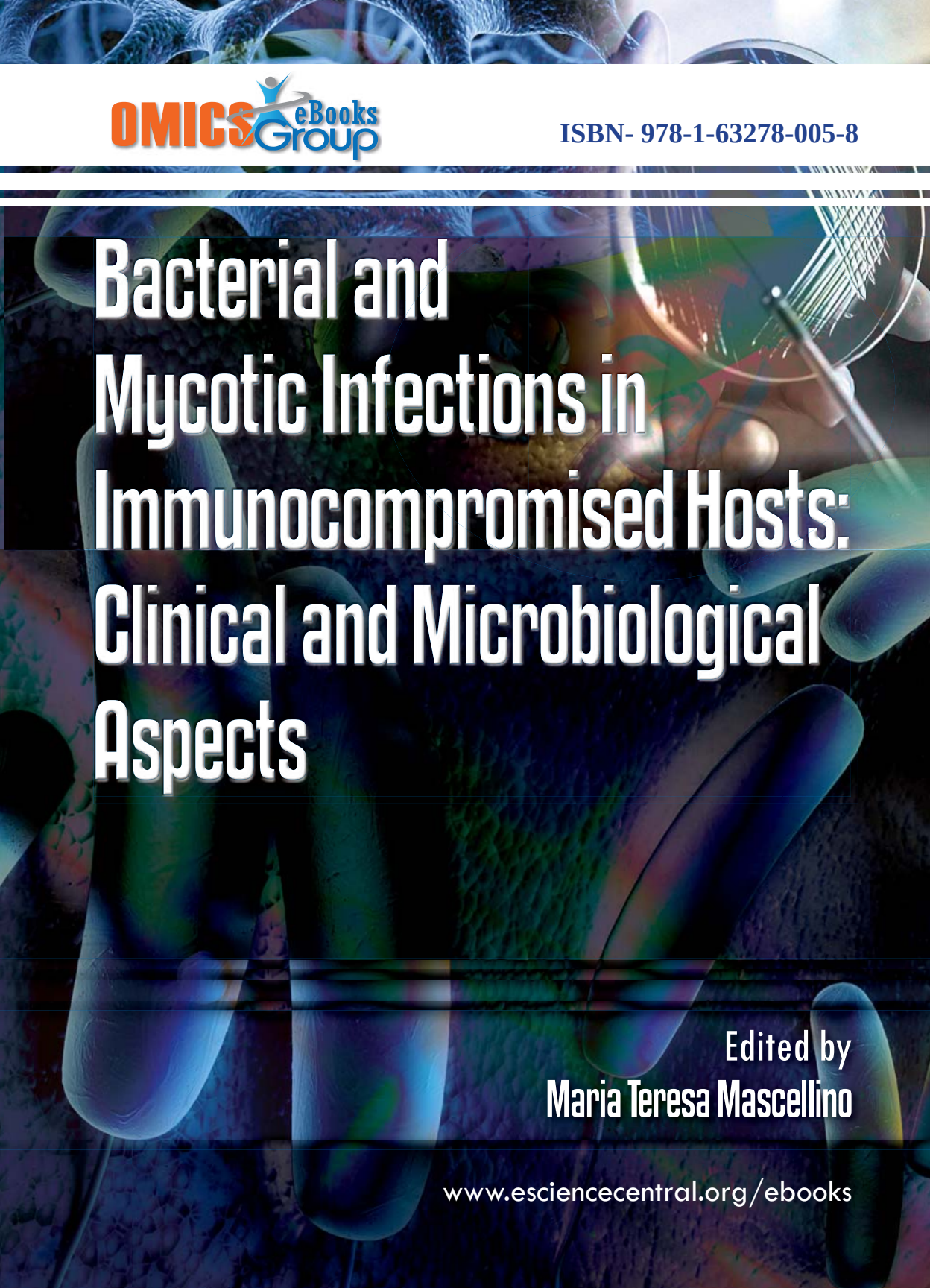
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Edited by
Maria Teresa Mascellino
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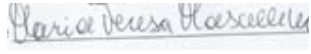
Preface

This book deals with bacterial and mycotic infections considering the particular aspects involved in the immunocompromised host. It is addressed to all people (microbiologists or pathologists) who for their activity have to face every day the clinical and microbiological features of these diseases.

The immunocompromised hosts are a real problem for the peculiar characteristics that these subjects show and for the problems arisen in their treatment. We tried to examine most of infectious diseases such as the gynecological infections, the sexually transmitted diseases, the sepsis, the Coagulase Negative Staphylococcus infections and so on, stressing the diagnosis, the management and the prevention of the diseases and considering the newest aspects i.e. the formation of the bacterial biofilm in the devices or prostheses and the consequent resistance to antimicrobial agents.



Thank you,

Maria Teresa Mascellino

About Editor

Maria Teresa Mascellino has completed her MD at the age of 25 years from University “La Sapienza” of Rome during the period of 1980 and specialization studies in Microbiolgy and Infectious Diseases from University “La Sapienza” of Rome (Italy). She is Responsible for the Simple Operative Unit “Microbiological Analyses in the immunocompromised hosts” in the Department of Public Health and Infectious Diseases at Policlinico Umberto I° of Rome. She has published more than 110 papers in reputed journals and has been serving as an editorial board member of repute. She is member of many scientific societies and has participated in relevant International Research Projects. Her research has included the following topics: Bacterial antibiotic resistance and multidrug resistant microorganisms, Tuberculosis, Chlamydia infections, MRSA (Methicillin Resistant Staphylococcus Aureus), Rapid laboratory methods (PCR), Helicobacter pylori: virulence determinants and pathology, Candidemiae: incidence and drugs susceptibility, Intracardiac devices infections, Sonication technique for bacterial biofilm, Klebsiella pneumoniae MDR in nosocomial infections and synergistic activity of double carbapenem.

Forewords

"With "Bacterial and Mycotic Infections in Immunocompromised Hosts: Clinical and Microbiological Aspects", Dr. Maria Teresa Mascellino has developed an excellent reference work for a very important field within medical microbiology. The expertly conceived and edited book advances understanding of the risks posed bacterial and fungal infectious agents to immunocompromised patients.

Dr. Mascellino is a leading international expert on global diseases and medical microbiology. Dr. Mascellino has contributed to over one hundred research papers. This includes important insights into Helicobacter pylori infection as well as some insightful studies into epidemiology in Italy. She is highly regarded as one of Europe's the foremost medical microbiologists, and is currently based at the University of Rome (La Sapienza)".

Tim Sande

Dr. Maria Teresa Mascellino presents a collaborative review of infectious conditions in immunocompromised patients. Dr. Mascellino has over 100 publications in recognized journals and textbooks, providing us with valuable information in several areas of infectious diseases, including Helicobacter pylori, MRSA, cardiac-device infections and HIV. It has been a privilege to contribute chapters in her recent eBook "Bacterial and Mycotic Infections in Immunocompromised Hosts: Clinical and Microbiological Aspects." As many authors who contribute to scholarly work seek to provide valuable information, the potpourri of fundamental clinical data in Maria Mascellino's eBook have achieved just that.

Ramzy Rimawi MD

I would like to thank the editor Dr. Maria Teresa Mascellino of the eBook "Bacterial and Mycotic Infections in Immunocompromised Hosts: Clinical and Microbiological Aspects," for giving an opportunity to contribute the book chapter "Yeasts: Candida and Cryptococcusis". She constantly supported and gave valuable scientific input while writing and also reviewing the book chapter. The eBook has come out very well as it covered various aspects of medically important microbes in immunocompromised human hosts. I am sure the scientific content covered in this book is very useful for the students and scientific community who are working in this area.

Thanks again for giving the opportunity to contribute book chapter in the book. I hope a long lasting scientific relationship with you.

Dr. Madhu Dyavaiah

Acknowledgement

First of all, I wish to thank the OMICS Group Incorporation for having given to me the possibility to edit the e-Book “Bacterial and Mycotic Infections in Immunocompromised Hosts: Clinical and Microbiological Aspects”. I want to send a particular thanks to all the authors that with their competence and expertise have contributed to the draft of this book bringing about important innovations and up-to-date topics. Both bacteriology and mycology subjects have been deeply treated with particular capability. Moreover I wish to thank my Department Director, Prof. Vincenzo Vullo, for having assisted and helped me with his valuable advice and his high expertise.

Introduction

This book concerns the immunocompromised host with his particular characteristics, features and problems. These patients, both for immunodeficiency or for other underlying diseases, are a real drawback for their treatment. In fact they require a particular attention for the management of the diseases due to the fact that their pathologies and infections show particular and different aspects. In this book we have examined some of the principal bacterial and mycotic illness regarding such subjects.

The term "immunocompromised host" describes a patient who is at increased risk for life-threatening infection as a consequence of a congenital or acquired abnormality of the immune system. During the past few decades, the population of immunocompromised hosts has expanded enormously. In addition, Acquired Immunodeficiency Syndrome (AIDS) has resulted in the existence of many immunocompromised patients. Acquired conditions may also interfere directly with the immune system or may disrupt barrier function. These include HIV (Human Immunodeficiency Virus) infection, solid organ and bone marrow transplant, diabetes, cancer, alcoholism and cirrhosis, autoimmune diseases (treated with steroids), hemodialysis, spleenectomy and hypospleenism, immunosuppressive (chemotherapy) therapy, malnutrition, severe trauma and burns, surgeries, indwelling vascular devices and advancing age. Infections in immunocompromised host pose a diagnostic challenge for few reasons: 1) the existing etiologies of infections are different; 2) the inflammatory responses are impaired by immunosuppressive therapy that results in diminished clinical findings making an early diagnosis much more difficult; 3) drug interaction and drug toxicity are common.

Opportunistic infection is an unusual infection caused by normally non-pathogenic organisms in a host whose resistance has been decreased by disorders such as diabetes mellitus, HIV infection, cancer etc., creating opportunity for microorganisms which are not usually pathogenic to become pathogens.

The risk of infection in an immunocompromised host is determined by the interaction of two factors: the potential pathogens to which the individual is exposed (epidemiologic exposures) and a measure of the individual's susceptibility to infection, termed as the "net state of immunocompromised." Epidemiologic exposures in the immunocompromised hosts can be divided into two general categories: those occurring within the community and those occurring within the hospital. Exposures within the community vary based on such factors as geography and socio-economic status. Within the hospital if the air, food, equipment or potable water supply is contaminated with pathogens, clustering of infection cases may occur.

In this book we examined the principal bacterial and mycotic infections in immunocompromised hosts trying to deeply analyze the particular characteristics of these patients towards the pathogen and opportunistic bacteria. The microorganisms more commonly involved are the following: *Staphylococcus aureus, Staphylococcus epidermidis*, *Sreptococcus pyogenes* and *pneumoniae*, Gram-negative aerobic organisms e.g. *Escherichia coli, Pseudomonas aeruginosa* and *klebsiella* that arise from endogenous gastrointestinal, mucosal or cutaneous flora , *Burkolderia cepacia, Mycobacterium tuberculosis* and Non-Tuberculosis Mycobacteria (i.e. *Mycobacterium avium-complex), Rhodococcus, Haemophylus influenzae, Listeria, Vancomycin Resistant Enterococcus* (VRE). Among other microrganisms (mycetes and parasites) we found *Candida, Aspergillus, Cryptococcus, Cryptosporidium, Toxoplasma, Pneumocystis, Scedosporium* etc.

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Chapter: I

Pathological Basis and Morphological Features

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Introduction to Terminology

The term "immunocompromised host" describes a patient who is at increased risk for Life-threatening infection as a consequence of a congenital or acquired abnormality of the immune system. During the past few decades, the population of immunocompromised hosts has expanded enormously, attributed to the increased use of immunosuppressive agents for the treatment of tumors and collagen vascular disease and for the prevention of rejection in organ transplant recipients. In addition, Acquired Immunodeficiency Syndrome (AIDS) has resulted in the existence of many immunocompromised patients [1]. The congenital causes of immunocompromised include a number of defects in B cells, T cells, combined B and T cell function defects, macrophage and cytokine defects, phagocyte deficiency and complement deficiencies. Acquired conditions may also interfere directly with the immune system or may disrupt barrier function. These include HIV (Human Immunodeficiency Virus) infection, solid organ and bone marrow transplant, diabetes, cancer, alcoholism and cirrhosis, autoimmune diseases (treated with steroids), hemodialysis, spleenectomy and hypospleenism, immunosuppressive (chemotherapy) therapy, malnutrition, severe trauma and burns, surgeries, in dwelling vascular devices and advancing age [2].

Infections in immunocompromised hosts pose a diagnostic challenge for few reasons; firstly the existing etiologies of infections are diverse and the scenario is further complicated by the explosion of newer pathogens with newer manifestations, secondly the inflammatory responses are impaired by immunosuppressive therapy which results in diminished clinical and radiological findings. This makes an early diagnosis much more difficult, warranting invasive diagnostic procedures. Thirdly, also because drug interactions and drug toxicities are common. The initiation or cessation of antimicrobial therapies may alter the levels of calcineurin inhibitors, antifungal agents and other drugs. Lastly graft rejection and graft-versus-host disease may be confused with infections [3].

Opportunistic infection is an unusual infection caused by normally nonpathogenic organisms in a host whose resistance has been decreased by disorders such as diabetes mellitus, HIV infection, cancer, a surgical procedure such as a cerebrospinal fluid shunt, a cardiac or urinary tract catheterization or by immunosuppressive drugs. Long-term use of antibiotics or other drugs may also affect the immune system, creating opportunity for microorganisms which are not usually pathogenic to become pathogens.

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tumors, radiotherapy, chemotherapy, surgical diagnostic and therapeutic procedures and intravenous lines and other devices. The pathogens found in cases of altered anatomical barriers are similar to those encountered in patients with granulocytopenia. The third factor predisposing such patients to serious bacterial and nonbacterial infections is immunocompromised state especially in patients with lymphomas, multiple myelomas and chronic lymphatic leukemia [5]. Neutropenic hosts are susceptible to a number of infections including pyogenic bacteria, invasive aspergillosis, candidiasis, and mucormycosis. Among these one sees abundant organisms but not the expected purulent inflammatory reaction. Hosts with adequate numbers of neutrophils but defective neutrophil function (e.g., in diabetes) also are at risk for invasive infections with these fungi. Vascular invasion is typical of invasive aspergillosis, candidiasis, and mucormycosis, although this phenomenon is usually appreciated on histological sections rather than in the cytology laboratory [6]. Protracted neutropenia coupled with breaches in skin (e.g., from a central intravascular line) and bowel (e.g., from mucotoxic chemotherapy) predispose patients to candidemia and invasive aspergillosis [6].

Bacterial Infections Among Immunocompromised Hosts

Bacterial respiratory infections

Bacterial pneumonia is a common cause of morbidity in immunocompromised hosts. The high rates of bacterial pneumonia in immunocompromised hosts probably result from multiple factors, including qualitative B-cell defects that impair the ability to produce pathogen-specific antibody, impaired neutrophil function, numbers or both and the factors (e.g., injection drug use) that are also associated with HIV infection. The type of pulmonary infection depends upon the defects in host defense mechanisms [7].

Pathological basis: Bacterial pneumonia may be associated with neutropenia, solid tumor or multiple myeloma. Opportunistic infections are associated with steroids, lymphoproliferative disorders and hematopoietic stem-cell transplantation [8]. Table 2 [7-10] presents the immune defects and commonly associated pulmonary pathogens.

Although the majority of pulmonary complications in immunocompromised hosts are infectious in nature, noninfectious complications of the disease can account for 25%. *Pseudomonas aeruginosa* has been increasingly recognized as an important source of bacterial pneumonia in HIV hosts particularly in those with neutropenia, steroid use, multiple antibiotic therapy, myelosuppressive therapy and indwelling catheters.

Defect	*Measure*	*Pulmonary Pathogen*	*Underlying cause*
Immunoglobulin defect	Ig G less than 400 mg/dl	*Streptococcus pneumonia, Hemophilus infleunzae*	Common variable immune deficiency Multiple myeloma CLL
Neutropenia	Neutrophils less than 1000 cells/cumm	*Staphylococcus aureus* *Pseudomonas aeruginosa*	Acute leukemia Chemotherapy induced neutropenia Aplastic anemia Bone marrow/stem cell transplantation
T cell defect	CD 4 cells less than 500cells/ cumm	*Mycobacterium tuberculosis* *Legionella pneumophilia*	

Table 2: Immune defects and commonly associated pulmonary pathogens.

In non HIV immunocompromised hosts *S. aureus* and gram negative aerobes such as *Klebsiella, P. aeruginosa*, *Rhodococcus equi*, *E. coli*, *Proteus*, *Pseudomonas*, *Enterobacter* and *Serratia* are responsible. Renal transplant recipients and those on immunosuppressive drugs and steroids are prone to *Legionella pneumophilia* [10]. Bacterial pneumonia might also be the first manifestation of underlying HIV infection and can occur at any stage of HIV disease and at any $CD4^+$ count. Risk factors associated with an increased risk for bacterial pneumonia, include low $CD4^+$ count, injection-drug use and cigarette smoking

relative defect in IFN-gamma production in response to MAC may predispose an individual to localized but partially controlled lung disease whereas defects leading to reduced IL-12 and TNF-alpha production may allow the dissemination of MAC [18].

Morphological features: Histologically, the pathology often shows extensive areas of non-necrotizing epithelioid histiocytes and is highly characteristic. With progressive immunodeficiency, granulomas become poorly formed or can be completely absent [19,20]. The causative mycobacteria are indistinguishable from *M. tuberculosis* and only a culture or PCR can accurately establish the diagnosis. A recently described variant of MAC infection is known as "hot tub lung." The lung shows a micro granulomatous hypersensitivity pneumonitis that may be accompanied by necrotizing granulomatous inflammation. An unusual presentation of MAC infection in the immunosuppressed host is the pseudosarcomatous nodule. This can develop in the lung or in soft tissues and hematopoietic tissues. The nodules are composed of spindle cells and may be mistaken for a low-grade spindle-cell neoplasm. However, examination reveals the foamy appearance of the spindle cells which proves to be CD 68-positive macrophages containing large numbers of ingested mycobacteria and the absence of mitotic activity [20]. Histopathologic examination of liver, spleen, bone marrow or intestine from patients with AIDS-related disseminated MAC shows high-grade infection with lack of inflammatory infiltrate or tissue necrosis.

Bacterial enteric infections

Incidence rates of gram-negative bacterial enteric infections are 20- to 100 fold higher among immunocompromised HIV-infected adults than in the general population. The most common causes in the United States are *Salmonella, Shigella, Spirochetosis, Mycobacterium avium intracellulare* and *Campylobacter* [21].

Pathological basis: The probable source for most infections is ingestion of contaminated food or water. Sexual activity with the potential for fecal-oral exposure also increases risk for infections especially with *Shigella* and *Campylobacter*. Acquisition of enteric bacterial infections might be facilitated by HIV-associated gastric achlorhydria by treatment with agents that decrease gastric acid secretion such as immunosuppressive drugs and by immunocompromised states associated with alterations in mucosal immunity. The risk for more profound illness increases with the degree of immunosuppression. Relapses in infections with *Salmonella* and other gram-negative bacterial enteric pathogens after appropriate treatment have been well-documented. *Salmonella* is a particularly common cause of septicemia which is prone to relapse. Recurrent *Salmonella* septicemia constitutes an AIDS-defining illness and might require chronic suppressive therapy. The development of antimicrobial resistance during therapy, often associated with clinical deterioration or relapse, can also occur among HIV-infected hosts with gram-negative enteritis [21]. Adults with diabetes, long term steroid medication, hematological malignancy, advanced or disseminated solid cancers, autoimmune disease, liver disease (particularly alcoholic), renal transplantation and those taking immunosuppressive drugs are susceptible to NTS bacteremia [22]. Patients with these underlying conditions are more likely to present with primary bacteremia and an absence of diarrheal disease, as confirmed in several populations and lack of diarrhea is a useful marker for underlying immunosuppression and high mortality [23]. *Salmonella* infection in humans cause a range of diseases including diarrheal disease caused by a large number of non-typhoidal *Salmonella* serovars. NTS have a broad vertebrate host range, epidemiology that often involves food animals, and have a dramatically more severe and invasive presentation in immunocompromised hosts. Immunocompromise among adults, including severe or progressive disease, chronic granulomatous disease, defects or blockade of specific cytokines (particularly IL-12/IL-23/IL-17 and TNF) and HIV, is associated with suppurative foci and with primary bacteremia disease, which may be recurrent. These patients have markedly increased mortality [23]. Intestinal spirochetosis is yet another emerging infection among immunocompromised hosts that involves the colon

patients are more likely to develop different and severe life-threatening disease. *Bartonella* are fastidious hemotropic facultative intracellular Gram-negative bacteria of the alpha-2 subgroup of Proteobacteria [30].

Pathological basis: *B. henselae* is a zoonosis transmitted from the natural reservoir cats usually via cat scratch or bite and less commonly by a vector such as cat fleas or ticks. Disease caused by *B. quintana* has dramatically decreased and now occurs primarily in small epidemics in conditions characterized by crowding and poor sanitation. Humans are likely the natural reservoir for *B. quintana* which is spread by human body lice. Most of the research in infection involving immunocompromised hosts has focused on the stimulatory role of angiogenesis by these organisms. However, understanding of the immune defense against *Bartonella* species among immunocompromised hosts has been hampered by the lack of good animal model [31]. Since the response in the immune competent system involves Th1 and innate immunity through macrophages, a logical inference can be made that HIV-positive and other hosts deficient in these immunological areas would have difficulty limiting the infection and thus would develop systemic manifestations. *Bartonella* has several characteristics that lead to immune evasion such as: *Bartonella* Lipopolysaccaride (LPS) is composed of a unique combination of lipid A and long-chain fatty acids which likely contributes to the bacteria's ability to evade the immune system as its surface molecules are not recognized by "Toll–Like Receptor-4" (TLR) on dendritic cells or macrophages. This allows for establishment of what might lead to persistent infection. Furthermore, *B. henselae* can avoid lysosomal fusion and acidification after the bacteria invades phagocytes such as endothelial cells and macrophages [31].

Morphological features: Bacillary angiomatosis is the most common sequel of *B. quintana* and *B. henselae* infections in patients with cell-mediated immunodeficiency such as HIV and post-transplant patients on immunosuppressive therapy [32]. Both the said species induce their characteristic vasoproliferative lesion, bacillary angiomatosis, or peliosis, in immunocompromised hosts by direct or indirect effects on endothelial cells. Both infections persist within periendothelial extracellular matrix resulting in sustained, localized bacterial replication within collagen tissue [33]. The lesions appear as cutaneous nodular vascular lesions but may also be found in a variety of organs including the GI-tract where they may cause hematemesis, genitourinary system and other organs including heart, spleen, bones and central nervous system [34]. The differential diagnosis for bacillary angiomatosis includes Kaposi's sarcoma, angiosarcoma and pyogenic granuloma [30].

Bacterial skin infections

***Mycobacterium haemophilum* infection:** *Mycobacterium haemophilum* causes mainly skin lesions in immunocompromised patients especially in patients with lymphoma or HIV and in organ transplant recipients [35,36]. Multiple skin lesions tend to occur and can present as erythematous papules, plaques, nodules, necrotic abscesses or chronic ulcers. Lesions are found most frequently over joints in the extremities and less commonly over trunk and face. Skin lesions typically evolve from papules to asymptomatic pustules and eventually to very painful deep-seated ulcers. The erythematous or violaceous papules and/or nodules are usually painless at first, but they can develop into potentially very painful abscesses. Several cases of septicemia and pneumonitis which occur due to M. *haemophilum* have been reported with disseminated and pulmonary infections. Rare cases of polymyositis, ophthalmic infections, central venous catheter infections, epididymal infections and osteomyelitis have also been reported [35].

Other emerging bacterial pathogens

Corynebacterium species are normal flora of skin and mucous membrane. In recent years, coryneforms have emerged as important opportunistic pathogens among immunocompromised hosts. Majority of the *Corynebacterium macginleyi* isolates are from

aspergillosis [46]. Invasive pulmonary aspergillosis is a disease that occurs in severely immunocompromised patients including patients with prolonged neutropenia, hematopoietic stem cell, solid organ transplant recipients, patients with AIDS, premature newborns and patients with chronic granulomatous disease [47]. Aspergillosis in immunocompromised hosts most frequently involves the respiratory tract with signs and symptoms of fever, cough, dyspnea and hemoptysis. It may also involve sinuses, tracheobronchial tree, heart bone or central nervous system [48].

Morphological features: *Aspergillus* are usually described as thin (3- to 12- m), septate, acute-angle (45 degree) or dichotomous branching hyphae (Figure 1 A). Vesicles with conidia can be observed when the fungi are present in cavitary lesions or sinuses [49] (Figure 1 C). Although a branching septate mold in tissue is most commonly *Aspergillus*, other organisms including hyaline and dematiaceous molds may be morphologically indistinguishable from *Aspergillus* species. By tissue morphology alone, *Aspergillus* cannot be definitively differentiated from other hyalohyphal fungi unless conidia-bearing fruiting heads are seen [50]. Allergic bronchopulmonary aspergillosis and rhinosinusitis are characterized by allergic mucous with eosinophils, Curshmann's spirals, Charcot-Leyden crystals; mucosa with suppurative and granulomatous inflammation, vasculitis and fibrosis. In chronic pulmonary aspergillosis the wall surrounding the fungus ball consists of fibrosis and in chronic necrotizing pulmonary aspergillosis the wall surrounding the fungus ball consists of a layer of necrosis, granulation tissue, granulomatous inflammation and fibrosis. In invasive disease there is angioinvasion by hyphae with consequent necrosis or hemorrhage of surrounding tissue [49]. The organisms can be seen with Hematoxylin and Eosin (H & E), Grocott's Methenamine Silver (GMS) (Figure 1 B and C) and Periodic Acid-Schiff (PAS) stains.

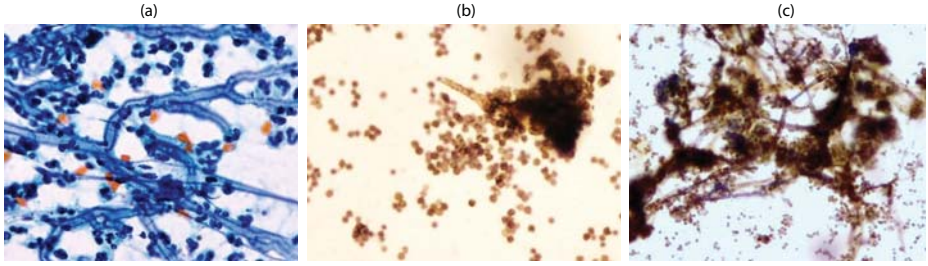


Figure 1: Cytological smear of bronchoalveolar lavage from a 75 year old diabetic female patient with recurrent carcinoma of the breast.
A: 60 X, Papanicolaou stain showing a cluster of dichotomous branching hyphae.
B: 60 X, Grocott's methenamine silver stain showing the branching hyphae.
C: 60 X, Grocott's methenamine silver stain showing conidiophores containing the typical fruiting heads of Aspergillus.

Candidiasis

Candida is a frequently occurring mycotic pathogen among immunocompromised hosts [51]. *Candida albicans* colonizes the human oropharynx, vagina and a small number of viable organisms can be cultured from these surfaces. C. *albicans* is the most frequent species isolated from the blood accounting for one-third to two-thirds of bloodstream isolates [51,52].

Pathological basis: Among the immunocompromised individuals candidiasis may cause superficial or invasive disease. The routes of invasion of *Candida* are given endogenous or exogenous. The endogenous route is the most important as *Candida* infections originate predominantly from the patient's own colonizing organisms from the gastrointestinal tract and skin. Infection however requires some defect in the normal host immunity. Breakdowns

Morphological features: Characteristics that set candidiasis apart are that in tissues *Candida* organisms appear as mats of yeasts measuring 3 to 5 m in diameter intermingled with pseudohyphae (also referred to as filaments) (Figure 2 A and B). The filaments may show periodic constrictions giving "Sheesh Kabab" appearance. The organisms can be seen with H & E, GMS and PAS stains (Figure 2 C and D). Histopathologic examination of specimens is very important to define invasion of tissues and vessels since growth from skin, lung and the gastrointestinal or genitourinary tract is only indicative of colonization [55]. The usual host reaction whether in superficial or invasive candidiasis consists primarily of neutrophilic inflammation with some lymphocytes, macrophages, fibrin and coagulative necrosis. Giant cells and granulomas can be seen but are sparse. As *Candida* organisms invade blood vessels, they can cause mycotic aneurysms or thrombophlebitis. Necrotizing vasculitis has been described in candidemia but organisms are not observed in the necrotic vessels supporting the concept that *Candida* soluble fractions cause the necrotizing lesions [49,56]. Morphologically superficial infections show minimal to suppurative inflammation [49]. Invasive disease shows various inflammatory responses; primarily suppurative inflammation with rare granulomas, invasion of blood vessels with necrotizing vasculitis [49].

Cryptococcosis

Human *Cryptococcosis* is caused by several *Cryptococcus* species including *C. neoformans* and *C. gattii*. *C. neoformans* is responsible for the majority of infections found in immunocompromised individuals [57].

Pathological basis: The most frequent predisposing immunocompromised factor for cryptococcal disease is HIV infection; however, other immunocompromised conditions associated with *Cryptococcosis* are underlying lung, liver or renal disease, immunosuppressive agents, malignancies and autoimmune diseases. From the lung, cryptococci can disseminate to the central nervous system (producing meningitis or cryptococcomas), skin, bones or other tissues. *C. gattii* is associated with a higher incidence of solid lesions in the lungs and brain. The frequency of disseminated disease is dependent on the immune status of the patient. An immunocompromised patient commonly presents with central nervous system involvement [58,59].

Morphological features: Histologically *Cryptococcosis* appear as encapsulated, spherical to oval yeast that measure 5 to 10 m in diameter and have narrow-based budding (Figure 3 A and B). A thick polysaccharide capsule gives these organisms the characteristic appearance of having a clear space around them that can be seen in tissue sections with hematoxylin and eosin stains. When testing Cerebrospinal Fluid (CSF), India ink can be used as a negative stain to highlight the capsule (Figure 3 C). Because of the capsule, the buds appear separate from the mother cells. The polysaccharide capsule stains with Alcian blue, Mayer's or Southgate's mucicarmine stain. As with all other yeasts, the wall of the organism stains with GMS and PAS stains [49,57,58]. Cryptococcoma is a granuloma with various degrees of necrosis and fibrosis seen more frequently with *C. gattii* among immunocompromised hosts. Cryptococcal pleural effusion is also common among these patients. Disseminated cryptococcal disease is characterized by abundant extracellular yeasts which efface tissue architecture by necrosis frequently extending to involve the CNS producing meningitis or cryptococcomas, skin, bones or other tissues [49].

Blastomycosis

Blastomycosis is a potentially fatal systemic and cutaneous fungal infection of humans and animals caused by *Blastomyces dermatitidis*, a thermally dimorphic fungus that exists in mycelial form in the soil of warm, moist, wooded areas that are rich in organic debris [60,61]. Due to widespread disease in Chicago, Blastomycosis is also referred to as "Chicago disease" [62].

Aspergillus, ranging from allergic responses in sinuses and lungs with colonization of lung cavities and formation of fungal balls to invasive pulmonary and disseminated organ involvement. Disseminated disease is seen primarily in patients with AIDS, primary immunodeficiencies, hematologic malignancies and transplants and/or corticosteroid recipients. In tissue the features of hyaline septate molds are similar to those seen with aspergillosis .The fungi invade vessels and cause thrombosis and necrosis of the surrounding tissues. Microscopically the hyphae are septate showing acute angle branching and are not pigmented [67].

Pneumocystis jirovecii

Pneumocystis is an interesting organism that has both protozoan and fungal characteristics and has been classified as both at different times in history. In addition, the species that is pathogenic to humans (previously named *P. carinii*) has recently been renamed *P. jirovecii* (PJ). Although the mode of transmission of Pneumocystis is not known, an airborne mechanism is suspected since the majority of infected patients present with pneumonia. Extra pulmonary infections result from dissemination from lungs to other organs, such as lymph nodes, spleen, bone marrow, liver, kidneys, heart, brain, pancreas and skin [69].

Pathological basis: *P. jirovecii* Pneumonia (PJP) has been the leading cause of morbidity and mortality in people with HIV for many years but with the widespread use of ART ((Anti-Retroviral Therapy) and anti-Pneumocystis prophylaxis. The incidence of PJP has declined particularly in high-resource countries where individuals have access to these treatments [69,70]. In contrast to the case for the HIV-infected population, the number of non-HIV-infected immunocompromised patients at risk for PJP has been increasing with the increasing use of organ transplantation, and(with) the introduction of anti-tumor necrosis factor alpha (anti-TNF-α) agents and other immunosuppressive agents or increasing number of patients with altered immune status [49,69,71,72]. Although the genetic susceptibility of HIV hosts to PJP has not been widely examined, polymorphism in the gene encoding the Fc segment of IgG (Fc γ R II a) has been shown to influence the risk of PJP [73]. The strongest risk factor for PJP in HIV patients is a $CD4^+$ cell count below 200 cells/μ l with the risk increasing the lower the $CD4^+$ count declines below this level. Previous PJP, oral candidiasis and persistent fevers are also risk factors for PJP [70]. Mortality in the non-HIV-infected immunosuppressed population is generally higher than that in HIV infection [71]. The $CD4^+$ cell count is less helpful in determining PJP risk in this population, although many patients do have low counts at the time of illness [74]. An imbalance of pro-inflammatory and anti-inflammatory cytokines in BALF was found in PJP of non-AIDS immunocompromised patients. BALF levels of IL-8, IL-8/IL-10 ratio, IL-1β/IL-10 ratio, IL-1β/TGF-β1 ratio, MCP-1/TGF-β1 ratio and IL-8/TGF-β1 ratio are been suggested to be of value in assessing the severity of PJP and in predicting the outcome of the patients [75].

Morphological features: *P. jiroveci* in a Papanicolaou-stained sample of BAL fluid shows a bubbly appearance of the "exudates" (Figure 4 A). The so-called foamy exudate is actually not an exudates but rather a massive collection of cysts and extra cystic forms of the fungus [76]. In tissue sections stained with H & E, pneumocystis pneumonia presents as foamy intra-alveolar eosinophilic exudate with minimal inflammatory infiltrate. In T cell–immunocompromised hosts the inflammatory response to the organism is minimal usually consisting of macrophages, lymphocytes and plasma cells. In pneumonia and disseminated disease affecting immunocompromised hosts there is minimal reaction with rare atypical reactions such as fibrosis and granulomas [76]. In Papanicolaou stained respiratory cytology specimens the organisms blend into the mucous blue-green background. GMS staining demonstrates that the foamy material in tissue sections or cytological specimens corresponds to multiple organisms which are thin-walled spheres of 2 to 5 m that have an intra-cystic focus (capsular dot) (Figure 4 B). Collapsed organisms are usually found

14. Tewari S, Chatterji R, Varghese S, Prasad B, Garg A (2001) Malakoplakia of lung in an immunocompromised patient. Medical Journal Armed Forces India 57: 63-65.

15. Piersimoni C (2012) Nontuberculous mycobacteria infection in solid organ transplant recipients. Eur J Clin Microbiol Infect Dis 31: 397-403.

16. Field SK, Fisher D, Cowie RL (2004) Mycobacterium avium complex pulmonary disease in patients without HIV infection. Chest 126: 566-581.

17. Corti M, Palmero D (2008) Mycobacterium avium complex infection in HIV/AIDS patients. Expert Rev Anti Infect Ther 6: 351-363.

18. Sadek M, Yue FY, Lee EY, Gyenes G, Jones RB, et al. (2008) Clinical and immunologic features of an atypical intracranial mycobacterium avium complex (MAC) infection compared with those of pulmonary MAC infections. Clin Vaccine Immunol 15: 1580-1589.

19. Aris F, Naim C, Bessissow T, Amre R, Artho GP (2011) AIRP Best Cases in Radiologic-Pathologic Correlation: Mycobacterium avium-intracellulare Complex Enteritis. Radiographics 31: 825-830.

20. Jarzembowski JA, Young MB (2008) Nontuberculous Mycobacterial Infections. Arch Pathol Lab Med 132: 1333-1341.

21. Sanchez TH, Brooks JT, Sullivan PS, Juhasz M, Mintz E, et al. (2005) Bacterial diarrhea in persons with HIV infection, United States, 1992-2002. Clin Infect Dis 41: 1621-1627.

22. Brown M, Eykyn SJ (2000) Non-typhoidal salmonella bacteremia without gastroenteritis: a marker of underlying immunosuppression. Review of cases at St. Thomas' hospital 1970–1999. J Infect 41: 256-259.

23. Gordon MA (2008) Salmonella infections in immunocompromised adults. J Infect 413-422.

24. Takahito Takezawa, Shunji Hayashi, Yoshikazu Adachi, Keijiro Sunada, Yoshikazu Hayashi, et al. (2012) Human intestinal spirochetosis in an immunocompromised host: evaluation of eradication therapy by endoscopy, histopathology and bacteriology. Clinical Journal of Gastroenterology 5: 69-73.

25. Dasgupta A, Singh N, Bhatia A (2009) Abdominal Tuberculosis: A Histopathological Study with Special Reference to Intestinal Perforation and Mesenteric Vasculopathy. J Lab Physicians 1: 56-61.

26. Beck A, McNeil C, Abdelsayed G, Chin-Lue R, Kassis S, et al. (2007) Salmonella pseudomembranous colitis. Conn Med 71: 339-342.

27. Papaconstantinou HT, Thomas JS (2007) Bacterial Colitis. Clin Colon Rectal Surg 20: 18-27.

28. SC Li (2000) Biopsy diagnosis of intestinal infectious diseases. Current Diagnostic Pathology 6: 206-212.

29. Lamps LW (2007) Infective disorders of the gastrointestinal tract. Histopathology 50: 55-63.

30. Mosepele M, Mazo D, Cohn J (2012) Bartonella Infection in Immunocompromised Hosts: Immunology of Vascular Infection and Vasoproliferation Clin Dev Immunol 2012: 612809.

31. Minnick MF, Battisti JM (2009) Review Pestilence, persistence and pathogenicity: infection strategies of Bartonella. Future Microbiol 4: 743-758.

32. Resto-Ruiz S, Burgess A, Anderson BE (2003) Review The role of the host immune response in pathogenesis of Bartonella henselae. DNA Cell Biol 22: 431-440.

33. Chiaraviglio L, Duong S, Brown DA, Birtles RJ, Kirby JE (2010) An immunocompromised murine model of chronic Bartonella infection. Am J Pathol 176: 2753-2763.

34. Chetty R, Sabaratnam RM (2003) Upper gastrointestinal bacillary angiomatosis causing hematemesis: a case report. Int J Surg Pathol 11: 241-244.

35. Lindeboom JA, Bruijnesteijn van Coppenraet LE, van Soolingen D, Prins JM, Kuijper EJ (2011) Clinical manifestations, diagnosis, and treatment of Mycobacterium haemophilum infections. Clin Microbiol Rev 24: 701-717.

36. Geisler WM, Harrington RD, Wallis CK, Harnisch JP, Liles WC (2002) Broad spectrum of dermatologic manifestations caused by Mycobacterium haemophilum infection. Arch Dermatol 138: 229-230.

37. Dias M, Shreevidya K, Rao SD, Shet D (2010) Corynebacterium macginleyi a rare bacteria causing infection in an immunocompromised patient. J Cancer Res Ther 6: 374-375.

61. McKinnell JA, Pappas PG (2009) Blastomycosis: new insights into diagnosis, prevention, and treatment. Clin Chest Med 30: 227-239.

62. Patel AJ, Gattuso P, Reddy VB (2010) Diagnosis of blastomycosis in surgical pathology and cytopathology: correlation with microbiologic culture. Am J Surg Pathol 34: 256-261.

63. Crampton TL, Light RB, Berg GM, Meyers MP, Schroeder GC, et al. (2002) Epidemiology and clinical spectrum of blastomycosis diagnosed at Manitoba hospitals. Clin Infect Dis 34: 1310-1316.

64. Bariola JR, Perry P, Pappas PG, Proia L, Shealey W, et al. (2010) Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. Clin Infect Dis 50: 797-804.

65. Lemos LB, Guo M, Baliga M (2000) Blastomycosis: organ involvement and etiologic diagnosis. A review of 123 patients from Mississippi. Ann Diagn Pathol 4: 391-406.

66. Naggie S, Perfect JR (2009) Molds: hyalohyphomycosis, phaeohyphomycosis, and zygomycosis. Clin Chest Med 30: 337-353.

67. Nucci M, Anaissie E (2007) Fusarium infections in immunocompromised patients. Clin Microbiol Rev 20: 695-704.

68. Busemann C, Krüger W, Schwesinger G, Kallinich B, Schröder G, et al. (2009) Myocardial and aortal involvement in a case of disseminated infection with Fusarium solani after allogeneic stem cell transplantation: report of a case. Mycoses 52: 372-376.

69. Kelley CF, Checkley W, Mannino DM, Franco-Paredes C, Del Rio C, et al. (2009) Trends in hospitalizations for AIDS-associated Pneumocystis jirovecii pneumonia in the United States (1986 to 2005). Chest 136: 190-197.

70. Morris A, Norris KA. Colonization by Pneumocystis jirovecii and its role in disease. (2012) Clin Microbiol Rev 25: 297-317.

71. Green H, Paul M, Vidal L, Leibovici L (2007) Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients: systematic review and meta-analysis of randomized controlled trials. Mayo Clin Proc 82: 1052-1059.

72. Krajicek BJ, Thomas CF Jr, Limper AH (2009) Pneumocystis pneumonia: current concepts in pathogenesis, diagnosis, and treatment. Clin Chest Med 30: 265-278.

73. Forthal DN, Landucci G, Bream J, Jacobson LP, Phan TB, et al. (2007) Fcgamma RIIa genotype predicts progression of HIV infection. J Immunol 179: 7916-7923.

74. Enomoto T, Azuma A, Kohno A, Kaneko K, Saito H, et al. (2010) Differences in the clinical characteristics of Pneumocystis jirovecii pneumonia in immunocompromised patients with and without HIV infection. Respirology 15: 126-131.

75. Chou CW, Lin FC, Tsai HC, Chang SC (2013) The importance of pro-inflammatory and anti-inflammatory cytokines in Pneumocystis jirovecii pneumonia. Med Mycol [Epub ahead of print].

76. Thomas CF Jr, Limper AH (2004) Pneumocystis pneumonia. N Engl J Med 350: 2487-2498.

77. Hartel PH, Shilo K, Klassen-Fischer M, Neafie RC, Ozbudak IH, et al. (2010) Granulomatous reaction to Pneumocystis jirovecii: clinicopathologic review of 20 cases. Am J Surg Pathol 34: 730-734.

Micro-elements: are those required in the neighborhood of 10-6 m or less and they include Iron,Copper, Manganese, Zinc and Molybdenum. Other elements that have been found to be essential are; Scandium, Vanadium and Gallium. They also require Vitamins for growth such as VitaminB1 (thiamin), B2 (biotin), B3 (nicotinic acid), B5 (pantothenic acid), B12 (cyano) Niacin, Pyridoxine and P-Aminobenzoic acid. Those fungi that require vitamin for growth are described as auxotropic fungi and those that can synthesize the vitamin they need are described asprototropic fungi.

In humans, mycosis often occurs in immunocompromised patients. And fungi are common problems in the immunocompromised patient as the causative agents of skin, nail or infections especially the *Aspergillus* species.

Aspergillus species are found worldwide, and widely distributed in the environment. *Aspergillus* belongs to the phylum Ascomycota. They are aerobic organisms found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate. These species are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. In addition to growth on carbon sources, many of them demonstrate oligotrophy where they are capable of growing in nutrient-depleted environments, or environments in which there is a complete lack of key nutrients. Example is *Aspergillus niger*.

Aspergillus is important medically and commercially. Some species can cause infection in humans and other animals. More than 60 *Aspergillus* species are medically relevant pathogens.

Some *Aspergillus* species cause serious disease in humans and animals. The most common causing pathogenic species are *Aspergillus fumigatus* and *Aspergillus flavus*. *Aspergillus flavus* produces aflatoxin which is both a toxin and a carcinogen, and which can contaminate foods such as nuts. The most common causing allergic diseases are *Aspergillus fumigatus* and *Aspergillus clavatus*. Other species are important as agricultural pathogens. They also cause diseases on many grain crops. Other species less commonly isolated as opportunistic pathogens are an *Aspergillus glaucus* group, *Aspergillus nidulans, Aspergillus oryzae*, *Aspergillus terreus* and *Aspergillus versicolor.*

The most common etiologic agents of Aspergillosis are *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *A. nidulans* and *A. terreus.* They occur chiefly in immunocompromised persons. Invasive infections caused by *Aspergillus* have been associated with high rates of morbidity and mortality especially in immune compromised individuals. They are the second most recovered fungal pathogens after *Candida*. These groups of diseases caused by *Aspergillus,* is known as **Aspergillosis**. Immune compromised individuals, suffer a lot of Aspergillosis.

There are several forms of Aspergillosis which include:

Allergic Bronchopulmonary Aspergillosis or ABPA: This type is caused by an allergic reaction to the spores the fungus. It usually develops in people who have lung problems (such as asthma, sinusitis and cystic fibrosis). It is the mildest form of aspergillosis.

Aspergilloma: It is a growth (fungus ball) that develops in an area of past lung disease (Such as tuberculosis or lung abscess) or that can form within the lungs. It is common in people who have cystic fibrosis or cavities (spaces) in their lungs. Coughing up blood (hemoptysis) is one of the most common symptoms of aspergilloma. *Aspergillus* fungi enter the lungs and group together to form a dense knot of fungi, called a fungal ball. Aspergilloma usually only affects people who have an existing lung condition, such as tuberculosis (TB), which means that they have cavities or damage in their lungs.

Invasıve Aspergillosıs: This type is a serious infection with pneumonia that can spread to other parts of the body. It is more common with people with a weakened immune system

Treatment

A fungus ball is usually not treated (with antifungal medicines) unless there is bleeding into the lung tissue. In that case, surgery is needed. Invasive aspergillosis is treated with several weeks of an antifungal drug called voriconazole. It can be given by mouth or directly into a vein (IV). Amphotericin B, echinocandins, oritraconazole can also be used.

Endocarditis caused by *Aspergillus* is treated by surgically removing the infected heart valves.

Long-term antifungal therapy is also needed.

Antifungal drugs alone do not help people with allergic aspergillosis. Allergic aspergillosis is

Treated with drugs that suppress the immune system (immunosuppressive drugs) -- most often

Prednisone taken by mouth.

Prevention of Aspergillosis

Be careful when using medications that suppress the immune system. Preventing AIDS also prevents certain diseases, including aspergillosis, that are associated with a damaged or weakened immune system.

- Mycotic infections are classified by the tissue levels that are colonized.**Superficial infections** are generally limited to the outer layers of the skin and hair.
- **Cutaneous infections** are located deeper in the epidermis, hair and nails.
- **Subcutaneous infections** involve the dermis, subcutaneous tissues and muscle.

References

1. Aspergillosis (2012) Retrieved on 2012- 04- 07.
2. Aspergillosis (2012) Retrieved on 2012- 11- 11.
3. The Free Encyclopedia Retrieved on 2011- 12- 12.
4. The Free Encyclopedia Retrieved on 2012- 03- 08.
5. The Free Encyclopedia Retrieved on 2012- 13- 10.

Gynecologic infections are a microbial phenomenon characterized by an inflammatory response to the presence of microorganisms or invasion of normally sterile host tissue. In many cases, these infections are transmitted via sexual intercourse. When unrecognized, or severe, these infections may result in bacteremia, sepsis, septic shock and death. The clinical manifestations of sepsis are caused by the body's inflammatory response to toxins and other components of microorganisms [1].

Immunocompetent women seeking medical care have the advantage of having these infections readily diagnosed given their clinical manifestations and treated with prompt antimicrobial therapy. Immunocompetent hosts also have the advantage of clearing the infections more readily resulting in quicker recovery periods with less complicating sequelae.

Uncommon cases, such as those presenting in immunocompromised hosts, often fail to mount a proper immune response to gynecologic infections. This results in difficulty in early detection and an infectious process that may go unrecognized without a careful assessment of clues available through physical examination and laboratory testing. A recent study found that hydrogen peroxide plays a role in the immune system in certain fish and humans [2]. In the study, scientists found that hydrogen peroxide within cells increased after tissues were damaged in zebra fish, which is thought to act as a signal to white blood cells to converge on the site and initiate the healing process. When the genes required to produce hydrogen peroxide were disabled, white blood cells did not accumulate at the site of damage. The experiments were conducted on fish; however, because fish are genetically similar to humans, the same process is speculated to occur in humans. This explains why immunocompromised patients are less likely to clear many infections so readily, specifically gynecological infections. However, even with an intact functioning immune system, these vulnerable hosts may come in contact with virulent pathogens that can lead to severe infections, septic shock and death, despite acting apprehensively and appropriately.

Unfortunately, pregnancy itself harbors an immunocompromised state, thereby, increasing the likelihood of severe infections when a virulent microorganism is paired with an infectious procedure complication such as induced abortions, vaginal delivery, more especially with episiotomies and cesarean deliveries. Gynecological procedures, such as hysterectomies, with concomitant medical co-morbidities (i.e. diabetes mellitus) may further increase the likelihood of severe infections to occur [3]. Clinicians often rely on the basics of history and physical examination to guide further diagnostic workup and treatment of women presenting with complaints after these procedures.

The association between preterm birth and infection is well known and long established. Systemic infections such as pyelonephritis and pneumonia are associated with preterm delivery, but ascending vaginal infection may be the most common and critical pathway. Ascending microbes reach the membranes and amniotic fluid by ascending into the cervix and uterus from the vagina and incite an inflammatory response. This maternal immune activation (MIA) can result in unwanted consequences, such as preterm birth, neonatal brain injury and low birth weight [4].

Homelessness is a major global problem, with estimates of up to 100 million people worldwide being vagrant, of which at least 600,000 are in the United States. Homeless women are at a high risk for rape, commercial sex working and abusing illicit drugs, such as needles from other immunocompromised hosts with HIV and hepatitis [5]. In addition to becoming immunocompromised, they are less likely to seek medical attention for gynecological infections. This consequentially places them at a higher risk of complications, including bacteremia, sepsis, septic shock and death.

Pathophysiology for Gynecological Infections

It is important to understand the fundamental stages of infection, defined as a microbial

and *Trichomonas vaginalis* [9]. Other normally inhabiting microorganisms consist of *Aerobic* gram-negative and gram-positive bacteria, some of which harbor devastating toxins. Although these microorganisms tend to keep their toxins inactivated, severe gynecological infections can occur if they are activated.

Each year, over 6 million healthcare visits are due to some form of vaginitis, with more than a billion dollars spent annually on assessment and treatment. Bacterial vaginosis is perhaps the most common form of vaginitis, accounting for approximately 12-50% of occurrences [10]. According to the National Health and Nutrition Examination Survey (NHSES), a national population-based study of 12,000 women aged 14-49 years old who self-collected their own vaginal swabs were analyzed with the Nugent Gram stain criteria [11]. The prevalence of bacterial vaginosis was 29.2%. Non-Hispanic blacks accounted for 51.6%, Mexican Americans accounted for 32.1% and non-Hispanic Whites accounted for 23.2%. Despite adequate therapy, recurrent bacterial vaginosis is common, with up to 80% in some studies.

Etiologic Causes of Gynecological Infections in Immunocompromised Hosts

Aerobic gram-negative bacteria harbor endotoxins within a disaccharide core consisting of a lipoidal acylated glucosamide. Two highly conserved endotoxins found within gram-negative bacteria are lipid A and lipopolysaccharide (LPS) release cytokines (i.e. tumor necrosis factor α) and other immune modulators that mediate the clinical manifestations of sepsis. *Aerobic* gram-positive organisms mediate sepsis by 2 main mechanisms: (1) cell wall components, such as peptidoglycan, teichoic acids and surface proteins; (2) release of various toxins, both endotoxins and exotoxins, such as toxic shock syndrome toxin-1 released by *Staphylococcus aureus* [12].

In addition to organisms that may typically infect normal hosts, immunocompromised patients have increased susceptibility to infections with organisms of little native virulence in normal individuals. *Group A streptococcus* (GAS) is rarely present, but may harbor, the normal vaginal flora from inoculation of the women's own pharynx or from a close contact source [13]. GAS is known to harbor a variety of virulence factors, such as M-protein, streptokinase, streptolysin S and O and streptococcal chemokine protease enzymes, which stimulate the release of interleukin-1, tumor necrosis factor α, interleukin-6 and arachidonate metabolites from human monocytes. These virulence factors result in clinical invasive GAS gynecological infections, such postpartum endometritis, wound infections, necrotizing fasciitis and toxic shock syndrome . The differential diagnoses of the potential microbes that lead to gynecological infections in immunocompromised host are broad and may include:

I. Encapsulated bacteria, such as *Group B streptococcus* (GBS) is of great concern, specifically during the immunocompromised state of pregnancy, which is known to cause infections in both the mother and her newborn child. In the mother, GBS is associated with vaginitis, cervicitis, endometritis, sepsis, meningitis and pneumonia, whereas in the newborn, GBS is associated with conjunctivitis, meningitis, pneumonia and sepsis. During pregnancy, chorioamnionitis can also be seen with GBS.

II. *Aerobic* bacteria— *Neisseria gonorrhoeae, Staphylococcus, Streptococcus, E. coli, Mycoplasma genitalium, Haemophilus ducreyi and Klebsiella granulomatis*

III. *AnAerobic* bacteria – *Chlamydia trachomatis, Trichomonas vaginalis, Listeria monocytogenes, Gardnerella vaginalis, Peptostreptococcus, Prevotella brevis, Mobiluncus, Mycoplasma species , Clostridium, Haemophilus ducreyi, Actinomyces israelii and Bacteroides*

IV. Spirochete - *Treponema pallidum*

plays a role [17]. BV is therefore considered to be a sexually "related" disease rather than sexually transmitted disease. Untreated or immunocompromised women are more likely to suffer from significant adverse sequelae due to BV, such as pelvic inflammatory disease, particularly after induced abortions and hysterectomies, resulting in postoperative cuff infections and abnormal cervical cytology. Pregnant women, especially those severely immunocompromised, are at high risk for premature rupture of membranes, preterm labor and delivery, chorioamnionitis and post-cesarean endometritis [18]. *See Section 4.4 for diagnostic algorithm and Section 4.5 for treatment.*

Trichomonas vaginitis is a sexually transmitted disease associated with an *AnAerobic* flagellated parasitic microorganism known as *Trichomonas vaginalis*. In many occasions, immunocompromised patients who contract this infection often have other concomitant gynecological infections, including BV, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, syphilis and HIV [19]. As with BV, immunocompromised patients with *Trichomonas* vaginitis left untreated or undertreated are at an increased risk of postoperative cuff cellulitis following a hysterectomy. Every patient scheduled to have a hysterectomy, whether via vaginal, abdominal, or minimally invasive technique, should have testing and treatment of *Trichomonas* and BV prior to their surgery. Immunocompromised pregnant patients also carry an additional risk of premature rupture of membranes and preterm delivery. Studies have shown that patients who acquire *Trichomonas* have a three times increased likelihood of acquiring HIV. Immunocompromised patients also carry an additional risk of acquiring chronic HPV infections, which can ultimately increase their risk of cervical cancer [20]. According to the CDC guidelines, all immunocompromised hosts should be tested for *Trichomonas* annually [21]. Immunocompromised hosts, such as those with HIV, who are infected with *Trichomonas vaginalis*, should be treated with a 7-day course of metronidazole; rather than a one-time dose of 2 grams orally as done with HIV-negative patients. For non-pregnant patients, a test of cure should be performed 3 months after receiving therapy, as well as testing, diagnosing and treating all partners involved. Immunocompromised hosts who have an allergy to metronidazole should be treated with a desensitization protocol; whereby, they are hospitalized and treated with escalating doses of metronidazole or tinidazole under close observation. *See Section 4.4 for diagnostic algorithm and Section 4.5 for treatment.*

Vulvovaginal candidiasis (VVC) is very common in immunocompromised patients, with estimates of at least 75% of hosts experiencing at least one episode during their lifetimes and over 50% of these hosts having recurrent infections even after adequate therapy [22]. Immunocompetent patients have the advantage of clearing their primary infection more readily with few having recurrent infection. *Candida albicans*, a dimorphic fungus that exists as a blastospore, has the ability to transform into mycelia as a result of blastospore germination. This transformation gives this fungus the ability to colonize the lower genital tracts of many immunocompromised hosts without any symptoms; however, tissue invasion may ultimately incur. When the invasion process occurs, these fungi are capable of releasing extracellular toxins, which accounts for the symptoms of pruritus and inflammation surrounding their lower genital tracts [23]. *Candida albicans* is responsible for at least 90% of vaginal yeast infections, which respond well to most over-the-counter antifungal vaginal suppository agents. For this reason, it is critical that a proper work-up with fungal cultures be performed when immunocompromised patients present with symptoms of VCC. *Candida glabrata* and *Candida kruseii* have been known to be resistant to many traditional antifungal agents. The most vulnerable immunocompromised hosts that suffer from recurrent symptomatic disease, defined as ≥ 4 per year, include patients with advanced or uncontrolled HIV, diabetes mellitus, pregnancy and chronic antibiotic use. Most experts agree that the main reason for their recurrent disease is their inability to mount a proper cell-mediated immunity, thereby, leading to a higher incidence of candidiasis [24]. *See Section 4.4 for diagnostic algorithm and Section 4.5 for treatment.*

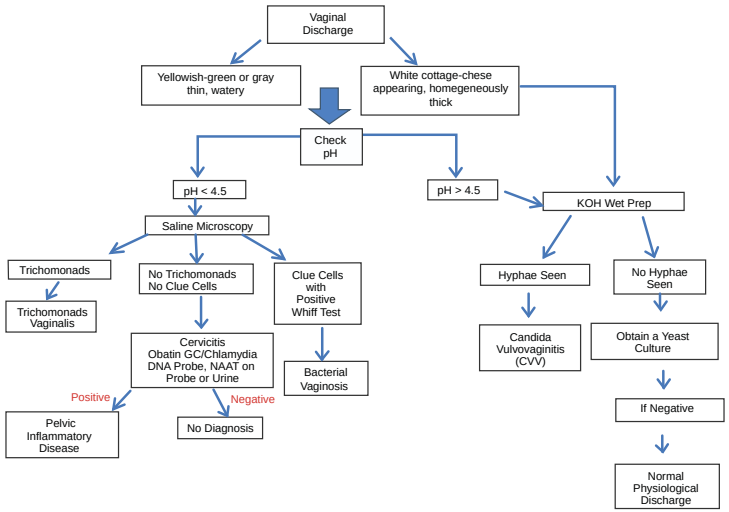
causes of genital ulcers caused by *herpes Simplex* virus (HSV) and syphilis in the United States. Immunocompromised patients also have the added list of acquiring additional ulcers, such as chancroid, caused by *Haemophilus ducreyi*, lymphogranuloma venereum (LGV) caused by *Chlamydia trachomatis* serovars L1, L2, L2a or L3, and granuloma inguinale (donovanosis) caused by *Klebsiella granulomatis* [31]. A thorough physical examination and history is crucial when assessing a patient with a genital ulcer(s). Optimally, the evaluation of a patient with a genital ulcer should include dark-field microscopy or direct immunofluorescence testin for *Treponema pallidum*, culture of antigen testing for HSV, and culture for *Haemophilus ducreyi*. Many immunocompromised hosts, particularly those who are homeless or seek very little medical attention, develop genital ulcers begin as small ulcers with time and expand and encompass nearly their entire genital area with time. Of note, approximately ¼ of these diagnoses remain unconfirmed.

Herpes Simplex is a viral disease from the *herpes*viridae family caused by both *Herpes Simplex* Virus type 1 (HSV-1) and type 2 (HSV-2). Infection with the *herpes* virus is categorized into one of several distinct disorders based on the site of infection. Transmission may also occur via skin contact during periods of asymptomatic shedding. Condom contraception has been shown to decrease transmission rate [31]. Recurrent infections may occur more especially with immunocompromised hosts. These hosts also have higher tendencies to have more outbreaks related to a variety of stimuli and stressors, such as more frequent bacterial and viral infections, other sexually transmitted diseases and longer recovery periods from prior infections [32]. Grouped vesicles mixed with small ulcers, particularly with a history of such lesions, are almost always pathognomonic of genital *herpes*. Due to the broader range of tissue, the psychological aspect that follows such ulcers alters their self-image and affects their perceived ability to enter new sexual relationships and bear children. Of all the tests available for making a diagnosis of HSV, culture remains the most sensitive and specific test, with sensitivities reaching as high as 100% and specificities as high as 89% in the pustular stage. However, outside the pustular stage, genital PCR remains the test of choice in making the diagnosis of HSV [33].

Immunocompromised patients may have higher false-negative results with HSV cultures, especially with recurrent disease. Therefore, the use of type-specific immunoglobulin assays (IgG and IgM) for both HSV 1 and 2 is more useful in making the diagnosis of genital *herpes*. Direct fluorescent antibody testing (DFA) can also be used in establishing a diagnosis, which has similar sensitivities to a culture. However, the sensitivity and specificity drops by at least 50% in patients who have genital ulcers 3 or more days after the initial eruption of the lesions. Studies have shown that immunocompromised hosts are at high risk for resistant HSV to standard therapy with either acyclovir or valacyclovir, with reports indicating at least 5-15% of HIV positive patients (1% of the entire population) having resistance [34]. Sensitivities for acyclovir, ganciclovir, foscarnet and cidofovir should be tested in cases with suspected HSV resistance. *See Section 4.5 for treatment.*

Another common sexually transmitted infection more commonly seen in immunocompromised hosts is syphilis. Women who acquire syphilis, caused by the spirochete bacterium *Treponema pallidum*, during pregnancy, particularly during the first trimester are at higher risk for transmitting the infection to the fetus, resulting in congenital syphilis [35]. With four different stages of syphilis, the signs and symptoms vary with each stage. The primary stage classically presents with a single firm, painless, non-itchy chancre. Secondary syphilis involves a diffuse rash frequently involving the palms and soles. Latent syphilis may present with little to no symptoms while tertiary syphilis may present with gummas, neurological, or cardiac symptoms. There are two available sensitive tests used for diagnosing this infection, a nontreponemal rapid plasma reagin test (RPR) and a venereal disease research laboratory (VDRL) test. If any of these tests are positive, a confirmation should be followed, using either a fluorescent treponemal antibody absorption test (FTA ABS) or a microhemagglutinin-*T. pallidum* test (MHA TP) [36]. Patients with an elevated RPR

for other types of HPV strains that may be present, these immunocompromised hosts are at high risk of developing gynecological malignancies, including vulvar, vaginal and cervical cancers. These hosts are also at higher risk for recurrences secondary to a reactivation of the subclinical infection [42]. Treatment typically consists of excision, as eradication of the viral infection is not possible. Practicing safe sexual behavior can decrease the risk of primary and recurrent HPV infections. *See Section 4.5 for treatment.*



Section 4.4: Diagnostic Algorithm for Gynecological Infection.

Vaginal Infections			
Disease	**Microorganism(s) Involved**	**Preferred Treatment**	**Alternative Treatment**
Bacterial Vaginosis	*1) Gardnella vaginalis* *2) Prevotella* *3) Peptostreptococcus* *4) Mobiluncus* *5) Bacteroides* *6) Mycoplasma Species*	Metronidazole 500 mg PO BID x 7 days Metronidazole Gel 0.25%, one applicator (5 gm) intravaginally once or twice daily for 5 days	Clindamycin 300 mg PO BID x 7 days Clindamycin Cream 2%, one applicator (5 gm) intravaginally for 7 days Clindamycin Ovules 100 mg, intravaginally every night for 3 days Clindamycin Bioadhesive Cream 2%, 100 mg intravaginally in a single dose
******Trichomonas*	*Trichomonas vaginalis*	Metronidazole 500 mg PO BID x 7 days Metronidazole Gel is not effective against *Trichomonas*	Metronidazole 2 gms Po daily x 5 days Tinidazole 2gms PO daily x 5 days
Vulvovaginal Candidiasis	*1) Candida albicans* *2) Candida glabrata* *3) Candida tropicalis*	Fluconazole 150 mg PO x 1 dose *give an additional dose 72 hours after first dose for complicated cases plus hydrocortisone cream 1% to help relieve external irritation	Topical Agents Clotrimazole - 1% cream, 5 gm intravaginally for 7-14 days - 100 mg vaginal tablet for 7 days - 100 mg vaginal tablet, 2 per day for 3 days - 500 mg vaginal tablet x 1 dose Butoconazole - 2% cream, 5 gm intravaginally for 3 days - 2% cream, 5 m BI-BSR, single intravaginal application Nystatin - 100,000 units vaginal tablet Daily for 14 days Ticonazole - 6.5% ointment, 5 gm intravaginally x 1 dose Terconazole - 0.4% cream, 5 gm intravaginally for 7 days - 0.8% cream, 5 gm intravaginally for 3 days

tuberculosis and *Pneumocystis jirovecii* pneumonia, formally known as Pneumocystis carinii pneumonia (PCP). As a result of this broad spectrum of infections, the initial evaluation of an HIV-positive woman includes screening for diseases associated with HIV, administration of recommended vaccinations (hepatitis A/B, pneumococcal and influenza), and behavioral and psychosocial counseling. HPV lesions, as with intraepithelial neoplasia, occur more frequently in HIV-positive women. For this reasoning, annual pap smears starting at the age of 21 years old is recommended [44]. Some non-randomized, non-blinded studies found that HIV-positive women who are virologically suppressed follow a similar course of HPV related infectious progression in the genital tract [45]. However, current recommendations still recommend annual pap smears in all HIV-positive women, irregardless of their HIV viral load [46]. As mentioned in the previous sections, immunocompromised hosts, such as those with HIV, tend to have a decreased ability to clear most acute gynecological infections readily and adequately, and in turn, face higher risks of complications secondary to disease-progression sequelae. In general, all gynecological infections should always be addressed and treated promptly to decrease morbidity and mortality, keeping in mind that recurrences are very common in patients who have a dysfunctional immune system.

Conclusion

With an intact functioning immune status, the majority of gynecologic infections can be diagnosed and treated effectively with few recurrences. However, immunocompromised hosts with untreated gynecologic infections have an increased morbidity and mortality rate. The major advantage of an intact immune system is the ability to clear an infection readily and adequately. The inability to mount a proper cell-mediated immune response can be detrimental to immunocompromised hosts. Healthcare providers should be keen to the likelihood of infections with ordinarily less virulent organisms during immunocompromised states and be able to adequately manage their infections in order to reduce relapses, complications, morbidity and mortality.

References

1. Bone RC (1996) The sepsis syndrome. Definition and general approach to management. Clin Chest Med 17: 175-181.
2. Martin P, Feng Y (2009) Inflammation: Wound healing in zebrafish. Nature 459: 921-923.
3. Soper DE (2012) Early recognition of serious infections in obstetrics and gynecology. Clin Obstet Gynecol 55: 858-863.
4. Romero R, Mazor M, Munoz H, Gomez R, Galasso M, et al. (1994) The preterm labor syndrome. Ann N Y Acad Sci 734: 414–429.
5. Rimawi BH, Mirdamadi M, John JF (2013) Infections in the homeless and the rise and risk in displaced homeless women. The Global Journal (In Press).
6. Soper DE (2007) Genitourinary Infections and sexually transmitted diseases. (14thedn), Lipponcott Williams and Wilkins, USA.
7. Vasquez A, Jakobsson T, Ahrne S, Forsum U, Molin G (2002) Vaginal lactobacillus flora of healthy Swedish women. J Clin Microbiol 40: 2746–2749.
8. Eschenbach DA, Davick PR, Williams BL, Klebanoff SJ, Young-Smith K (1989) Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with vaginal vaginosis. J Clin Microbiol 27: 251-256.
9. Larsen B (1994) Obstetric and gynecologic infectious disease. Raven Press, New York, USA.
10. Kent HL (1991) Epidemiology of vaginitis. Am J Obstet Gynecol 165: 1168-1176.
11. Allsworth JE, Peipert JF (2007) Prevalence of bacterial vaginosis: 2001-2004 Prevalence of Bacterial Vaginosis: 2001–2004 National Health and Nutrition Examination Survey Data. Obstet Gynecol 109: 114-120.
12. Spiegel CA, Amsel R, Eschenbach D, Schoenknecht F, Holmes KK (1980) *AnAerobic* bacteria in nonspecific vaginitis. N Engl J Med 303: 601-607.

39. Rapini, Ronald P, Bolognia, Jean L, Jorizzo, et al. (2007) Dermatology: 2-Volume Set. St. Louis: Mosby.

40. Jeffrey DK, Edward WH III (2007) CURRENT Diagnosis & Treatment of Sexually Transmitted Diseases. McGraw-Hill Companies, Inc., New York, USA.

41. O'Farrell N (2002) Donovanosis. Sex Transm Infect 78: 452–457.

42. Beutner KR, Reitano MV, Richwald GA, Wiley DJ (1998) External genital warts: report of the American Medical Association Consensus Conference. AMA Expert Panel on External Genital Warts. Clin Infect Dis 27: 796-806.

43. Centers for Disease Control and Prevention (CDC) (2004) Diagnoses of HIV/AIDS--32 States, 2000-2003. MMWR Morb Mortal Wkly Rep 53: 1106-1110.

44. United States Preventive Services Task Force (2012) Guide to Clinical Preventive Services. (2ndedn), Williams and Wilkins, Baltimore, USA.

45. Mbizvo MT, Chou D, Shaw D (2013) Today's evidence, tomorrow's agenda: Implantation of strategies to improve global reproductive health. Int J Gynaecol Obstet 121: 3-8.

46. Pokharel S, Rimawi RH, Siraj D (2013) Medical Care of Pregnant Women in Eastern North Carolina with *Human immunodeficiency virus*. South Med J 106: 196-200.

2010, including 390,000 among children less than 15. Thus, HIV represents a major health and economic burden to the global society.

HIV renders the host immunocompromised and more susceptible to get other STIs. Both STDs and HIV infection are behaviorally and biologically intertwined and facilitate the sexual transmission of one another. Such interrelationship is known as epidemiologic synergy and has been studied previously. It has been shown that HIV infection increases the prevalence of some STDs at the community level and prior STI/STD increases HIV transmission risk by approximately 3-5 folds [2-4]. People with some bacterial STIs, such as Gonorrhea, Chlamydia, or Syphilis are at increased risk for HIV as well. Prevalent *herpes* simplex virus-2 (HSV-2) infection is also known to be associated with a 3 fold increased risk of HIV acquisition among both men and women in the general population [5]. Some STDs may promote HIV transmission by a variety of biological mechanisms that affect both HIV infectiousness and susceptibility. For example, other STDs facilitate HIV shedding in the genital tract, which probably promotes HIV infectiousness. This has been demonstrated by testing genital secretions for the presence and concentrations of HIV. Moreover, STDs potentially increase the HIV susceptible inflammatory cells in the genital tract and disrupts mucosal barriers that may increase the susceptibility to HIV [6]. Given the increase in such epidemiological synergy, it is particularly important to diagnose, treat, and prevent such STIs or STDs.

STD caused by Bacteria

Some of the most common bacteria that cause STD or STI are *Chlamydia trachomatis* that causes Chlamydia, *Neisseria gonorrhoeae* that causes Gonorrhea, and *Treponema pallidum* that causes Syphilis. An estimated 78 to 330 million new cases of genitourinary tract infections due to *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Ureaplasma urealyiticum* and *Mycoplasma genitalium* are diagnosed each year worldwide1. Of these, 5 to 12 million are diagnosed in North America alone [7].

Chlamydia

C. trachomatis infection is the most commonly reported notifiable disease worldwide including developed countries like the United States (U.S.) [1], United Kingdom [8], and Australia [9]. It is among the most prevalent of all STDs, and since 1994, has comprised the largest proportion of all STDs reported to CDC. Studies also demonstrate the high prevalence of chlamydial infections in the general population especially sexually active younger females [1,8-11]. Based on estimates from national surveys, Chlamydia accounts for more than 50% of all reported cases of infectious disease in Canada and the U.S. [9,12]. Chlamydia can lead to serious health consequences in females, such as pelvic inflammatory disease, infertility, ectopic pregnancy and chronic pelvic pain [13,14]. It also causes increased risks of HIV transmission and acquisition [1,6,15], eye and lung disease in newborn infants [16,17], and epididymo-orchitis in men [9]. In some cases, *C. trachomatis* may also cause pneumonia in adult immunocompromised and HIV positive hosts [18,19].

Syphilis

Syphilis is a genital ulcerative disease that is caused by Gram-negative bacteria, *Treponema pallidum*, and is categorized into primary, secondary, and tertiary stages. Syphilis causes significant complications if untreated and facilitates the transmission and acquisition of HIV infection [2,4]. Many individuals with early syphilis are either truly asymptomatic or undiagnosed; in the absence of diagnosis and treatment, approximately one-third of individuals will progress to neurological complications, visual and hearing

STIs are now shown in young population, sex education in school can help prevent such infections. According to a WHO report, a large majority of school-based sex education and HIV education interventions reduced the number of reported risky sexual behaviors in developing countries [31]. Furthermore, comprehensive mass media, such as radio, television, etc. are also valuable in teaching young population about appropriate sexual behavior and preventive measures [32].

Emerging scientific interventions

Historically, STDs and STIs have been managed through various behavioral interventions methods mentioned above. With the new advances in science, however, we have developed several potential emerging technologies, including microbicides and prophylactic vaccines to fight against some STIs. The vaccine against hepatitis B and Human Papillomavirus (HPV) are currently available and are recommended by the U.S. Centers for Disease Control and Prevention. However, many research efforts are underway to develop vaccines against other bacterial and viral pathogens, such as *N. gonorrhea*, *C. trachomatis*, *herpes* simplex virus (HSV), and HIV [33,34].

The development of effective STI vaccines has been particularly difficult because STI pathogenesis generally does not involve hematogenous spread of the organism similar to those for measles and hepatitis B virus. The pathogenesis of most STIs involves local replication with spread, when it occurs, along contiguous surfaces (e.g., spread from the vagina to the uterus and fallopian tubes) or by non-hematogenous routes (e.g., intraneuronal spread of HSV) [35]. Traditionally, vaccines have not been very effective at providing durable protection against mucosal infection. New advances in immunology, however, may overcome this hurdle and develop more effective vaccines against such infections and reduce their occurrence. Due to high health and economic costs of these STIs, development of such vaccines will be a valuable source in prevention and controls of such infections and diseases.

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References

1. Sexually transmitted disease surveillance 2010 (2011) Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, USA.
2. Wasserheit JN (1992) Epidemiological synergy. interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. Sex Transm Dis 19: 61-77.
3. Pepin J, Plummer FA, Brunham RC, Piot P, Cameron DW, et al. (1989) The interaction of HIV infection and other sexually transmitted diseases: An opportunity for intervention. AIDS 1989 3: 3-9.
4. Farhi D, Dupin N (2010) Management of syphilis in the HIV-infected patient: Facts and controversies. Clin Dermatol 28: 539-545.
5. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, et al. (2006) *Herpes* simplex virus 2 infection increases HIV acquisition in men and women: Systematic review and meta-analysis of longitudinal studies. AIDS 20: 73-83.
6. Fleming DT, Wasserheit JN (1999) From epidemiological synergy to public health policy and practice: The contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 75: 3-17.
7. Lee SR, Chung JM, Kim YG (2007) Rapid one step detection of pathogenic bacteria in urine with sexually transmitted disease (STD) and prostatitis patient by multiplex PCR assay (mPCR). J Microbiol 45: 453-459.
8. Adams EJ, Charlett A, Edmunds WJ, Hughes G (2004) *Chlamydia trachomatis* in the united kingdom: A systematic review and analysis of prevalence studies. Sex Transm Infect 80: 354-362.
9. Lewis D, Newton DC, Guy RJ, Ali H, Chen MY, et al. (2012) The prevalence of *Chlamydia trachomatis* infection in australia: A systematic review and meta-analysis. BMC Infect Dis 14: 113.

31. Kirby D, Obasi A, Laris B (2006) The effectiveness of sex education and HIV education interventions in schools in developing countries. World Health Organ Tech Rep Ser 938: 103-150.

32. Bertrand JT, Anhang R (2006) The effectiveness of mass media in changing HIV/AIDS-related behaviour among young people in developing countries. World Health Organ Tech Rep Ser 938: 205-241.

33. Zimet GD, Mays RM, Fortenberry DJ (2000) Vaccines against sexually transmitted infections: Promise and problems of the magic bullets for prevention and control. Sex Transm Dis 27: 49-52.

34. Jovanović M, Karadaglić Đ, Golušin Z, Brkić S, Poljački M (2009) Experimental vaccines for sexually transmitted infections. Med Pregl 62: 42-48.

35. Liddon N, Zimet G, Stanberry L (2007) STI vaccines: Status of development, potential impact, and important factors for implementation. Springer, USA.

to a higher incidence of life-threatening CNS infections, but also widens the spectrum of infectious etiologies they can acquire [3,4]. It is of utmost prominence that these infections are recognized and treated early with appropriate antimicrobial agents.

Etiology

The spectrums of infectious etiologies associated with CNS infections in immunocompromised patients are vast (Table 1). Despite extensive diagnostic testing and advancements in microbiologic modalities to obtain a microorganism(s), 32%-75% cases do not reveal the etiology and are considered idiopathic [1,4].

Epidemiology

CNS infections are a major global cause of morbidity and mortality. The World Health Organization (WHO) reported an estimated worldwide incidence of 700,000 cases of meningitis in 2004 with approximately 340,000 related deaths [5]. In the United States (US), estimated deaths related to meningitis were approximately 13,000/year. The most common (58%) cause of bacterial meningitis in the US is *Streptococcus pneumoniae* [5,6]. Despite appropriate antibiotic therapy, the mortality rate for bacterial meningitis remains between 16-26% [5,6]. The incidence of bacterial meningitis over last 2 decades has declined [6-8]. (Table 2) Age is an important risk factor regardless of the immune status. While pneumococcal meningitis is common in all age groups, *Listeria* is more commonly seen in the elderly (>50 years of age) [5,8]. Exposure to heavily populated areas such as Saudi Arabia during the time of Hajj can increase the risk of meningitis [9,10]. Immigration from Latin America is associated with *Taenia solium* related brain abscesses. Residence in India has been associated with acquisition of tuberculomas [1,6]. Immunosuppressive disorders are a major risk factor in the development of focal CNS infections such as brain abscess due to *Nocardia*, fungal organisms (*Aspergillus*, *Mucor*, *Cryptococcus*, *Candida*) and parasites (*Toxoplasma*). The incidence of focal bacterial brain abscesses in the US is approximately 0.3-1.3 /100,000 persons/year [5,8].

Predisposing Factors

Common predisposing factors associated with bacterial meningitis include human

Meningitis	Bacterial	Encapsulated organisms: *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenza* Non-capsulated organisms: *Listeria monocytogenes, Streptococcus agalactiae* *Aerobic gram-negative organisms: Klebsiella spp., Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter baumannii, Salmonella spp.* Other bacterial organisms: *Mycobacteria tuberculosis, Treponema pallidum and Borrelia burgdoferi*
	Viral	*Enterovirus, Herpesviruses (Varicella-zoster virus (VZV), Herpes simplex virus (HSV), Cytomegalovirus (CMV), Ebstein-Barr virus (EBV), Human herpesvirus-6), acute* HIV, *Arbovirus, Lymphocytic choriomeningitis virus (LCM), Rabies, Influenzae*
	Fungal	*Cryptococcus neoformans, Coccidioides immitis.*
	Parasitic	*Naegleria fowleri, Angiostrongylus catonensis*
Encephalitis	Bacterial	*Listeria monocytogens, Rickettsia spp., Ehrlichia spp., Bartonella spp., Mycoplasma pneumoniae*
	Viral	*HSV, CMV, VZV*, Arboviruses (i.e. West Nile virus, eastern equine, St. Louis, California, Japanese encephalitis); HIV, *Rabies virus, Enterovirus*
	Parasitic	*Toxoplasma gondii, Cryptococcus neoformans*
Focal infections (abscess/ empyema)	Bacterial	*Streptococcus spp., Enterobacteriaceae,* anaerobes, *Staphylococci, Nocardia spp., Mycobacterium tuberculosis* (tuberculoma)
	Fungal	*Aspergillus spp., Candida spp., Mucor spp., Cryptococcus neoformans, Coccidioides immitis*
	Parasitic	*Taenia solium* (neurocysticercosis), *Toxoplasma gondii, Echinococcus spp.*
Septic thrombophlebitis	Bacterial	*Staphylococcus* (60-70%), *Streptococcus* (~17%, of which approximately 5% are *S. pneumococcus*), gram-negative bacilli (5%), *Bacteriodes* spp.

Table 1: Etiologic Organisms Associated With Central Nervous System Infections in Immunocompromised Hosts.

to additional CNS infections [12]. In patients with CD4 counts over 200 cells per cubic millimeter of blood, the most common cause of bacterial meningitis is the same agent responsible for bacterial meningitis in patients with a normal immune status (*Streptococcus pneumoniae*) [12]. Risk factors associated with HIV infection include intravenous drug use, which places this group at risk for acquiring epidural and/or other brain abscesses [3]. AIDS (CD4 count <200 cells/ml) patients are at a higher risk of acquiring cryptococcal infection and toxoplasmosis than those with normal immunity [3,12]. While disseminated cryptococcosis usually presents as meningitis, it can also present as focal infection (cryptococcoma). Toxoplasmosis is the most common cause of ring-enhancing single or multiple lesions in this subset of patients [3,12]. In HIV patients, regardless of the CD4 counts, living in Mycobacterium tuberculosis (TB)-endemic areas, extra-pulmonary TB including tuberculous meningitis and tuberculoma are seen more often [3]. When the CD4 count falls below 50 cells/ml, AIDS patients are at a higher risk of developing CMV encephalitis and progressive multifocal leucoencephalopathy (PML) from JC virus [12]. Cases of endemic fungus as *Histoplasma* or filamentous fungus such as *Aspergillus* presenting as a focal brain infection are rarely reported with decreased immunity from AIDS [3].

Diagnostic Approach

The clinical suspicion of meningitis should be followed by prompt action toward diagnosis and management, as a delay will increase the mortality and morbidity [13]. Lumbar puncture is an effective tool in diagnosing meningitis regardless of the immune status. Computed tomography (CT) of head is recommended prior to lumbar puncture in immunocompromised states, history of seizure or cranial diseases (i.e. mass, cerebrovascular accident), acute focal neurologic deficits or papillaedema [3,14]. Cerebro-spinal fluid (CSF) analysis should include cell count with differential; glucose concentration (with concurrent serum glucose concentration), protein level, and bacterial culture with gram stain [15]. Depending on the special clinical indication, other tests include viral cultures and polymerase chain reaction (PCR) as for HSV, EBV, VZV, *Enterovirus*, fungal cultures, acid-fast bacilli smears and cultures, venereal disease research laboratory (VDRL), *serum* and *CSF cryptococcal* antibody, cytology and flow cytometry. For West Nile virus encephalitis, CSF viral immunoglobulin M is the recommended diagnostic modality [14-16]. Latex agglutination is a rapid, simple test with sensitivities of 67-100% to *S. pneumoniae*, 50-93% to *N. meningitidis*, 69-100% to *S. agalactiae*, and 78-100% to *H. influenzae* antigens [15]. It serves useful in patients who have been treated with antibiotics prior to getting a lumbar puncture. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) are instrumental tools in diagnosing focal infections such as brain abscess, subdural empyema and infectious thrombophlebitis. Biopsy or aspiration via stereotactic guidance is needed for microbial diagnosis. In ring-enhancing focal lesions, empirical therapy for toxoplasmosis is recommended. Follow-up imaging is recommended after two weeks of therapy to assess for resolution or reduction in the lesion size, further supporting the diagnosis of toxoplasmosis rather than malignancy and/or abscess.

Management

Many of the CNS infections, including acute bacterial meningitis, are considered medical emergencies that necessitate emergent empiric antibiotics. Delay in sterilization of CSF can result in neurological sequel, including deafness [17,18]. The choice of appropriate empiric antibiotics for bacterial meningitis depends on age and predisposing conditions. (Table 4) Other important factors include the rapidity, efficiency and effectiveness of penetrating the CSF barrier to eradicate the meningeal pathogens [2]. Once the organism is identified, the antibiotics can be tailored based on the *in-vitro* susceptibility reports, minimal inhibitory concentration (MIC) and up-to-date evidence-based literature.

For patients suspected to have encephalitis, empirical therapy for herpes encephalitis

4. Zunt JR (2002) Central nervous system infection during immunosuppression. Neurol Clin 20: 1-22.

5. Mathers C, Boerma T, Ma Fat D (2009) Global and regional causes of death Br Med Bull 92: 7-32.

6. Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, et al. (1997) Bacterial meningitis in the United States in 1995. Active Surveillance Team. N Engl J Med 337: 970-976.

7. Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, et al. (2009) Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. N Engl J Med 360: 244-256.

8. Lehmann D, Willis J, Moore HC, Giele C, Murphy D, et al. (2010) The changing epidemiology of invasive pneumococcal disease in aboriginal and non-aboriginal western Australians from 1997 through 2007 and emergence of nonvaccine serotypes. Clin Infect Dis 50: 1477-1486.

9. Ahmed QA, Barbeschi M, Memish ZA (2009) The quest for public health security at Hajj: the WHO guidelines on communicable disease alert and response during mass gatherings. Travel Med Infect Dis 7: 226-230.

10. Aguilera JF, Perrocheau A, Meffre C, Hahné S (2002) Outbreak of serogroup W135 meningococcal disease after the Hajj pilgrimage, Europe, 2000. Emerg Infect Dis 2002. 8:761-767.

11. Glaser CA, Honarmand S, Anderson LJ, Schnurr DP, Forghani B, et al. Beyond viruses: Clinical profiles and etiologies associated with encephalitis. Clin Infect Dis 43: 1565-1577.

12. Tan IL, Smith BR, von Geldern G, Mateen FJ, McArthur JC (2012) HIV-associated opportunistic infections of the CNS. Lancet Neurol 11: 605-617.

13. Tunkel AR (2001) Bacterial Meningitis. Lippincott Williams & Wilkins, Philadelphia, USA.

14. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, (2004) Practice Guidelines for the Management of Bacterial Meningitis. Clin Infect Dis 39: 1267-1284.

15. James Molton, Denis Spelman (2012) Intensive care Central nervous system infections in intensive care patients learning objectives. Anaesthesia and Intensive Care Medicine Volume 13: 176-180.

16. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, et al. (2008) The Management of Encephalitis: Clinical Practice Guidelines by the Infectious Diseases Society of America. Clin Infect Dis 47: 303-327.

17. Aronin SI, Peduzzi P, Quagliarello VJ (1998) Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. Ann Intern Med 129: 862–9.

18. Begg N, Cartwright KA, Cohen J, Kaczmarski EB, Innes JA, et al. (1999) Consensus statement on diagnosis, investigation, treatment, and prevention of acute bacterial meningitis in immunocompetent adults. J Infect 39: 1-15.

19. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, et al. (2010) Practice Guidelines for the Management of Cryptococcal Disease. Clin Infect Dis 50: 291-322.

20. Conti DJ, Rubin RH (1988) Infection of the central nervous system in organ transplant recipients. Neurol Clin 6: 241–260.

number of mast cells and macrophages whose primary role is to phagocytose incoming pathogens. The skin has low moisture content and acidic pH (5.5). It produces sebum which is rich in the degradative enzyme lysozyme all of which afford a hostile environment for microbial growth [2]. Sebaceous glands secrete the sebum but are themselves prone to infection. Their shaft-like shape makes them prone to accumulating both bacteria and other debris that can result in localised irritation. Humoral immune responses to microorganisms that have penetrated the skin are rapid due to its extensive vasculature, and such responses rely on antigen presentation by the Langerhans cells. IgA and IgG are the major immunoglobulins that mediate this response and are produced by eccrine cells on the surface of the skin. Small antimicrobial peptides called defensins are secreted by the keratinocytes and contribute to innate immunity by non-specifically embedding into the surface of microorganisms, mediating lysis by the efflux of cellular constituents and simultaneous influx of extracellular material [3]. Keratinocytes are also known to secrete numerous cytokines that mediate an inflammatory response to infection. However, it should be noted that in the healthy host skin infections are less uncommon and usually seen following trauma.

Human Skin Microbiota

Despite the seemingly hostile environment, numerous microorganisms successfully colonise the skin and form part of the commensal microbiota. The human microbiome project has revealed a diverse ecosystem that is associated with human skin. It is widely accepted that these so called normal inhabitants are not only especially adapted to their environment but also offer protection against colonisation by exogenous microorganisms [4,5]. In addition to protection offered by the immune system, the resident microbiota is thought to contribute to defence against infection and experimental evidence has shown that removal of the indigenous microbiota results in proliferation of species that are ordinarily suppressed [6]. Within the microbial community competition for space and nutrients is rife and most microorganisms are especially well adapted to their particular niche meaning that newcomers can find it difficult to colonise, which is one of the reasons why most pathogens form only part of the transient microbiota. Numerous members of the skin microbiota secrete inhibitory substances that prevent the growth of other microorganisms; these include bacteriocins, peptides and antibiotics [4]. While in evolutionary terms these substances are produced purely for the benefit of the microorganism, they provide additional protection to the human host and as such the indigenous microbiota is becoming recognised being an important protective mechanism against infection.

The skin microbiota exists in specific niches influenced by localised skin temperature and humidity. Warmer areas of the skin support the growth of higher numbers of microorganisms and include areas such as the groin and axillae. Sweat is an excellent source of nutrients for microorganisms, consisting of amino acids, minerals and water which further promote the growth of microorganisms. Since sweat is generally produced in greater abundance on warmer areas of the body, this explains the higher abundance and a more diverse range of microorganisms are found at these sites. Principally, microorganisms of the skin are divided into two groups, the resident and transient microbiota. The former are irreversibly attached to the keratinocytes whereas the latter rarely grow on the skin and are lost following a relatively short period of time.

The most abundant group of bacteria associated with the skin are Gram-positive and include the following genera: *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Propionibacterium* and *Brevibacterium*. Less abundant Gram negative microorganisms include *Acinetobacter* and *Pseudomonas*. These microorganisms tend to form microcolonies or biofilms comprised of multiple species on the surface of the skin. Over the course of an individual's life the skin microbiota changes, in the very young the predominant microbiota tend to be Gram negative microorganisms or coryneforms or micrococci. The distribution of microorganisms is quite

***Acinetobacter*:** Bacteria of this genus are members of the gamma-proteobacteria, and the precise designation of these microorganisms has changed considerably since their first discovery as part of the human skin microbiota in the early 1960s. *Acinetobacter spp* is largely regarded as being part of the commensal microbiota and are most commonly found on the hands, face, in between the toes and in the groin. Despite their designation as commensal microorganisms, *Acinetobacter baumanii* has emerged as an opportunistic pathogen capable of causing serious and life threatening infection [14,15]. It is a greater cause for concern for immunocompromised individuals and is a key source of nosocomial infection, especially in intensive care units. It can enter the body through open wounds, catheters and respiratory ventilators resulting in wound infection, bloodstream infection and pneumonia (ventilator pneumonia). Numerous reports of *A. baumanii* infection in veterans return from Iraq have earned this microorganism the nickname of "Iraqibacter". Interestingly *A. baumanii* has been isolated from wounds where it had successfully colonised but failed to cause infection.

***Corynebacterium*:** Otherwise known as coryneforms or diphtheroids, this group consists of any non-pathogenic strain of the genus *Corynebacterium*. These commensal microorganisms are typically found associated with the sebaceous glands and are recognised as opportunistic pathogens in the immunocompromised or those with underlying medical conditions. Erythrasma caused by *C. minutissimum* is often seen in diabetics, the pathology of infection manifestation often means that it is confused with fungal infection. Pitted keratolysis is a superficial skin infection that commonly affects the feet and is restricted to the stratum corneum [16]. Infection is associated with wearing occlusive footwear and warm, moist conditions. Rare extra-pulmonary infection by *C. ulcerans* mimics cutaneous diphtheria and can result in ulceration of the skin ultimately causing a wound infection that is difficult to treat [17].

***Pseudomonas aeruginosa*:** *P. aeruginosa* is an opportunistic pathogen that does not ordinarily colonise the skin of healthy individuals. Burn wound patients are especially predisposed to wound infection by *P. aeruginosa* which is notoriously difficult to treat; infection remains one of the main causes of death amongst burn patients [18]. Infection can also be acquired as a consequence of skin trauma, post-operatively, following skin-grafts or from hot tubs (cutaneous folliculitis). Once *P. aeruginosa* infections are established they often become chronic, a state that is associated with bacterial biofilms. It adheres strongly to numerous human tissue proteins that are associated with the wound bed, including collagen and fibrinogen; multidrug resistant variants are especially difficult to treat.

***Candida*:** *Candida spp.*, typically Candida albicans can grow on moist areas of the skin such as the groin or armpits. It is a yeast-like dimorphic fungus that is found as a transient member of the skin microbiota. *C. albicans* is considered to be a commensal microorganism, but is an opportunistic pathogen in the immunocompromised. Its pathogenicity is thought to be linked to a phenotypic 'switch' [19]. Candidiasis is a superficial infection of the skin and mucous membranes resulting in rashes, scaling, itching and swelling. People who are taking long courses of systemic antibiotics are also predisposed to candidiasis as the normal bacterial microbiota is disrupted allowing *C. albicans* to flourish.

***Dermatophytes*:** These comprise a large group of fungi that can infect and degrade keratinised tissues such as the skin, more usually these organisms are regarded as being pathogens and like many other infectious agents, tend to form only part of the transient microbiota of the skin. The three main genera of this group include *Epidermophyton*, *Microsporum* and *Trichophyton* [20]. Dermatopytoses is commonly accompanied by localised blistering of the skin, with exudate if the blisters are broken. As the blisters dry they form a dry ring like lesion on the surface of the skin. Infection often remains localised and is rarely systemic due to the inflammatory response of the immune system.

surgeons are now beginning to look towards greater use of antiseptics to diminish reliance on antibiotics.

Neurotrophic ulcers

Approximately 15% of diabetics will develop a neurotrophic ulcer in their lifetime and these are susceptible to infection. As is the case for open fractures, these ulcers have an associated bacterial load, but this does not always result in infection. However, the presence of a bacterial load can hinder the proper reparative process, thus prolonging the time it takes for the ulcer to heal [27]. Most usually, bacteria isolated from neurotrophic ulcers are comprised of members of the normal skin flora and when the bacterial load is high, a chronic wound can emerge. Chronic wounds have been associated with the prevalence of bacterial biofilms and often respond poorly to treatment, recurring throughout the course of the patients' life.

Polymicrobial infection

The idea that a bacterial infection is caused by just one microorganism at a time is beginning to be replaced as a consequence of better understanding of microbial community and co-infection; it is now generally accepted that skin and soft tissue infections can be polymicrobial in nature [28]. It has become especially apparent that chronic wounds in particular contain complex polymicrobial communities that demonstrate an average of three different species of bacteria, the most commonly isolated being *Pseudomonas spp.*, *Streptococcus spp* and *Staphylococcus spp*. In the past polymicrobial infections have been largely overlooked, mainly due to the limitation of culture techniques. In particular biofilms tend to be comprised of multiple species of bacteria and are themselves inherently difficult to treat, the added complication of needing to eradicate multiple species of bacteria can make polymicrobial infections especially difficult to resolve. Molecular techniques that rely on peptide nucleic acid fluorescence *in situ* hybridisation (PNA FISH) have enabled polymicrobial biofilm communities that are associated with chronic wounds to be studied [29]. These have confirmed the predominance of *Pseudomonas* spp., *Streptococcus spp* and *Staphylococcus spp*. as well as *Micrococcus* spp. and have revealed clustering of each species within the biofilm.

Colonisation of wounds by bacteria

As previously stated wounds can become contaminated by both exogenous or endogenous microorganisms and potential wound pathogens are numerous including β-haemolytic *Streptococci* (group A and group G *Streptococci*), enterococci (Enterococcus faecalis and E. faecum), *Staphylococci* (*S. aureus* including MRSA; *S. epidermidis*), *P. aeruginosa*, *Acinetobacter spp.*, *Enterobacter spp.*, *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Bacteroides spp.*, *Clostridium spp.*, *Candida spp.* and *Aspergillus spp*. Different bacterial species have different fates within the wound, depending upon the location and type of wound. If the environment is not right and the immune system is not successfully evaded colonisation will only be transient and infection will not ensue. If a potential pathogen is successful it will grow and multiply within the wound eventually invading the underlying tissues, leading to a state of acute infection. To achieve the latter, the pathogen must not only evade the immune defences but also utilise the nutrients available at the wound site.

Wound colonisation and progression towards wound infection has been described as a spectrum that begins with initial contamination and proceeds to a state of critical colonisation after which the only outcome is infection [30]. Wounds with high microbial loads are most prone to infection when the bacterial loads exceeding 105 cfu g-2 [31,32] In 2001, this process was defined by Kingsley as the 'Infection Continuum' and described the shift from

and episodes are generally not recurrent. In severe cases scalded skin syndrome can lead to deeper skin infections such as cellulitis and occasionally septicaemia.

Toxic shock syndrome (TSS) associated with wound infection is primarily seen in young children with burn injuries and post-surgery may be caused by *S. aureus* or *S. pyogenes*. Symptoms manifest as vomiting, high fever, hypotension, erythema, confusion, renal failure and in severe cases shock and death. Toxigenic strains of *S. aureus* belong to phage group I and produce an exfoliative toxin known as enterotoxin F or toxic shock toxin-1 (TST-1). *S. pyogenes* TSS is associated with persons who have pre-existing skin infections as a consequence of this microorganism and there is a rapid onset of symptoms (described above) [37]. Streptococcal exotoxins A and B may mediate progression to fever and shock as they stimulate release of tumour necrosis factor-α in the host. Additionally it is thought that pyrogenic toxin (exotoxin C) or M proteins 1 and 3 might act as superantigens to mediate a severe immune reaction to infection.

Anaerobic wound infection

Anaerobic wound infections occur usually as a consequence of a traumatic wound (i.e. a puncture wound or as a SSI following bowel surgery). The major causes of such infections are the *Clostridium spp*. in particular *Clostridium perfringens* which is found ordinarily as part of the human intestinal microbiota and also in soil. The most serious and widely recognised soft tissue infection caused by *C. perfringens* is clostridial myonecrosis, otherwise known as gas gangrene [38]. Deep tissue traumas mean that the muscle becomes infected, and there is a limited supply of oxygen away from the surface of the skin, favouring the growth of anaerobes. Gas gangrene results in the extensive destruction of deep tissues leading to a localised blackening of the skin and soft tissue as they become necrotic; in addition to this *C. perfringens* produces a foul smelling gas that forms blisters under the skin. However, this is mostly seen where there are underlying conditions such as ischemic vascular disease or diabetes where vascularisation of tissues is sub-optimal. A less serious skin infection caused by *C. perfringens* is anaerobic cellulitis and does not involve the deeper tissues or muscle.

The Role of Biofilms in Skin and Soft Tissue Infection

It is now accepted that many microorganisms exist in nature as part of a complex community known as a biofilm. These exist on surfaces including the skin, and can be problematic in wounds. Bacteria in biofilms are embedded in an extracellular polysaccharide 'matrix' material that is secreted by members of the biofilm and which provides a protective layer that antibiotics or other types of antimicrobial cannot permeate. The presence of biofilms in wounds has been associated with chronicity and a failure to heal, but isolating biofilm microorganisms or indeed establishing the presence of a biofilm in the diagnostic lab is difficult. Often biofilms are polymicrobial in nature so as well as requiring much higher doses of antibiotics for treatment, also require broad-spectrum or combination therapies to successfully clear them.

Development of biofilms

Irrespective of the composition or location development of a bacterial biofilm proceeds through five distinct stages. The first of these is transient or reversible attachment to a given surface. This process is mediated by surface structures such as pili, and in the case of the human host might involve adhesion to proteins such as fibronectin or fibrinogen which are often found at wound sites or on the surface of keratinocytes [39]. During the second stage of biofilm development microorganisms become irreversibly attached and if motile, lose their flagellae; during this stage these so called pioneer colonisers begin to secrete the stick exopolysaccharide material. Pioneer colonisers begin to multiply and in doing so they provide additional binding sites for any new incoming microorganisms and microcolonies

microbiota such as *E. coli* and *Klebsiella*. The major reservoirs for these bacteria are colonised patients, healthcare workers and possibly the inanimate environment; routes of infection are the consequence of poor hand hygiene, or in some cases introduction of host microbiota to surgical sites following abdominal surgery. Data to support the spread of contamination and subsequent infection from the inanimate environment to patients remains poor. However both *S. aureus* and *P. aeruginosa* have been isolated from patient mattresses, and MRSA has been isolated from mops as well as gloves and gowns of health workers where they can remain viable for up to nine weeks [44].

Summary

Skin and soft tissue infections are commonplace in both the community and healthcare setting. They can be caused by numerous different microorganisms both of prokaryotic and eukaryotic origin and symptoms may vary from the superficial to life-threatening. The human microbiota is a significant cause of many skin and soft tissue infections and these types of infection are best prevented by good hygiene practice. While most skin and soft tissue infections are treatable with antibiotics those that lead to sepsis or severe tissue necrosis and systemic disease are associated with significant levels of morbidity and mortality. Infection rates have remain relatively constant, peaking in the warmer seasons and the major problem now facing microbiologists and clinicians is the emergence of antibiotics resistant pathogens that hinder treatment and recovery from infection.

References

1. Rajan S (2012) Skin and soft tissue infections: classifying and treating a spectrum. Cleve Clin J Med 79: 57-66.
2. Niyonsaba F, Ogawa H (2005) Protective roles of the skin against infection: implication of naturally occurring human antimicrobial agents beta-defensins, cathelicidin LL-37 and lysozyme. J Dermatol Sci 40: 147-158.
3. Shauber J, Gallo RL (2008) Antimicrobial peptides and the skin immune defense system. J Allergy Clin Immunol 122: 261-266.
4. Gallo RL, Kakatsuji T (2011) Microbial Symbiosis with the Innate Immune Defense System of the Skin. J Invest Dermatol 131: 1974-1980.
5. Grice EA, Segra JA (2011) The skin microbiome. Nature Rev Microbiol 9: 244-253.
6. Cogen AL, Nizet V and Gallo RL (2008) Skin microbiota; a source of disease or defense? Br J Dermatol 158: 442-445.
7. Roth RR and James WD (1988) Microbial ecology of the skin. Annu Rev Microbiol 42: 441-464.
8. Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, et al. (2008) A diversity profile of the human skin microbiota. Genome Res 18: 1043-1050.
9. Otto M (2010) *Staphylococcus* colonisation of the skin and antimicrobial peptides. Expert Rev Dermatol 5: 183-195.
10. Vuong C, Otto M (2002) *Staphylococcus epidermidis* infections. Microbes and Infection 4: 481-489.
11. Olsen RJ, Musser JM (2010) Molecular pathogenesis of necrotizing fasciitis. Annu Rev Pathol 5: 1-31.
12. Kloos W and Musselwhite MS (1975) Ditribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. Appl Microbiol 30: 381-395.
13. Jappa U (2003) Pathological mechanisms of acne with special emphasis on *Propionibacterium acnes* and related therapy. Acta Derm Venereol 83: 241-248.
14. Sebeny PJ, Riddle MS, Petersen K (2008) *Acinetobacter* baumannii Skin and Soft-Tissue Infection Associated with War Trauma. Clin Infect Dis 47: 444-449.
15. Guerrero DM, Perez F, Conger NG, Solomkin JS, Adams MD, et al. (2010) *Acinetobacter* baumannii-associated skin and soft tissue infections: recognizing a broadening spectrum of disease. Surg Infect 11: 49-57.
16. Blaise G, Nikkels AF, Hermanns-Le T, Nikkels-Tassoudji N, Pierard GE (2008) *Corynebacterium* associate skin infections. Int J Dermatol 47: 884-890.
17. Corti MA , Bloemberg GV, Borelli S, Kutzner H, Eich G, et al. (2012) Rare human skin infection with *Corynebacterium* ulcerans: transmission by a domestic cat. Infection 40: 575-578.
18. Tredget EE, Shankowsky HA, Rennie R, Burrell RE, Logsetty S (2004) *Pseudomonas* infections in the thermally injured patient. Burns 30: 3–26.
19. Calderone RA, Fonzi WA (2011) Virulence factors of Candida albicans. Trends Microbiol 9: 327-335.

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Chapter: VII

Sepsis in an Immunocompromised Host

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Abstract

Sepsis, severe sepsis and septic shock are major healthcare problems. Immunocompromised hosts are particularly more prone to infections. Though the initial management of severe sepsis and septic shock may not be greatly affected by the immune status, the spectrums of opportunistic offending infectious causes are greater. We present the etiologies, clinical manifestations, diagnostic criteria, microbiology and treatment options for severe sepsis in immunocompromised hosts.

Keywords: Sepsis; Septic shock; Immunocompromised; Critical care; Infections

Introduction

Sepsis is a life-threatening systemic host response to an infectious agent, which may consequentially lead to acute organ dysfunction (termed "severe sepsis") and hemodynamic instability (termed "septic shock"). Severe sepsis and septic shock are becoming increasingly prevalent, deleterious and financially burdensome. Severe sepsis is reported in 2.26 cases per 100 hospital discharges, 1 in 5 intensive care unit (ICU) admissions with a mortality rate between 28.6-50% [1,2]. Rapid initiation of aggressive care should begin as soon as sepsis is suspected, with subsequent management in an intensive care unit (ICU) setting as soon as possible. Immunodeficiency is state in which the host immune system is compromised to various degrees of severity. A primary immunodeficiency is a congenital immune system malfunction. A secondary immunodeficiency is acquired via medications (immunosuppressants, glucocorticoids), malnutrition, aging, malignancy and/or chronic infection such as human immunodeficiency virus (HIV). The spectrum of potential pathogens known to cause infections in immunocompromised patients has grown as a result of amplified immunosupppression, extended lifetime survival and enhanced diagnostic assays. Though the immunocompromised state may not mandate significant differences in the management of the physiologic and hemodynamic derangements of severe sepsis or septic shock (i.e. fluid resuscitation, vasopressors, mechanical ventilation), it does require consideration in regards to antibacterial, antiviral and antifungal therapy. This chapter will describe the etiologies, clinical manifestations, diagnostic criteria, microbiology and

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VII. Parasitic – *Toxoplasma*, *Strongyloides*

Management of Sepsis: Initial Resuscitation

In order to achieve the most optimal outcome, adequate resuscitation of blood pressure as well as efforts to restore tissue perfusion should be accomplished within the first 6 hours of presentation. Fluid resuscitation can be guided by central venous pressure measurement to a goal between 8-15 mmHg, ultrasonographic visualization of inferior vena cava filling or pulse-pressure variation. The adequately resuscitated patient will have a mean arterial pressure >65 mmHg, urine output is greater than 0.5 mL/kg/hr, central or mixed venous oxygenation saturation of 70% or 65%, respectively, and/or normalization of plasma lactate levels[3].

Management of sepsis: Infectious work-up in all septic patients

The most important consideration in the treatment of sepsis is aggressive and timely efforts to identify and control of the source of infection. Therapy should be implemented without delay. If the delay of microbiologic culture(s) acquisition is ≤ 45 minutes, cultures should be taken as clinically appropriate before antimicrobial therapy is initiated. At least two sets of aerobic and anaerobic blood cultures should be obtained. If a vascular access device was placed >48hours from presentation, blood cultures should be obtained from at least 1 percutaneous and 1 through each vascular access device [3]. If invasive candidiasis is suspected, use of 1,3 beta-D-glucan assay or mannan and anti-mannan antibody assays may be used. Imaging studies should be performed promptly to help identify the potential source of infection.

Management of sepsis: Infectious work-up in immunocompromised septic patients

In addition to the infectious workup described 4.1, patients with immunocompromised states should have additional diagnostic tests considered. Healthcare providers should also keep in mind that multiple simultaneous infectious processes may occur concomitantly. Invasive procedures such as bronchoscopy with biopsy are often necessary to establish a microbiologic diagnosis. Blood cultures may also be sent for fungus and acid-fast bacilli. Special antimicrobial susceptibility testing, including immunohistology and quantitative molecular assays, are often needed to establish a diagnosis. Serologic testing may demonstrate immunoglobulin-G antibodies consistent with prior exposure (i.e. *Strongyloides*, *Toxoplasma*). A tuberculin skin test response greater than 5 mm is considered positive in intensively immunocompromised patients. Opportunistic infections should be suspected in patients not receiving prophylaxis (i.e. *Pneumocystis, Mycobacterium avium intracellular, CMV*). Seasonal epidemiology may help in diagnosing potential viral etiologies (i.e. November-April=influenza, respiratory syncytial virus, human metapneumovirus; Fall and Spring=rhinovirus).

Antimicrobial Therapy in All Septic Patients

Effective, appropriate parenteral antimicrobials should be given within the first hour of recognition of severe sepsis or septic shock. Empiric therapy typically includes more than one drug against potential pathogens. Two gram-negative agents (i.e. extended spectrum beta-lactam and either an aminoglycoside or fluoroquinolone) may be used empirically in order to increase the likelihood of treating multidrug-resistant bacteria (i.e. *Pseudomonas*, *Acinetobacter*). A combination of beta-lactam and macrolide may be used in patients with septic shock from bacteremic *Streptococcus pneumonia* infections. Antimicrobial therapy should be reassessed daily for potential de-escalation in order to reduce healthcare cost,

drug-interactions with HAART (i.e. non-nucleoside reverse transcriptase inhibitors interacting with azoles and macrolides). Although the surviving sepsis campaign discusses the use of corticosteroids and recombinant human activated protein C, though no longer commercially available, in critically ill patients, HIV-infected patients with CD4 counts ≤ 50 cells/μL were excluded from their randomized controlled trials.

Special considerations: Stem cell transplant

The use of hematopoietic stem cell transplant (HSCT), whether allogenic or autologous, is increasing annually. The subsequent secondary immunodeficiency can cause a variety of infectious and non-infectious complications that may require ICU care. The post-transplant period is divided into three phases, each of which can cause unique complications much different from other immunodeficiencies: pre-engraftment (onset of conditioning therapy to 30 days), early post-engraftment (30-100 days) and late post-engraftment (>100 days) [12]. The pre-engraftment period includes neutropenia and mucositis, leading to dehydration from poor oral intake, airway compromise and gastrointestinal bleeding. Bacterial infections are common during this time of profound neutropenia and lymphopenia. Despite prophylactic therapy, 5-55% of HSCT recipients still require intensive care [13]. Opportunistic infections include gram-positive bacteremia (20-30%), facultative gram-negative bacteria (5-10%), *herpes* simplex virus (5-9%), *Candida spp.* (<5%) and *Aspergillus* spp. (<5%) [14]. Opportunistic infections in the early and late post-engraftment phase include CMV, BK virus, adenovirus, varicella zoster, late aspergillosis, PCP, toxoplasmosis, strongyloidiasis and cryptosporidiosis. Acute graft-versus-host disease may also be difficult to distinguish from an active infection.

Hemodynamic compromise secondary to sepsis with respiratory failure is the most common reason for intensive care, with >47% of patients requiring vasopressor support [12]. Although shock may be secondary to sepsis, hemodynamic instability may also result from hypovolemia secondary to dehydration or gastrointestinal bleeding secondary to mucositis. Hemodynamic instability is also complicated by multi-organ failure in 22-81% and death in 65% of HSCT recipients in ICU care. Bronchoscopy may provide the only conclusive result in the majority of the patients [15]. Other diagnostic modalities include blood and sputum cultures, *Aspergillus* serology, induced sputum or bronchioalveolar lavage for PCP, *Legionella* and *Streptococcus pneumonia* urinary antigens and CMV circulating antigen or polymerase-chain reaction. Additional diagnostic tests should be considered depending on epidemiology, co-morbidities and prognostic/risk factors. Like HIV, clinical trials for the International Surviving Sepsis Campaign excluded HSCT recipients and caution should be advised when applying such recommendations in these patients [3]. Expert consultation from Infectious Diseases and Surgical/Medical Oncology is strongly advised.

Special considerations: Solid organ transplant

Over 28,000 solid organ transplantations (SOT) are performed yearly in the United States [14]. Transplant recipients are not equally susceptible to all pathogens that normal hosts may acquire (i.e. enterovirus do not typically infect SOT recipients as they do in normal hosts, tuberculosis is rarely encountered at most transplantation centers in developing countries). Infections are most common and most diverse during the first 6-9 months of transplantation due to graft rejection, nosocomial infections and/or immunosuppressive drugs [16]. Factors that contribute to infection after SOT include pre-transplantation host factors (co-morbidities, medications, lack of immunity, prior colonization, effective pre-transplantation screening), transplantation factors (type of SOT, time spent in surgery, surgical complications), immunosuppression and allograft reactions (graft-versus-host or

Infectious Diseases and Critical Care on Antimicrobial Utilization and Patient Outcome. Crit Care Med *In press*.

8. Hartung HP, Mouthon L, Ahmed R, Jordan S, Laupland KB, et al. (2009) Clinical applications of intravenous immunoglobulins (IVIg) – beyond immunodeficiencies and neurology. Clin Exp Immunol 158: 23-33.
9. Iwashyna TJ, Ely EW, Smith DM, Langa KM (2010) Long-term cognitive impairment and functional disability among survivors of severe sepsis. JAMA 204: 1787-1794.
10. Vincent B, Timsit JF, Auburtin M, Schortgen F, Bouadma L, et al. (2004) Characteristics and outcomes of HIV-infected patients in the ICU: impact of highly active antiretroviral treatment era. Intensive Care Med 30: 859-866.
11. Gill JK, Greene L, Miller R, Pozniak A, Cartledge J, et al. (1999) ICU admission in patients infected with the human immunodeficiency virus – a multicentre survey. Anaethesia 54: 727-32.
12. Affesa B, Azoulay E (2010) Critical Care of Hematopoietic StemCell Transplant Recipient. Crit Care Clin 26: 133-150.
13. Kew AK, Couban S, Patrick W, Thompson K, White D (2006) Outcome of hematopoeitc stem cell transplant recipients admitted to the intensive care unit. Biol Blood Marrow Transplant 12: 301-305.
14. Young JH, Weisdorf DJ (2010) Infections in Recipients of Hematopoeitc Cell Transplantation. Mandell, Doughlas, and Bennett's Principles and Practice of Infectious Diseases. (7thedn), Elsevier, Philidelphia, USA.
15. Azoulay E, Mokart D, Rabbat A, Pene F, Kouatchet A, et al. (2008) Diagnostic bronchoscopy in hematology and oncology patients with acute respiratory failure: prospective multicenter data. Crit Care med 36: 100-107.
16. Fisherman JA (2007) Infection in solid-organ transplant recipients. N Engl J Med 357: 2601-2614.
17. http://www.cdc.gov/nchs/data/databriefs/db62.pdf
18. http://www.cdc.gov/nchs/data/nvsr/nvsr58/nvsr58_19.pdf
19. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, et al. (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 101: 1664-1655.
20. Edbrooke DL, Hibbert CL, Kingsley JM, Smith S, Bright NM, et al. (1999) The patient-related costs of care for sepsis patients in the United Kingdom adult general intensive care unit. Crit Care Med 27: 1760-1767.
21. Rosen MJ, Clayton K, Schneider RF, Fulkerson W, Rao AV, et al. (1997) Intensive care of patients with HIV infection: utilization, critical illness and outcomes. Pulmonary Complications of HIV Infection Study Group. Am J Respir Crit Care Med 155: 67-71.

with minimal mortality and substantial residual morbidity [7,10]. However in the era of globalization and international tourism, brucellosis has become a common imported disease in the developed world [7,9].

Landmarks in The History of Brucellosis

In the year 1853, Jeffery Allen Marston made the first accurate description of the disease in British army troops serving in Malta during the Crimean war [1]. In 1887, David Bruce isolated Gram negative coccobacilli, named later *Brucella melitensis* (*B. melitensis*), from spleens of fatal cases. In 1895, Bernard Bang isolated *B. abortus* from placental tissues of cattle. In 1897, M.L. Hughes published a review on the clinical and pathological features of the disease and suggested the name undulant fever (Table 1) [1-3]. In 1897, Wright and Semple succeeded in applying serum agglutination method for differentiating brucellosis from other febrile illnesses. In 1904, the Commission of Mediterranean Fever was established [1]. Between 1904 and 1907, several reports on epidemiology, bacteriology and pathology of brucellosis were published. In 1905, Themistocles Zammit identified a Maltese goat as the animal host of brucellosis. In 1918, Alice Evans published data on the antigenic relatedness between *B. melitensis* and *B. abortus*. Subsequently, the genus was named *Brucella* to honor David Bruce. In 1924, human infection with *B. abortus* was documented by Orpen in the U.K. Similar studies on *B. abortus* were performed by Morales-Otero in Puerto Rico [1]. In 1914, Jacob Traum isolated *B. suis* from an aborted swine fetus (Table 1) [1-3]. In 1909, Hutyra and Marek might have recovered the organism in Hungary [1]. In 1953, van Drimmelen made identification of *B. ovis* in sheep. In 1957, Stoenner and Lackman identified *B. neotomae* in rodents (Table 1) [2]. In 1964, Carmichael and Bruner identified *B. canis* in the canines [1,2]. In 1994, Ewalt, Ross and colleagues identified *B. pinnediae* and *B. cetacear* (provisionally) in Minke whales, dolphins, porpoises as well as seals (Table 1) [1,2]. In 1979, the WHO (world health organization) established a specialized program with a unit coordinating and managing activities (The Mediterranean Zoonoses Control Centre) operating from Athens in Greece [4]. In 1986, the WHO and FAO (food and agriculture organization) recommended treatment for acute brucellosis in adults with a combination regimen composed of rifampicin and doxycycline orally for 6 weeks [5-7]. In 2004, WHO/FAO/WOAH (world organization for animal health) joint consultation on emerging zoonotic diseases held in Geneva defined an emerging zoonosis as a pathogen that is newly recognized or newly evolved or that has occurred previously but shows an increase in incidence or expansion in geographical, host or vector range [8]. In November 2006, a panel of international experts met in Ioannina in Greece and they made a number of therapeutic recommendations that included treatment of uncomplicated brucellosis using a combination of oral doxycycline for 6 weeks and parenteral streptomycin for 2 to 3 weeks or oral rifampicin for 6 weeks [7].

Species	Biotype	Animal Hosts	First Description	Human Virulence
B. melitensis	1-3	Goats, sheep and camels	Bruce, 1887	High
B. abortus	1-6, 9	Cows, camels, yaks and buffalos	Bang, 1897	Intermediate
B. suis	1-5	Pigs, wild hares, caribou, reindeer, wild rodents	Traum, 1914	Low
B. canis	-	Dogs	Carmichael and Bruner, 1968	Low
B. ovis	-	Sheep	Van Drimmelen, 1953	None
B. neotomae	-	Rodents	Stoenner and Lackman, 1957	None
B. pinnipediae and B. cetaceae (provisional)	-	Minke whales, dolphins, porpoises and seals	Ewart and Ross 1994	None

Table 1: Basic Microbiological Details of *Brucella* Species.

LPS O-side chains, while *B. canis* is a naturally occurring rough strain [21]. The severity of brucellosis is variable, ranging from lethal to subclinical in humans as in most cases *Brucella*e establish long-term parasitic relationships with their human hosts. Survival and replication of *Brucella*e in macrophages is critical for maintenance of such chronic infections [21]. Survival of *Brucella*e within monocytes is the single most important aspect of pathogenesis contributing to persistence of bacteria in host tissues [22].

Virulence of *Brucella* species depends on survival and replication properties in the host cells. *Brucella* has developed specific strategies to influence antigen presentation mediated by cells and an evolutionary stealthy strategy to escape recognition by the innate immunity. It has also modulated not only the adaptive immunity of the host but also the signaling events during host adaptive immune response [23]. *Brucella* modulates both (the innate and the adaptive) functional arms of the human immune system leading to T-cell anergy and chronic infection [24]. *Brucella* periplasmic cyclic β-1,2-glucan plays an important role during bacterium-host interaction [25]. Cyclic β-1,2-glucan must be transported into the periplasmic space to exert its action as a virulence factor [26]. However, cyclic β-1,2-glucan succinylation is not required for virulence and no low-osmotic stress conditions must be overcome during infection [25].

In the past decade, the mechanisms of *Brucella* pathogenesis and host protective immunity against *Brucella* infections have been extensively investigated using the cutting edge systems biology and bioinformatics approaches [27]. Integrative experimental Omics and computational bioinformatics analyses have dramatically advanced our understanding of how *Brucella* species infect different host species, how *Brucella* gene expressions are regulated in cell cultures or inside host cells and how host cells respond to *Brucella* infections [27]. Acquired immunity to brucellosis has been studied through observations of naturally infected hosts e.g. cattle and goats, mouse models and human infection. New systems biology analyses of antigens recognized by human innate responses in brucellosis have identified large numbers of protein antigens with the potential of understanding mechanisms of pathogenesis and immune evasion and may point the way toward novel vaccines and diagnostic approaches [28].

CD80/CD28 costimulation enhances the interaction of antigen / major histocompatibility complex (MHC) and is critical for adequate induction and maintenance of the Th1 response [24,29]. In humans, brucellosis is characterized by an intense Th1cytokine production with strikingly high serum levels of interferon (IFN)-γ and evidence of defective monocyte function [30]. Although CD4 and CD8 cells are closely involved in the production of IFN-γ and despite that CD8 T cells may be cytotoxic, the role of natural killer (NK) cells and cytotoxicity in protective immunity to brucellosis have not been substantiated experimentally. Also, antibodies have been shown to have a limited role in passive transfer studies [31]. Although infected macrophages may persist in the presence of *Brucella*-specific T cells, CD8 T cells have been shown to have an important role in clearance of *Brucella*e following the peak of infection and may act by lysing chronically infected macrophages [32]. NK cells may be capable of modulating the development of *Brucella* infection in human beings by lysing infected host cells. Chronicity or elimination of *Brucella* infection depends upon the balance between the contradictory effects induced by the bacteria that favor either the host or the pathogen [31,33]. CD4 (+) invariant NK T cells have antibacterial activity and participate directly in the elimination of bacteria and/or in the control of bacterial growth by killing infected cells. These cells inhibit intramacrophagic growth of *Brucella*e by different mechanisms [34].

Human Vγ9Vδ2 T cells play a crucial role in the early immune response to intracellular pathogens. In brucellosis, these cells are drastically increased in the peripheral blood during the acute phase of infection. They are able to use a combination of mechanisms that reduce the total numbers of *B. suis* thus they may benefit the host by limiting the spread of *Brucella* species [35]. Interleukin (IL)-37 is a soluble factor responsible for part of the

hypervariable octameric oligonucleotide finger-prints (HOOF-Prints) technique is highly discriminatory among *Brucella* species, among previously characterized *Brucella* strains and among unrelated field isolates that cannot be differentiated by classical methods [55]. For human *Brucella* isolates, both MLVA and HOOF-Print assays are rapid, highly discriminatory and reproducible. They significantly contribute to *Brucella* epidemiology and may advance surveillance and control of human brucellosis [55,56]. Also, mixed-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-FOS-MS) is a rapid method for the analysis of biological samples. The accurate identification of *Brucella* species can be achieved with MALDI-FOS-MS by constructing a *Brucella* reference library based on the genetic relationships according to MLVA data [57].

The *Brucella* bioinformatics portal (BBP) is a gateway for *Brucella* researchers to search, analyze and curate *Brucella* genome data originating from public databases and medical literature. *Brucella* gene mutations and genetic interactions are annotated using Limix leading to better understanding of *Brucella* pathogenesis [58]. The sequence database provides a powerful dataset for addressing ongoing controversies in *Brucella* taxonomy and a tool for unambiguously placing atypical, phenotypically discordant and newly emerging *Brucella* isolates [59]. The identification of novel genes within previously described groups has added insight with regard to the regulatory elements, nutritional demands and mechanisms required for efficient intracellular growth and survival of the organism [60]. Four genes of *B. suis* that are necessary to resist specifically the action of γ9δ2 T cells have been identified. *B. suis* that induces chronic disease in humans might have developed specific strategies to subvert the immune system at the level of the innate secondary response [61].

Transposon mutagenesis is the most frequently used approach in the identification of genes involved in the virulence of bacterial pathogens [62]. A recent modification of the classical transposon mutagenesis technique, signature-tagged transposon mutagenesis (STM), allows the detection of a given mutant within a complex pool of mutants by hybridization with a probe obtained by polymerase chain reaction (PCR) with primers based on constant regions [62]. Very little is known about the genetic basis of *Brucella* virulence. However, stress response proteins and smooth LPS may be required for virulence in vitro and in animal models. A two-component system (Bvr AS) and a type IV secretion system (VirB) have recently been identified as essential virulence factors. More recently, the application of STM has allowed identification of *B. suis* genes affecting intracellular survival in an in vitro human macrophage infection model [62]. The *Brucella* genome contains an IS 711 transposon element that is often used in finger printing *Brucella* species samples. While *B. suis* 1330 and *B. suis* ATCC 23445 contain 7 and 13 IS71 copies respectively, *B. suis* VBI 22 has 8 copies. All 7 IS 711 loci in *B. suis* 1330 genome are observed in the genomes of ATCC 23445 and VBI 22 strains. *B. suis* VBI 22 has an additional IS 711 locus right after the stop codon of the BSB 122_A1627 gene, which has not yet been previously observed in any sequenced *Brucella* species [63]. Obtaining the complete *Brucella* genome and identification of the global expression genetic profile of *Brucella* species will be a great step in understanding: biology and evolution of the pathogen, virulence of the organism and the interaction between *Brucella* and its host at the molecular level in order to improve the development of vaccines and new antimicrobial therapies [64,65]. The STM technique is a powerful method that allows a large number of mutants to be screened for attenuation. The isolation of attenuated mutants in virB operon and manB, that are known *Brucella* virulence factors, has validated the application of STM in *Brucella* virulence studies [62].

The rough strains RB51, RB115 and B18 which are characterized by different antigenic and immunological properties show differences in genes involved in LPS synthesis. Specific genes affected by such mutations have been identified [66]. *Brucella* global expression profile studies can provide novel information on growth phase-specific gene expression. Further characterization of some genes that have been found to be differentially expressed in most invasive cultures will likely bring new insights into the initial molecular interactions

the M antigen is the major antigen in *B. melitensis*. Numerous outer and inner membrane, cytoplasmic and periplasmic proteins have also been characterized [3].

Transmission of Brucellosis and Means of Infection

Brucellosis is commonly transmitted by: (1) consumption of unpasteurized, contaminated animal dairy products, (2) direct contact with infected animal parts and (3) inhalation of infected aerosolized particles. Person to person transmission is less common, but has been reported [16-19]. The disease can be transmitted by blood transfusion. Reports of blood transfusion as a means of transmission of brucellosis in recipients of blood product transfusion existed as early as the year 1950. Screening of blood donors for brucellosis has revealed presence of *Brucella* antibodies in serum samples of 0.057 to 3.19% of blood donors [79-81]. In Saudi Arabia, the national seroprevalence of brucellosis is 15% [82]. Brucellosis can also be transmitted by transfusion of harvested bone marrow in recipients of hematopoietic stem cell transplantation [83]. Sexual transmission, although rare, has been reported in humans. In the reported cases, *Brucella* was either cultured from semen or its presence in serum was demonstrated by PCR [84,85].

Laboratory Exposure to Brucellosis and Bioterrorism

The potential use of *Brucella* as a bioweapon derives from its great infectivity (virulence), ability to incapacitate infected individuals (potential lethality), the stubborn persistent nature of human disease (ability to develop resistance to known antimicrobials) and the absence of a safe and an effective vaccine for use in humans. Both the USA and the former Soviet Union weaponized *Brucella* in 1945 [86-88]. *Brucella* is classified by the centers for disease control and prevention (CDC) as a category B pathogen that has potential for development as a bioweapon. Moreover, *Brucella* species are considered as the most common laboratory-acquired pathogen [13,20,89]. However, it is crucial to discriminate between true brucellosis and Y 09 infections that cause false positive serological reactions in testing for brucellosis [20]. In the management of bioterrorism, with the potential use of *Brucella* as a bioweapon, doxycycline should be considered a first-line antibiotic [87]. Discovery of a laboratory exposure to *Brucella*e should prompt an exhaustive investigation of the event and its circumstances, definition of the population at risk, enforcement of safe laboratory practices and administration of antimicrobial prophylaxis for exposed individuals [19,89,90].

Conclussions

Brucellosis is a common global re-emerging zoonosis that constitutes a major health and economic problem in many parts of the world. *Brucella*e are Gram negative, intracellular coccobacilli that predominantly affect organs with rich macrophage content. At least 6 species have been recognized species, 4 of them are human pathogens. Infection can be acquired by: consumption of unpasteurized dairy products, direct contact with animals, blood product transfusion and sexual transmission. In the era of globalization, international tourism and travel across free borders, brucellosis has become a common imported disease in developed countries. Laboratory exposure to the organism and possible utilization as a biological weapon add more to the pathogenic potential of the organism. The recent immunologic, genetic and genomic advances have translated into better understanding of the pathogenesis of brucellosis and are likely to be utilized well in the vaccination, prevention and therapy of this global infection.

References

1. Evans AS, Brachman PS (2009) Bacterial Infections of Humans Epidemiology and Control. (4thedn), Springer Science + Business Media, LLC, 233, New York, NY 10013, USA.

2. Pappas G, Akritidis N, Bosilkovski M, Tsianos E (2005) Brucellosis. N Engl J Med 352: 2325-2336.

25. Roset MS, Ciocchini AE, Ugalde RA, de Iannino NI (2006) The *Brucella* abortus cyclic β-1,2 glucan virulence factor is substituted with O-ester-linked succinyl residues. J Bacteriol 188: 5003-5013.

26. Roset MS, Ciocchini AE, Ugalde RA, Inon de Iannino N (2004) Molecular cloning and characterization of cgt, the *Brucella* abortus cyclic β 1,2 glucan transporter gene and its role in virulence. Infect Immun 72: 2263-2271.

27. He Y (2012) Analyses of *Brucella* pathogenesis, host immunity and vaccine targets using systems biology and bioinformatics. Front Cell Infect Microbiol 2: 2.

28. Cannela AP, Tsolis RM, Liang L, Felgner PL, Saito M, et al. (2012) Antigen-specific acquired immunity in human brucellosis: implications for diagnosis, prognosis and vaccine development. Front Cell Infect Microbiol 2: 1.

29. Skendros P, Boura P, Kamaria F, Raptopoulou-Gigi M (2006) CD80/CD28 co-stimulation in human brucellosis. Clin Exp Immunol 146: 400-408.

30. Rodriguez-Zapata M, Matias MJ, Prieto A, Jonde MA, Monserrat J, et al. (2010) Human brucellosis is characterized by an intense Th1 profile associated with a defective monocyte function. Infect Immun 78: 3272-3279.

31. Baldwin CL, Goenka R (2006) Host immune responses to the intracellular bacteria *Brucella*: Does the bacteria instruct the host to facilitate chronic infection? Crit Rev Immunol 26: 407-442.

32. Baldwin CL, Winter AJ (1994) Macrophages and *Brucella*. Immunol Ser 60: 363-380.

33. Dornard J, Gross A, Lafont V, Liautard J, Oliaro J, et al. (2002) The innate immune response against *Brucella* in humans. Vet Microbiol 90: 383-394.

34. Bessoles S, Dudal S, Besra GS, Sanchez F, Lafont V (2009) Human CD4+ invariant NKT cells are involved in antibacterial immunity against *Brucella* suis through CD1d-dependent but CD4-independent mechanisms. Eur J Immunol 39: 1025-1035.

35. Oliaro J, Dudal S, Liautard J, Andrault J-B, Liautard JP, et al. (2005) Vγ9Vδ2 T cells use a combination of mechanisms to limit the spread of the pathogenic bacteria *Brucell*a. J Leukocyte Biol 77: 652-660.

36. Dudal S, Turriere C, Bessoles S, Fontes P, Sanchez F, et al. (2006) Release of LL-37 by activated human Vγ9Vδ2 T cells: a microbicidal weapon against *Brucella* suis. J Immunol 177: 5533-5539.

37. Skyberg JA, Thornburg T, Rollins M, Huarte E, Jutila MA, et al. (2011) Murine and bovine γδ T cells enhance innate immunity against *Brucella* abortus infections. PLoS ONE 6: e21978.

38. Kilic SS, Akbulut HH, Ozden M, Bulut V (2009) Gamma/delta T cells in patients with brucellosis. Clin Exp Med 9: 101-104.

39. Atluri VL, Xavier MN, de Jong MF, den Hartigh AB, Tsolis RM (2011) Interactions of the human pathogenic *Brucella* species with their hosts. Annu Rev Microbiol 65: 523-541.

40. Dornand J, Lafont V, Oliaro J, Terraza A, Castaneda-Roldan E, et al. (2004) Impairement of intramacrophagic *Brucella* suis multiplication by human natural killer cells through a contact-dependent mechanism. Infect Immun 72: 2303-2311.

41. Grillo M-J, Blasco JM, Gorvel JP, Moriyon I, Moreno E (2012) What have we learned from brucellosis in the mouse model? Vet Res 43: 29.

42. Silva TMA, Costa EA, Paixao TA, Tsolis RM, Santos RL (2011) Laboratory animal models for brucellosis research. J Biomed Biotechol 2011: 518323.

43. Murphy EA, Sathiyaseelan J, Parent MA, Zou B, Baldwin CL (2001) Interferon-γ is crucial for surviving a *Brucella* abortus infection in both resistant C57BL/6 and susceptible BALB/c mice. Immunology 103: 511-518.

44. Brandao APMS, Oliveira F, Carvalho NB, Vieira LQ, Azevedo V, et al. (2012) Host susceptibility to *Brucella* abortus infection is more pronounced in IFN- γ knockout than IL-12/β2 microglobulin double-deficient mice. Clin Dev Immunol 2012: 589494.

45. Zhan Y, Liu Z, Cheers C (1996) Tumor necrosis factor alpha and interleukin-12 contribute to resistance to the intracellular bacterium *Brucella* abortus by different mechanisms. Infect Immun 64: 2782-2786.

46. Fernandez DM, Baldwin CL (1995) Interleukin-10 downregulates protective immunity to *Brucella* abortus. Infect Immun 63: 1130-1133.

67. Rajashekara G, Glasner JD, Glover DA, Splitter GA (2004) Comparative whole-genome hybridization reveals genomic islands in *Brucella* species. J Bacteriol 186: 5040-5051.

68. Del Vecchio VG, Kapartal V, Redkar RJ, Patra J, Mujer C, et al. (2002) The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. Proc Natl Acad Sci U.S.A 99: 443-448.

69. Kim KW, Sung S, Kim H (2011) A genomic-wide identification of genes potentially associated with host specificity of *Brucella* species. J Microbiol 49: 768-775.

70. Kulakov IuK, Zhelodkov MM (2006) Molecular basis of *Brucella* persistence. Zh Mikrobiol Epidemiol Immunobiol 4: 72-77.

71. Delrue RM, Lestrate P, Tibor A, Letesson JJ, De Bolle X (2004) *Brucella* pathogenesis, genes identified from random large-scale screens. FEMS Microbiol Lett 231: 1-12.

72. Rajashekara G, Krepps M, Eskra A, Mathison A, Montgomery A, et al. (2005) Unravelling *Brucella* genomics and pathogenesis in immunocompromised IRF-1-/- mice. Am J Reprod Immunol 54: 358-368.

73. Rajashekara G, Eskra A, Mathison A, Petersen E, Yu Q, et al. (2006) *Brucella*: functional genomics and host-pathogen interactions. Anim Health Res Rev 7: 1-11.

74. Seleem MN, Boyle SM, Sriranganathan N (2008) *Brucella*: a pathogen without classic virulence genes. Vet Microbiol 129: 1-14.

75. Vizcaino N, Cloeckaert A, Verger J, Grayon M, Fernandez-Logo L (2000) DNA polymorphism in the genus *Brucella*. Microbes Infect 2: 1089-1100.

76. Vizcaino N, Caro-Hernandez P, Cloeckaert A, Fernandez-Logo L (2004) DNA polymorphism in the omp25/om31 family of *Brucella* spp.: identification of a 1.7-kb inversion in *Brucella* cetaceae and of a 15.1-kb genomic island, absent from *Brucella* ovis, related to the synthesis of smooth lipopolysaccharide. Microbes Infect 6: 821-834.

77. Marianelli C, Ciuchini F, Tarantino M, Pasuali P, Adone R (2004) Genetic bases of the rifampicin resistance phenotype in *Brucella* spp. J Clin Microbiol 42: 5439-5443.

78. Halling SM, Jensen AE (2006) Intrinsic and selected resistance to antibiotics binding the ribosome: analysis of *Brucella* 23Srrn, L4, L22, EF-TuL, EF-Tu2, efflux and phylogenetic implications. BMC Microbiol 6: 84.

79. Economidou J, Kalafatas P, Vatopoulou T, Petropoulou D, Kattamis C (1976) Brucellosis in two thalassaemic patients infected by blood transfusions from the same donor. Acta Haematol 55: 244-249.

80. Akcakus M, Esel D, Cetin N, Kisaarslan AP, Kurtoğlu S (2005) *Brucella* mellitensis in blood cultures in two newborns due to exchange transfusion. Turk J Pediatr 47: 272-274.

81. Khorasgani MR, Esmaeili H, Pourkarim MR, Mankhian AR, Salehi TZ (2008) Anti-*Brucella* antibodies in blood donors in Boushehr, Iran. Comparative Clinical Pathology 17: 267-269.

82. Khan MY, Mah MW, Memish ZA (2001) Brucellosis in pregnant women. Clin Infect Dis 32: 1172-1177.

83. Ertem M, Kürekci AE, Aysev D, Unal E, Ikincioğullari A (2000) Brucellosis transmitted by bone marrow transplantation. Bone Marrow Transplant 26: 225-226.

84. Meltzer E, Sidi Y, Smolen G, Banai M, Bardenstein S, et al. (2010) Sexually transmitted brucellosis in humans. Clin Infect Dis 51: 12 - 15.

85. Vandercam B, Zech F, de Cooman S, Bughin C, Gigi J, et al. (1990) Isolation of *Brucella melitensis* from human semen. Eur J Clin Microbiol Infect Dis 9: 303-304.

86. Luther E. Lindler, Frank J. Lebeda, George W. Korch (2005) Biological Weapons Defence: Infectious Diseases and Counterbioterrorism. Humana Press, Totowa, New Jersey, USA.

87. Brouillard JE, Terriff CM, Tofan A, Garrison MW (2006) Antibiotic selection and resistance issues with flouroquinolones and doxycycline against bioterrorism agents. Pharmacotherapy 26: 3-14.

88. Burt Anderson, Herman Friedman, Mauro Bendinelli (2006) Microorganisms and Bioterrorism, Infectious Agents and Pathogenesis. Springer Science+Business Media, Inc., 233 Spring Street, New York, NY 10013, USA.

89. Centers for Disease Control and Prevention [CDC] (2008) Laboratory-acquired brucellosis---Indiana and Minnesota, 2006, MMWR Morb Mortal Wkly Rep, 57: 39-42.

90. Yagupsky P, Jo Baron E (2005) Laboratory exposures to *Brucella*e and implications for bioterrorism. Emerg Infect Dis 11: 1180-1185.

incubation period, the clinical features are protean and non-specific [1,2].

Presentation of *Brucellosis* is also very variable. It may present as an acute febrile illness or as a chronic infection. It can cause both localized infection as well as systemic and generalized disease. Severity of the infection is also very variable as it may be totally asymptomatic or can cause severe and potentially fatal illness. Relapses and complicated infections may also be encountered [1,2].

Fever is the commonest clinical feature. Insidious onset of fever, high grade and irregular, with chills lasting for days to weeks is the most usual presentation [1-3,5]. As the infection may be atypical in presentation, some cases present with fever of unknown origin (FUO) [2,5]. Other symptoms of *Brucellosis* include: night sweats, rigors, myalgia, arthralgia, low backache, anorexia, malaise, fatigue, weakness, weight loss, headache, dizziness, depressed mood, dyspepsia, nausea, vomiting, abdominal pain, cough, dyspnea, hemoptesis, testicular pain and burning micturition. Physical examination may reveal: swelling of joints, tenderness over joints and the lower back, splenomegaly, hepatomegaly, external lymphadenopathy, jaundice, mouth ulcerations, scrotal swelling and a variety of cutaneous eruptions [1-12]. The clinical manifestations and complications of *Brucellosis* included in 2 major retrospective studies and 1 meta-analysis are summarized in Table 1 [5,9,12].

Complications of *Brucellosis*

Osteoarticular complications

Osteoarticular disease is the most common complication of *Brucellosis* as it has been described in 10 to 85% of patients [13,14]. The spectrum of bone and joint involvement by *Brucellosis* includes: arthritis, bursitis, tenosynovitis, osteomyelitis, spondylitis and sacroiliitis [13-16]. Spondylitis is the most common and most important form of osteoarticular involvement by *Brucellosis* in adults as it has been reported in 6-58% of cases [16]. Spondylitis may be difficult to diagnose and can be complicated by potentially devastating neurological and vascular conditions. Back pain, fever and constitutional symptoms are the most common manifestations [13]. Spondylitis typically affects men over the age of 40 years. Areas of the spine that are involved include: lumber (60%), thoracic (19%) and cervical (12%). Tuberculosis of the spine should be included in the differential diagnosis [16]. Laboratory abnormalities in *Brucella sp*ondylitis include: elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), leucopenia or leucocytosis (at times with pus formation), anemia, thrombocytopenia and moderate elevation of liver enzymes (Table 2). Blood cultures may be positive in up to 85% of cases. Coomb's test is invariably positive and agglutination tests may be positive in up to 90% of cases [13,16]. Plain X-rays, computerized axial tomography (CAT) scans, bone scans and magnetic resonance imaging (MRI) are useful in the diagnosis of spinal *Brucellosis*. Although bone scans are very useful, MRI is the method of choice for diagnosis and follow-up in spondylitis caused by *Brucellosis* [13,16-19]. The radiological features appear 3 to 5 weeks after the onset of the clinical manifestations [16]. The radiological abnormalities are variable and they include: focal spondylitis, epiphysitis, diskitis with disc collapse, paravertebral abscesses and osteomyelitis (bone destruction) that may involve more than one bone or vertebral level and that may occur in 6-50% of patients (Figure 1) [13-16,18,20,21]. Treatment of spondylitis includes a combination of antimicrobials in addition to other measures such as analgesia, immobilization to reduce pain and drainage of abscesses if present. The best combination therapy includes doxycycline and aminoglycoside and response rate using this regimen ranges between 60 and 90% of treated patients. Doxycycline and rifampicin combination can be given alternatively while the combination of co-trimoxazole and ciprofloxacin is not recommended as it has been reported to yield a poor outcome. Longer duration of treatment is usually required. While some studies have shown that 6 to 12 weeks may be the optimal

Laboratory abnormality	Percentage
Elevated ESR	69.4
Elevated CRP	57.5
Anemia	26.4
Positive rheumatoid factor	12.7
Leucopenia	10.7
Leucocytosis	8.4
Thrombocytopenia	7.03
Thrombocytosis	1.1

ESR: erythrocyte sedimentation rate;CRP: C-reactive protein

Table 2: Laboratory abnormalities in 1239 patients with brucellosis.

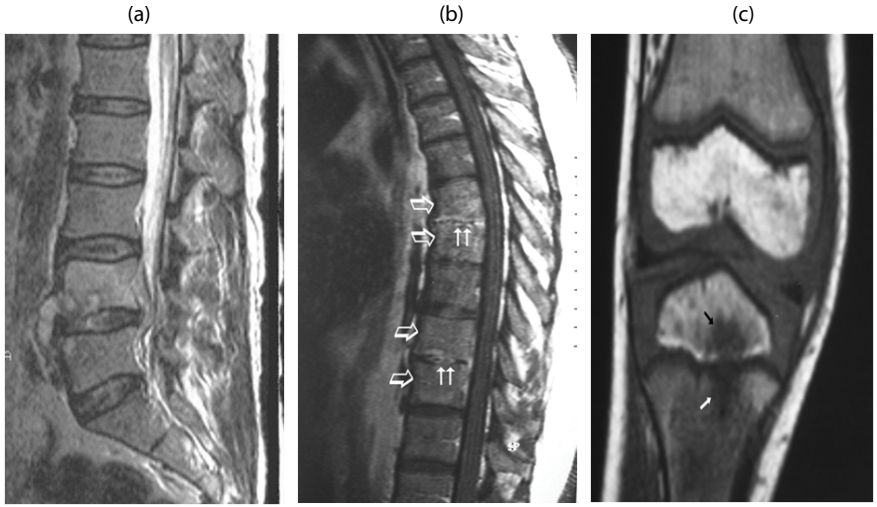

Figure 1: (A): MRI of lumbar spine in a patient with brucellosis showing evidence of paravertebral and epidural abscess; (B): MRI of thoracic spine in a patient with brucellosis showing evidence of multifocal spondylodiskitis; (C): MRI of tibia in a patient with brucellosis showing evidence of osteomyelitis.

into: central, peripheral, focal or diffuse. The onset can be acute or chronic [26,29,30]. The clinical manifestations of neuro*Brucellosis* include: (1) meningitis that can be acute or chronic with acute *Brucella* meningitis being the commonest presentation of neuro*Brucellosis*, (2) meningoencephalitis, (3) signs of meningeal irritation such as neck stiffness, (3) headache and isolated intracranial hypertension, (4) polyradiculoneuritis: peripheral and cranial nerve palsies, sensory and motor abnormalities in addition to paraplegia and quadriplegia, (5) brain and epidural abscesses, (6) subdural hematomas, intracerebral hemorrhages, subarachnoidal hemorrhages, transient ischemic attacks and hemiplegia, (7) convulsions, coma and stupor, (8) cerebellar dysfunction, gait abnormalities and spastic paraplegia, (9) myelitis and chorea, (10) depression, psychosis and dementia, (11) backache, areflexia and hearing loss, (12) mycotic aneurysms and (13) Guillain-Barre syndrome [26-30].

Cerebrospinal fluid (CSF) analysis usually shows: leucocytosis with predominant lymphocytosis, elevated CSF protein and low CSF glucose [26,27,29]. CSF cultures for *Brucella* may be positive. *Brucella* serology and blood cultures are usually positive. Pus cultures from brain abscess may be positive for *Brucella sp*ecies and brain biopsy usually reveals granuloma formation with central necrosis [26]. MRI may show: brain parenchymal swelling causing decreased lateral ventricular volume, hydrocephalus with periventricular edema, meningeal enhancement in posterior fossa, multiple hypodense periventricular

monocytic or lymphocytic infiltration, interstitial pneumonitis, pulmonary granulomas or solitary nodules, bilateral glass round opacities and military mottling, empyema, pneumothorax, hilar and paratracheal lymphadenopathy, perivascular and peribronchial thickening, centri-acinar emphysematous changes and atelectasis [37-39]. *Brucella sp*ecies can be cultured from pleural fluid, but the yield from sputum or bronchoalveolar lavage is usually poor. Treatment is usually in the form of combination therapy that includes two of the following agents: doxycycline, rifampicin, streptomycin and co-trimoxazole. Pulmonary involvement by *Brucellosis* has good prognosis provided combined antimicrobial therapy is administered early in the course of the infection [37-39]. Chest wall involvement is a rare manifestation of *Brucellosis*. Presentation is usually with parasternal masses and nodular lesions over the chest wall that may be misdiagnosed as tuberculosis or malignancy [40].

Cutaneous involvement

Cutaneous lesions are usually considered non-specific findings in patients with *Brucellosis*. Cutaneous involvement has been reported in 1 to 14% of patients with *Brucellosis*. Skin lesions may appear at presentation, during the course of the illness or at relapse [41-45]. A variety of skin manifestations have been reported including: disseminated erythema, diffuse maculopapular eruption, papulonodular lesions, erythema nodosum-like eruption, psoriasiform eruption, malar eruption, palmar erythema and eczema, ecchymoses, purpura, leucocytoclastic vasulitis, panniculitis and multiple skin abscesses [41-46]. Hematogenous spread of the organisms can be the most important pathogenic mechanism [41,42]. Serological tests for *Brucellosis* may be positive and blood cultures may be positive in up to 77% of patients, with B.mellitensis being the commonest species cultured [42,43,46]. Skin biopsy is usually positive and may facilitate the diagnosis of *Brucellosis*. A variety of histopathological features have been described including: dermal inflammatory infiltrates with dominance of lymphocytes and histiocytes, perivascular and periadnexal arrangement of infiltrates and focal granulomatous changes. Cultures of skin lesions, particularly in case of abscess formation, may be positive for *Brucella sp*ecies [41-43,46].

Hematological abnormalities

Leucopenia is more frequently encountered in acute *Brucellosis*. Lymphopenia significantly correlates with the severity of clinical manifestations e.g. bleeding and hepatic involvement. Relative lymphocytosis may occasionally be encountered. Pancytopenia is commonly seen, often at presentation. Anemia is also a common consequence of *Brucella* infection and may be severe. Thrombocytopenia is occasionally encountered and may be severe (Table 2). Bleeding diathesis and even disseminated intravascular coagulation may occur. Hemolytic anemia that may be acute and Coomb's positive can also be seen. Elevation of ESR and CRP may also be seen (Table 2) [3,47-54]. Bone marrow examination in patients with *Brucellosis* usually shows: hypercellular or normocellular marrow, epitheliod giant cell granulomas that are usually small with indistinct borders mimicking granulomas of tuberculosis and sarcoidosis. Histiocytic phagocytosis may be seen with or without peripheral pancytopenia [47,48,54,55].

Microangiopathic hemolytic anemias, e.g. thrombotic thrombocytopenic purpura, have been reported in patients with acute *Brucellosis*. Despite the severity of this rare complication, complete recovery has been encountered with early and prompt therapy using plasma exchange, antimicrobial therapy for *Brucellosis* and corticosteroids [56-59]. Thrombocytopenic purpura has also been reported in patients with *Brucellosis*. Early recognition of this complication is essential as central nervous system (CNS) hemorrhage is associated with high mortality rates. Nevertheless, treatment of *Brucellosis* in addition to corticosteroid therapy can control both disorders [60,61].

Capillary leak syndrome has been reported in patients with *Brucellosis* and pancytopenia. Patients may present with: fever, sweats, weakness, hepatosplenomegaly, peripheral edema,

Other rare complications of *Brucellosis*

1. Gastrointestinal involvement: *Brucellosis* may be complicated by: acute cholecystitis, spontaneous bacterial peritonitis, ileitis, colitis and diverticulitis presenting with acute abdomen [85-88].
2. Eye complications: Uveitis is the most frequent ocular presentation of *Brucellosis* and posterior uveitis is the most frequent uveal syndrome. Other ophthalomological complications include: keratoconjunctivitis, endophthalmitis, choroiditis, iridocyclitis, corneal ulcers, nummular keratitis, papilledema, cataract, glaucoma, diplopia, ophthalmoplegia, optic neuritis and atrophy in addition to phthisis bulbi [2,89]. It is important to rule out uveitis through an ophthalmic examination in every suspicious case of *Brucellosis*, as uveitis is a potentially blinding complication [89,90].
3. *Brucellosis* does not appear to be associated with hearing loss, while mastitis is a very rare presentation of *Brucellosis* in female patients [91,92].
4. Acute renal failure and spontaneous splenic rupture have also been reported [93,94].

Relapse of *Brucellosis*

Relapses occur in 4.7 to 29% of patients with *Brucellosis* [2,6,9,95]. Relapses usually occur within 3 to 12 months of discontinuation of the antimicrobial therapy [1,2,6]. The risk factors for relapse include: male sex, old age, lymphopenia, deficient immunologic response such as in associated human immunodeficiency virus (HIV) infection, presence of an aggressive disease or a chronic infection, positive blood cultures during initial infection, an inadequate choice of antibiotics, monotherapy rather than combination treatment, shortened duration of therapy, localized foci of infection, positive family history, living in endemic area and rarely resistance to antimicrobial therapy [1,2,6,95-98]. Relapse is not usually associated with: the initial or subsequent antibiotic susceptibility, the specific antimicrobial regimen used to control the initial infection or having a high risk occupation [1,2,6,98.99]. Clinical differentiation between relapse and re-infection in areas of ongoing exposure can be difficult [2]. The highest relapse rates have been encountered in patients with complications such as osteoarticular involvement [9,95]. Independent predictors of relapse include: positive blood cultures at baseline, temperature of ≥ 38 0C and duration of symptoms less than 10 days [2]. During relapse: blood cultures may be positive, ESR and CRP are usually elevated [1,96,97]. A repeat, but a longer course of a standard therapeutic regimen such as the combination of doxycycline, streptomycin and/or rifampicin and surgical intervention in case of localized foci of infection are usually successful in most relapses [1,2,6,9].

Chronic *Brucellosis*

Although no uniform definition has been agreed upon, chronic *Brucellosis* traditionally refers to the persistence of clinical manifestations for at least one year after establishment of the diagnosis of *Brucellosis* [1,2]. Chronic *Brucellosis* is characterized by: (1) localized infection such as spondylitis, osteomyelitis, tissue abscess or uveitis producing recurrent bouts of fever and other clinical manifestations, (2) relapse in patients with an objective evidence of infection such as high 1gG antibody titers and/or recovery of *Brucella*e from blood or tissues and (3) manifestations such as chronic fatigue syndrome and psychoneurosis [1,2,6].

The clinical manifestations of chronic *Brucellosis* include: sweating, fatigue, malaise, tiredness, weakness, anorexia, depression, arthralgia, myalgia, headache, lower backache, pain in temporomandibular joints, irritability, insomnia, abdominal pain, diarrhea, constipation, arthritis, and lymphadenopathy [2,100-102]. There are 2 types of chronic *Brucellosis*: (1) chronic *Brucellosis* with clear history of acute infection, where *Brucella* symptomatology continues after the acute attack. (2) chronic *Brucellosis* without clear

rifampicin or cotrimoxazole is inadequate and is associated with high relapse rates [117]. The treatment of choice should be effective and should have minimal adverse effects [117]. In pregnant women presenting with febrile illness, the beneficial effects of treatment are usually encountered [107]. The optimal duration of antimicrobial therapy is 6 weeks and the success rate of antibiotic treatment for *Brucellosis* in pregnant women may reach 90% [109,117,120]. Health education of the target population is advisable to prevent the disease and its complications. Screening programs for *Brucellosis* in pregnant women living in endemic areas is recommended [108,124]. *Brucellosis* should also be kept in the differential diagnosis of fever in pregnant females living in endemic areas as early diagnosis and prompt therapy have been shown to improve the outcome considerably [108,124].

Congenital and neonatal *Brucellosis*

Congenital *Brucellosis* is very rare and its diagnosis indicates a substantial local endemic activity [125]. Congenital and neonatal *Brucellosis* are usually associated with the presence of untreated maternal *Brucellosis* or *Brucella* bacteremia in the pregnant mother [125,126]. Neonates become congenitally infected with *Brucellosis* by the following means: transplacental transmission from an infected mother, exposure to blood, urine or genital secretions during delivery, breast feeding and blood or exchange transfusion in the early neonatal period [117,125-128]. The clinical manifestation include: fever, anemic symptoms or features of bone marrow failure, jaundice, respiratory distress, vomiting, irritability, convulsions, hepatosplenomegaly, septic shock, multiorgan involvement, meningitis and endocarditis [125,127-129]. The diagnosis is made in the presence of: a compatible clinical picture, positive *Brucella* serology or positive cultures for *Brucella* obtained from blood or breast milk [125,127-129]. The choice of drug therapy is very limited due to side effects such as fluoroquinolone induced cartilage damage, teeth staining and fatty necrosis of liver caused by tetracyclines and cotrimoxazole induced kernicterus [125,127-129]. However, the teratogenic potential of rifampicin, fluoroquinolones and cotrimoxazole is simply unknown [130]. In individual cases, cotrimoxazole has been used successfully and there have been no reports of gentamicin toxicity [130]. The combination of an aminoglycoside e.g. gentamicin or amikacin plus rifampicin or cotrimoxazole is usually successful and may prevent complications [125,127-129].

Brucellosis in children

The incidence of childhood *Brucellosis* varies according to the geographic location and the strain of *Brucella sp*ecies. Where B. abortus is endemic, childhood *Brucellosis* is relatively uncommon while in areas that are endemic for B. *melitensis*, children represent 20-25% of all cases of *Brucellosis* [131,132]. Childhood *Brucellosis* is more frequently encountered in males than in females [132-134]. The main sources of *Brucella* infection in childhood are: consumption of raw milk and dairy products, close animal contact and recent history of travel to endemic areas [131,132,134-142]. The clinical manifestations of *Brucellosis* in children include: fever, sweating, lethargy, clinical evidence of bleeding e.g. epistaxis or hematuria, nasopharyngitis, arthralgia, myalgia, hepatosplenomegaly and weight loss [131,133-145]. Fever, which may be prolonged, is the commonest clinical feature as it is encountered in 75-100% of cases while arthritis is usually seen in 50-75% of patients [135,137-140,142]. Arthritis is usually oligoarticular with predominant involvement of lower limb joints. Unlike in adults, axial skeletal involvement is rarely encountered [137]. Childhood *Brucellosis* may be complicated by: neuro*Brucellosis*, endomyocarditis, osteomyelitis, skin lesions, bacteremia and relapse [131,132,135,136,146-149]. Various skin eruptions have been reported and lesions include: maculopapular eruptions, petechiae, purpura, impetiginous and psoriasiform lesions, papules and Pityriasis alba [131,135,146].

Involvement of the nervous system is rare in childhood *Brucellosis* [131,147]. Childhooh neurobrucllosis may present with meningitis, meningoencephalitis, meningomyelitis, cerebellar ataxia, acute facial palsy, peripheral neuritis and chorea [131,134,147]. CSF

presentation of acute leukemia or even if leukemia is under control. Early diagnosis of *Brucellosis* and prompt administration of appropriate antimicrobial therapy usually improve the outcome in such immunocompromised individuals. Presentation is usually with fever and pancytopenia. Antimicrobial therapy can be administered simultaneously with cytotoxic chemotherapy to control both *Brucellosis* and leukemia but in case of bacteremia, prompt antimicrobial therapy may become essential [154-156]. *Brucellosis* and even *Brucella* bacteremia can develop in recipients of hematopoietic stem cell transplantation (HSCT) living in endemic areas at any stage of their illness. Like in patients with acute leukemia, presentation is usually with fever and cytopenias. Prompt therapy with appropriate antimicrobials such as streptomycin, doxycycline and ciprofloxacin will control the infection and prevent further complications [157].

Brucellosis and renal disease

Brucellosis has been reported in patients with end stage renal disease (ESRD) living in endemic areas. Such cases usually present with FUO but if left untreated, the infection may be complicated by serious complications such as neuro*Brucellosis*, paravertebral and epidural abscesses and peripheral arthritis [158,159]. On the other hand, *Brucellosis* has been reported to cause ESRD. Renal biopsies in patients having *Brucellosis* as a cause of ESRD have shown: mesangial and diffuse proliferative glomerulonephritis, rapidly progressive and focal segmental glomerulonephritis as well as exudative glomerulonephritis. Other renal complications of *Brucellosis* include: pyelonephritis, interstitial nephritis, mixed cryoglobulinemia and IgA nephropathy. Antimicrobial therapy given for *Brucellosis* usually improves the renal involvement [160].

Brucellosis coexisting with other infections and medical illnesses

Brucellosis has been reported in patients having other infections such as leishmaniasis, hepatitis C infection, HIV and viral hemorrhagic fevers e.g. dengue fever. In such patients, cytopenias are major complications. Treatment of both infections and supportive care will prevent further complications [161-166].

Brucellosis has also been reported in patients having chronic medical illnesses such as polycythemia rubra vera, chronic osteoarthritis, liver cirrhosis, pulmonary fibrosis and rheumatoid arthritis receiving infliximab therapy. Early institution of appropriate antimicrobial therapy is essential to control *Brucella* infection and to prevent evolution of complications [167,168].

Brucellosis and chronic neurological disorders

Approximately 40% of patients with infections caused by *Brucella sp*ecies develop systemic and chronic manifestations indistinguishable from chronic fatigue syndrome (CFS) or myalgic encephalomyelitis (ME). Approximately 10% of patients with CFS/ME have been found to have presence of *Brucella sp*ecies infections as shown by PCR [169]. Patients infected with *Brucellosis* may present with neurological manifestations compatible with multiple sclerosis. However, old and new literature provides conflicting data on the association between *Brucellosis* and multiple sclerosis [170-172].

Brucellosis and solid tumors

It has been postulated that chronic *Brucellosis* may be associated with tumor formation. *Brucella sp*ecies DNA, not *Brucella sp*ecies organism, has been identified in CNS tumors such as medulloblastomas. Further studies are needed to explore the true association between *Brucella sp*ecies DNA positivity and CNS tumor formation [173].

Brucellosis may also simultaneously present with other solid tumors e.g. ovarian

8. Arizona Department of Health Services (2004) *Brucellosis*: Bioterrorism Agent Profiles for Health Care Workers, Office of Public Health Emergency Preparedness and Response, 5.1-5.4.

9. Mantur BG, Amarnath SK (2008) *Brucellosis* in India-a review. J Biosci 33: 539-547.

10. Evans AS, Brachman PS (2009) Bacterial Infections of Humans Epidemiology and Control. (4thedn), Springer Science + Business Media, LLC, 233, New York, NY 10013, USA.

11. Pappas G, Akritidis N, Bosilkovski M, Tsianos E (2005). *Brucellosis*. N Engl J Med 352: 2325-2336.

12. Donev D, Karadzovski A, Kasapinov B, Lazarevik V (2010) Epidemiological and public health aspects of *Brucellosis* in the republic of Macedonia. Prilozi 31: 33-54.

13. Buzgan T, Karahocagil MK, Irmak H, Baran AI, Karsen H, et al. (2010) Clinical manifestations and complications in 1028 cases of *Brucellosis*: a retrospective evaluation and review of the literature. Int J Infect Dis 14: 469-478.

14. Demiroğlu YZ, Turunc T, Aliskan H, Colakoğlu S, Arslan H (2007) *Brucellosis*: retrospective evaluation of the clinical, laboratory and epidemiological features of 151 cases. Mikrobiyol Bul 41: 517-527.

15. Lulu AR, Araj GF, Khateeb MI, Mustafa MY, Yusuf AR, et al. (1988) Human *Brucellosis* in Kuwait: a prospective study of 400 cases. Quart J Med 66: 39-54.

16. Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, et al (2012) Clinical manifestations of human *Brucellosis*: a systematic review and meta-analysis. PLOS Negl Trop Dis 6: 1929.

17. Solera J, Lozano E, Martinez-Alfaro E, Espinosa A, Castillejos ML, et al. (1999) *Brucella*r spondylitis: review of 35 cases and literature survey. Clin Infect Dis 29: 1440-1449.

18. Weil Y, Mattan Y, Liebergall M, Rahav G (2003) *Brucella* prosthetic joint infection: a report of 3 cases and a review of the literature. Clin Infect Dis 36: 81- 86.

19. Namiduru M, Karaoglan I, Gursoy S, Bayazit N, Sirikci A (2004) *Brucellosis* of the spine: evaluation of the clinical laboratory and radiological findings of 14 patients. Rheumatol Int 24: 125-129.

20. Bouaziz MC, Ladeb MF, Chakroun M, Chaabane S (2008) Spinal *Brucellosis*: a review. Skeletal Radiol 37: 785-790.

21. Pourbagher A, Pourbogher MA, Savas L, Turunc T, Demiroglu YZ, et al. (2006) Epidemiologic, clinical and imaging findings in *Brucellosis* patients with osteoarticular involvement. Am J Radiol 187: 873-880.

22. Ozaksoy D, Yücesoy K, Yücesoy M, Karonlikaya I, Yüce A, et al. (2001) *Brucella*r spondylitis: MRI findings. Eur Spine J 10: 529-533.

23. Aydin A, Fuat Yapar A, Savas L, Reyhan M, Pourbagher A, et al. (2005) Scintigraphic findings in osteoarticular *Brucellosis*. Nucl Med Commun 26: 639-647.

24. Colmenero JD, Ruiz-Mesa JD, Plata A, Bermudez P, Martin-Rice P, et al. (2008) Clinical findings, therapeutic approach and outcome of *Brucella*r vertebral osteomyelitis. Clin Infect Dis 46: 426-433.

25. Kim D-H, Cho Y-D (2008) A case of spondylodiscitis with spinal epidural abscess due to *Brucella*. J Korean Neurosurg Soc 43: 37-40.

26. Alkhateeb MI, Araj GF, Majeed SA, Lulu AR (1990) *Brucella* arthritis: a study of 96 cases in Kuwait. Ann Rheum Dis 49: 994-998.

27. Bayindir Y, Sonmez E, Aladag A, Buyukberber N (2003) Comparison of five antimicrobial regimens for the treatment of *Brucella*r spondylitis: a prospective, randomized study. J Chemother 15: 466-471.

28. Turgut M, Turgut AT, Kosar U (2006) Spinal *Brucellosis*: Turkish experience based on 452 cases published during the last century. Acta Neurochir (Wien) 148: 1033-1044.

29. Santiago T, Rovisco J, Silva J, Pereira da Silva JA (2011) Osteoarticular *Brucellosis*, a ten year restrospective analysis. Acta Rheumatol Port 36: 120-125.

30. Haji-Abdolbagi M, Rasooli-Nejad M, Jafari S, Hasibi M, Soudbakhsh A (2008) Clinical and laboratory findings in neuro*Brucellosis*: review of 31 cases. Arch Iranian Med 11: 21-25.

31. Yetkin MA, Bulut C, Erdinc FC, Oral B, Tulek N (2006) Evaluation of the clinical presentations in neuro*Brucellosis*. Int J Infect Dis 10: 446-452.

55. Abdi-Liae Z, Soudbakhsh S, Jafari S, Emadi H, Tomaj K (2007) Haematological manifestations of *Brucellosis*. Acta Med Iranica 45: 145-148.

56. Crosby E, Liosa L, Miro Quesada M, Carrillo C, Gotuzzo E (1984) Hematological changes in *Brucellosis*. J Infect Dis 150: 419-424.

57. Sari I, Kocyigit I, Altuntas F, Kaynar L, Eser B (2008) An unusual case of acute *Brucellosis* presenting with Coombs-positive autoimmune hemolytic anemia. Inter Med 47: 1043-1045.

58. Martin-Moreno S, Soto-Guzman O, Bernaldo-de-Quirös J, Reverte-Cejudo D, Bascones-Casas C (1983) Pancytopenia due to hemophagocytosis in patients with *Brucellosis*: a report of four cases. J Infect Dis 147: 445-449.

59. Hovde RF, Sundberg RD (1950) Granulomatous lesions in the bone marrow in infectious mononucleosis: a comparison of the changes in the bone marrow in infectious mononucleosis with those in *Brucellosis*, tuberculosis, sarcoidosis and lymphatic leukemia. Blood 5: 209-232.

60. Kuperman AA, Baidousi A, Nasser M, Braester A, Nassar F (2010) Microangiopathic anemia of acute *Brucellosis*-is it a true TTP? Medit J Hemat Infect Dis 2: 2010031.

61. Altuntas F, Eser B, Sari I, Yildiz O, Cetin M, Ünal A (2005) Severe thrombotic microangiopathy associated with *Brucellosis*: successful treatment with plasmapheresis. Clin Appl Thromb Hemost 11: 105-108.

62. Erdem F, Kiki I, Gundoğdu M, Kaya H (2007) Thrombotic thrombocytopenic purpura in a patient with *Brucella* infection is highly responsive to combined plasma infusion and antimicrobial therapy. Med Princ Pract 16: 324-326.

63. Di Mario A, Sica S, Zini G, Salutari P, Leone G (1995) Microagiopathic hemolytic anemia and severe thrombocytopenia in *Brucella* infection. Ann Hematol 70: 59-60.

64. Young EJ, Tarry A, Genta RM, Ayden A, Gutuzoo E (2000) Thrombocytopenic purpura associated with *Brucellosis*: report of 2 cases and literature review. Clin Infect Dis 31: 904-909.

65. Sevinc A, Buyukberber N, Camci C, Buyukberber S, Karsligil T (2005) Thrombocytopenia in *Brucellosis*: case report and literature review. J Natl Med Assoc 97: 290-293.

66. Erkurt MA, Sari I, Gul HC, Coskun O, Eyigun CP, et al. (2008) The first documented case of *Brucellosis* manifested with pancytopenia and capillary leak syndrome. Inter Med 47: 863-865.

67. Bakri FG, Al-Bsoul NM, Magableh AY, Shehabi A, Tarawneh M, et al. (2010) *Brucellosis* presenting as myelofibrosis: first case report. Int J Infect Dis 14: 158-160.

68. Li JJ, Sheng Z-K, Tu S, Bi S, Shen X-M, et al. (2012) Acute *Brucellosis* with myelodysplastic syndrome presenting as pancytopenia and fever of unknown origin. Med Princ Pract 21: 183-185.

69. Gur A, Geyik MF, Dikici B, Nas K, Cevik R, et al. (2003) Complications of *Brucellosis* in different age groups: a study of 283 cases in southeastern Anatolia of Turkey. Yonsei Med J 44: 33-44.

70. Ariza J, Pigrau C, Canas C, Marron A, Martinez F, et al. (2001) Current understanding and management of chronic hepatosplenic suppurative *Brucellosis*. Clin Infect Dis 32: 1024-1033.

71. Diaz R, Ariza J, Alberola I, Casanova I, Rubio MF (2006) Secondary serological responses of patients with chronic hepatosplenic suppurative *Brucellosis*. Clin Vaccine Immunol 13: 1190-1196.

72. Colmenero JD, Suarez-Munöz MA, Queipo-ortuño MI, Reguera JM, Morata P (2002) Late reactivation of calcified granuloma in a patient with chronic suppurative *Brucellosis*. Eur J Clin Microbiol Infect Dis 21: 897-899.

73. Young EY (1979) *Brucella melitensis* hepatitis: the absence of granulomas. Ann Intern Med 19: 414-415.

74. Young EY (1980) *Brucella* hepatitis. Ann Intern Med 92: 708-709.

75. Bruguera M, Cervantes F (1980) Hepatic granulomas in *Brucellosis*. Ann Intern Med 92: 571-572.

76. Jordans HGM, De Bruin KD (1980) Granulomas in *Brucella melitensis* infection. Ann Intern Med 92: 264-265.

77. Halimi C, Bringard N, Boyer N, Vilgrain V, Panis Y, et al (1999) Hepatic brucelloma: 2 cases and a review of the literature. Gastroenterol Clin Biol 23: 513-517.

78. Ennibi K, Rabhi M, Chemsi M, Elouennass M, Chaari J, et al (2009) Nodular liver lesions with fever in a Moroccan man: hepatic brucelloma. Med Trop (Mars) 69: 509-511.

103. Ariza J, Bosch J, Gudiol F, Linanes J, Fernandez Viladrich P, et al. (1986) Relevance of in vitro antimicrobial susceptibility of *Brucella melitensis* to relapse rate in human *Brucellosis*. Antimicrob Agents Chemother 30: 958-960.

104. Editorial (1974) Chronic *Brucellosis*. Br Med J 1: 299.

105. Sacks N, Van Rensburg AJ (1976) Clinical aspects of chronic *Brucellosis*. S Afr Med J 50: 725-728.

106. McDevitt MG (1973) Symptomatology of chronic *Brucellosis*. Br J Indust Med 30: 385-389.

107. White RG (1978) Immunoglobulin profiles of the chronic antibody response: discussion in relation to *Brucellosis* infections. Postgrad Med J 54: 595-602.

108. Aroca MJC, Garcia EN, Santos JS (2011) Association between *Brucella melitensis* DNA and *Brucella spp*. antibodies. Clin Vaccine Immunol 18: 892.

109. Castano MJ, Solera J (2009) Chronic *Brucellosis* and persistence of *Brucella melitensis* DNA. J Clin Microbiol 47: 2084-2089.

110. Nimri LF (2003) Diagnosis of recent and relapsed cases of human *Brucellosis* by PCR assay. BMC Infect Dis 3: 1-7.

111. Khan MY, Mah MW, Memish ZA (2001) *Brucellosis* in pregnant women. Clin Infect Dis 32: 1172-1177.

112. Kurdoglu M, Adali E, Kurdoglu Z, Karahocagil MK, Kolusari A et al (2010) *Brucellosis* in pregnancy: a 6-year clinical analysis. Arch Gynecol Obstet 281: 201-206.

113. Mohammad KI, El Ghazaly MM, Zaalouk TK, Morsy AT (2011) Maternal *Brucellosis* and human pregnancy. J Egypt Soc Parasitol 41: 485-496.

114. Schreyer P, Caspi E, Leiba Y, Eshchar Y, Simpolinski D (1980) *Brucella* septicemia in pregnancy. Eur J Obstet Reprod Biol 10: 99-107.

115. Jay Gloeb D, Lupi C, Jo O'Sullivan (1994) Neuro*Brucellosis* complicating pregnancy: a case report. Infect Dis Obstet Gynecol 1: 285-289.

116. Gulsun S, Aslan S, Satici O, Gul T (2011) *Brucellosis* in pregnancy. Trop Doct 41: 82-84.

117. Roushan MR, Baiani M, Asnafi N, Saedi F (2011) Outcome of 19 pregnant women with *Brucellosis* in Babol. Trans R Soc Trop Med Hyg 105: 540-542.

118. Hackmon R, Bar-David J, Bashiri A, Mazor M (1998) *Brucellosis* in pregnancy. Harefuah 135: 3-7,88.

119. Elshami M, Amien Ahmed I (2008) The effects of maternal *Brucellosis* on pregnancy outcome. J Infect Developing Countries 2: 230-234.

120. Nuri P, Volkan T, Mete E, Ozgur Y (2011) *Brucellosis* in adolescent pregnancy-case report and review of literature. Ginecol Pol 82: 226-229.

121. Karcaaltincaba D, Senan I, Kandemir O, Guvendag-Guven ES, Yalvac S (2010) Does *Brucellosis* in human pregnancy increase abortion risk? Presentation of two cases and review of literature. J Obstet Gynaecol Res 36: 418-423.

122. Makhseed M, Harouny A, Araj G, Moussa MA, Sharma P (1998) Obstetric and gynecologic implications of *Brucellosis* in Kuwait. J Perinatol 18: 196-199.

123. Al-Tawfiq JA, Memish ZA (2013) Pregnancy associated *Brucellosis*. Recent Pat Antiinfect Drug Discov 8: 47-50.

124. Abo-Shehada MN, Abu-Halaweh M (2011) Seroprevalence of *Brucella sp*ecies among women with miscarriage in Jordan. East MediterrHealth J 17: 871-874.

125. Nassaji M, Rahbar N, Ghorbani R, Lavaf S (2008) The role of *Brucella* infection among women with spontaneous abortion in an endemic region. J Turkish-German Gynecol Assoc 9: 20-23.

126. Sharif A, Reyes Z, Thomassen P (1990) Screening for *Brucellosis* in pregnant women. J Trop Med Hyg 93: 42-43.

127. Figueroa Damian R, Rojas Rodriguez L, Marcano Tochon ES (1995) *Brucellosis* in pregnancy: course and perinatal results. Ginecol Obstet Mex 63: 190-195.

154. Al-Eissa Y, Al-Nasser M (1993) Haematological manifestations of childhood *Brucellosis*. Infection 21: 23-26.

155. Khuri-Bulos NA, Daoud AH, Azab SM (1993) Treatment of childhood *Brucellosis*: results of a prospective trial on 113 children. Pediatr Infect Dis J 12: 377-381.

156. Roushan MR, Mohraz M, Janmohammadi N, Hajiahmadi M (2006) Efficacy of cotrimoxazole and rifampicin for 6 or 8 weeks of therapy in childhood *Brucellosis*. Pediatr Infect Dis J 25: 544-545.

157. Lubani MM, Dudin KI, Sharda DC, Mana Ndhar DS, Aray GF, et al. (1989) A multicenter therapeutic study of 1100 children with *Brucellosis*. Pediatr Infect Dis 8: 75-78.

158. Al-Anazi KA, Al-Jasser AM (2007) *Brucella* bacteremia in patients with acute leukemia: a case series. J Med Case Reports 1: 144.

159. Eser B, Altuntas F, Soyuer I, Er O, Canoz O, et al. (2006) Acute lymphoblastic leukemia associated with *Brucellosis* in two patients with fever and pancytopenia. Yonsei Med J 47: 741-744.

160. Bay A, Oner AF, Dogan M, Acikgoz M, Dilek I (2007) *Brucellosis* concomitant with acute leukemia. Indian J Pediatr 74: 790-792.

161. Al-Anazi KA, Abu Jafar S, Al-Jasser AM, Al-Omar H, Al-Mohareb FI (2009) *Brucella* bacteremia in a recipient of an allogeneic hematopoietic stem cell transplant: a case report. Cases J 2: 91.

162. Turunc T, Demiroğlu YZ, Aliskan H, Colakoglu S, Timurkaynak F, et al. (2008) *Brucellosis* in cases of end-stage renal disease. Nephrol Dial Transplant 23: 2344-2349.

163. Kantartzi K, Panagoutsos S, Kokkinou V, Passadakis P, Vargemezis V (2009) Unexpectedly high incidence of *Brucellosis* in one university dialysis unit of North East Greece. Nephrol Dial Transplant 24: 2003-2004.

164. Ghanei E, Miladipour A, Nasrollahi A, Homayuni M (2009) *Brucellosis* with kidney failure. Iran J Kidney Dis 3: 109-111.

165. M. Fakhar M, E. Banimustafavi E, M.M. Motazedian MH, Q. Asgari Q (2009) Co-infection of *Leishmania infantum* and *Brucella sp*p. in Iran. Com Clin Pathol 18: 91-94.

166. Kurtaran B, Oto OA, Candevir A, Inal AS, Sirin Y (2011) A case of HIV infection with thrombocytopenia: association of HIV, thrombotic thrombocytopenic purpura and *Brucellosis*. Indian J Hematol Blood Transfus 27: 35-38.

167. Abou El Azm AR, Abou-Ali L, Kobtan AR, Mansour N, Tawfik S (2013) Can *Brucellosis* influence the course of hepatitis C in dual infection? Arch Virol 158 (3): 543-547.

168. Cocchi S, Bisi L,Codeluppi M, Venturelli C, Di Benedetto F, et al (2010) *Brucellosis* in a patient with end-stage liver disease undergoing liver transplantation: successful treatment with tigecycline. Liver Transplant 16: 1215-1216.

169. Bzeizi KI, Benmousa A, Sanai FM (2010) Coincidence of acute *Brucella* hepatitis and dengue fever or serologic cross-reactivity? Saudi J Gastroenterol 16: 299-301.

170. Ayyub M, Al-Juhani NR, Alfi AY, Al-Ukayli S (2006) *Brucellosis* and dengue fever- a co-infection or cross reactivity? Biomedica 22: 80-83.

171. Jacob NR, Rodriguez CG, Binaghi MA, Scapellato PG, Rosales Ostriz MB, et al. (2008) *Brucellosis* complicating chronic non-infectious disorders: diagnostic and therapeutic dilemmas. J Med Microbiol 57: 1161-1166.

172. Akgul O, Ozgocmen S (2011) Infliximab and *Brucellosis*: not the usual suspects, this time. Mod Rheumatol 21: 313-315.

173. Nicolson GL, J. Haier J (2010) Role of chronic bacterial and viral infections in neurodegenerative, neurobehavioural, psychiatric, autoimmune and fatiguing illnesses: part 2. Br J Med Practit 3: 301-310.

174. Eisele CW, McCullough NB, Beal GA (1950) *Brucellosis* and multiple sclerosis. JAMA 143: 1473-1474.

175. Spicknail CG, Kurland LT, Carle BN, Terry LL (1950) Relation of *Brucellosis* and multiple sclerosis. JAMA 143: 1470-1473.

176. Murrell TG, Mathews BJ (1990) Multiple sclerosis--one manifestation of neuro*Brucellosis*? Med Hypotheses 33: 43-48.

177. Zhang B, Izadjoo M, Horkayne-Szakaly I, Morrison A, Wear DJ (2011) Medulloblastoma and *Brucellosis*-

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Chapter: X

Brucellosis: A Global Re-emerging Zoonosis Diagnosis, Treatment and Prevention

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Abbreviations: *B: Brucella*; BCAP: anti-*Brucella* Immunocapture Agglutination Test; ELISA: Enzyme-Linked Immunosorbent Assay; FAO: Food and Agriculture Organization; FPA: Flouresence Polarization Assay; FUO: Fever of Unknown Origin; IFN-γ: Interferon-Gamma; Ig: Immunoglobulin; LFA: Lateral Flow Assay; LPS: Lipopolysaccharide; MAT: Microagglutination Test; MET: Mercaptoethanol; OPS: O polysaccharide; PCR: Polymerase Chain Reaction; PGK: Phosphoglycerate Kinase; RBT: Rose Bengal Test; RSAT: Rapid Slide Agglutination Test; SAT: Standard Tube Agglutination; WHO: World Health Organization

Introduction

Globally, brucellosis is one of the most common zoonotic diseases [1]. It is endemic and reemerging infection in rural areas in Mediterranean, Middle East and Latin American countries [2]. The disease is caused by *Brucella* species and 4 species of the genus *Brucella* are human pathogens. Brucellosis is transmitted from animals to humans by direct contact with infected animals or consumption of raw unpasteurized dairy products [1,2].

Brucellae are small Gram-negative strictly aerobic, nonencapsulated and nonmotile coccobacilli that can be isolated from the genitourinary tract flora of various wild and domestic animals. *Brucellae* evade the host defence mechanisms and are capable of affecting any organ system in the body thus causing various acute and chronic clinical manifestations, prolonged morbidity, relapses and long-term sequelae. The diagnosis is often delayed or may be missed and successful management requires prolonged treatment using a combination of antimicrobials [2].

Laboratory Diagnosis of Brucellosis

Brucella cultures

Blood cultures in brucellosis: In patients with brucellosis, positive blood cultures may

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subacute infection (31 vs 21%), chronic brucellosis (0.0 vs 8.0%) and the overall yield rates were 38% compared to 34% [4]. Several studies also showed that bone marrow cultures using conventional biphasic techniques may be superior to blood cultures in chronic cases of brucellosis and in patients with history of prior use of antibiotics where blood cultures may be negative [3,4,19]. In addition to bone marrow cultures, it is also worthwhile to try to culture aspirates or fluids obtained from: liver, lymph nodes, pleural space, bronchoalveolar lavage, joints and skin lesions in areas that lack advanced technology to diagnose brucellosis [19].

Serology in the diagnosis of brucellosis

Following *Brucella* infection, specific immunoglobulin (IgM) antibodies appear within one week, they reach a peak within 3 months and they remain elevated for weeks to months. Other specific immunoglobulin (IgG) antibodies appear within 3 weeks of infection, they reach a peak within 6 to 8 weeks and they remain present, albeit at low levels, for months to years after the recovery of patients [20-22].

Despite having a plethora of serological tests for the diagnosis of brucellosis, none of these tests is 100% reliable or perfect. So, serological test results should always be considered or interpreted in conjunction with patient history, clinical manifestations and other laboratory findings [23]. Other specific agglutinating antibodies are detected by Rose Bengal test (RBT) and standard tube agglutination (SAT) test. Coombs test, SAT in the presence of reducing agent and more recently enzyme linked immunosorbent assay [ELISA] have been used for the detection of specific IgG antibodies. Coombs test and IgG ELISA are particularly useful in the diagnosis of chronic infection and monitoring of disease relapse. Specific IgM ELISA antibodies are particularly useful in the diagnosis of acute brucellosis [20,24,25]. In acute brucellosis, *Brucella* Capt and Coombs test have positive titers of <1:160 [21]. When the titers are lower, they increase significantly in the following 30 days regardless the levels of SAT titers. In chronic brucellosis, titers of *Brucella* Capt and Coombs test are always high (≥ 1:640), whether SAT titers are higher or lower than 1:160 [21]. The diagnosis of relapse is particularly difficult and is most often based on the presence of a positive Coombs test with high titers [24].

a. Rose Bengal test (RBT)

The RBT is a rapid, slide-type agglutination assay performed with a stained *B. abortus* suspension at PH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources [26]. The drawbacks of RBT include: low sensitivity particularly in chronic cases, relatively low specificity in endemic areas and prozones make strongly positive sera appear negative in RBT [26]. The overall sensitivity is 92.9%, so the use of RBT should be considered carefully in endemic areas, particularly in individuals exposed to brucellosis and those having history of *Brucella* infection [27]. RBT is an agglutination test that is based on reactivity of antibodies against smooth lipopolysaccharide (LPS). As sensitivity is high, false negative results are rarely encountered. To increase specificity, the test may be applied to a serial dilution [1:2 through 1:64] of the serum samples [24,26,27]. The present world health organization (WHO) guidelines recommend the confirmation of the RBT by other assays such as serum agglutination tests [26,27].

b. ELISA assays

The available ELISA assays include: (1) IgG, IgA and IgM antibody test, (2) SAT and (3) 2- mercaptoethanol test (2-MET). However, the SAT is the gold standard assay [28,29]. ELISA has become popular as a standard assay for the diagnosis of brucellosis, serologically. It measures IgG, IgA and IgM antibodies and this allows a better interpretation of the clinical situation. The diagnosis of brucellosis is based on the detection of antibodies against the smooth LPS. Detection of IgG antibodies is more sensitive than detection of IgM antibodies for diagnosing cases of brucellosis but specificity is comparable [29-31]. Compared to the

a. Coombs test

This is the most suitable and sensitive test for confirmation of relapsing patients with persistent disease [24]. It is an extension of the SAT test i.e if the SAT test yields negative results due to the presence of blocking antibodies, Coombs test may be used instead. Agglutination can be determined visually, as for SAT, by using an agglutinoscope or a drop on a slide examined under the microscope [29]. Coombs test is used for detection of incomplete, blocking or non-agglutinating IgG. It is time consuming, technically difficult, requires skilled personnel and not routinely performed in clinical laboratories. It is good for complicated and chronic cases but misses about 7% of cases compared with ELISA [24,29,37].

b. Dipstick assay

The IgM dipstick assay is one of the tests that have been adapted to detect IgM antibodies to the smooth LPS. The assay has shown high sensitivity for patients with disease lasting less than 3 months [38-41]. IgM dipstick assay offers higher sensitivity and easier manipulation than IgM ELISA to detect IgM antibodies to *Brucella* species and improves the interpretation of results thus establishing cut-off points. IgM dipstick assay could be used as a rapid and simple alternative to the ELISA IgM for the serodiagnosis of patients with acute brucellosis. The combined results of SAT and IgM dipstick assays can provide an indication of the stage of disease for those patients in whom the onset of clinical manifestations is not known [38,41].

c. Fluorescence polarization assay (FPA)

The FPA was initially developed for testing serum. However, the technology has been extended to testing whole blood and milk samples from individual animals. FPA is based on the rotational differences between a small soluble antigen molecule in solution and the antigen molecule complex with its antibody [24,42]. It measures the size of a fluorescent tagged molecule such as an antigen. The utilization of the O-side chain of LPS from *Brucella* species has shown encouraging results. The test is a valuable alternative to conventional serological tests. Sensitivity of FPA is 96% for culture-confirmed human brucellosis and specificity is about 98% [24,42].

d. Immunocapture agglutination test; *Brucella* Capt (BCAP)

Recently, new immunocapture agglutination for anti-*Brucella* (BCAP) assay has been developed to detect agglutinating and non-agglutinating antibodies with high sensitivity [24]. It is based on the sandwich ELISA system, where a microwell is covered with Coombs antibodies against human origin IgG, IgA and IgM antibodies [22]. This *Brucella* agglutination test occurs in a microwell and is performed with Coombs antibodies and determines the 3 antibodies that form against brucellosis. It has been suggested as a possible substitute for Coombs test and a better marker for disease activity [22]. Compared to Coombs test, it has similar sensitivity and specificity but both can remain positive for long time after treatment in cured patients. BCAP determines blocking antibodies at diagnosis and during follow up for patients having brucellosis [22,43]. It is easier to carry out in 24 hours without a second step necessary as in Coombs test [43]. In comparison with other tests: it is more complex, expensive and slow. It can hardly replace rapid screening tests such as RBT and dipstick as a screening or first diagnostic test. However, it could help to diagnose disease in patients with longstanding evolution of brucellosis that is not detected by SAT. So, like Coombs test, *Brucella* Capt which is based on the immunocapture-agglutination of the total anti-*Brucella* antibodies, could be a second level serological test [22,24,37,40].

e. Lateral flow assay (LFA)

An immunochromatographic *Brucella* IgM / IgG lateral flow assay is a simplified version of the ELISA test and has a great potential as a rapid point-of-care assay. The test has high

by certain authoritative sources are contradictory to each other. All these controversies and differences make the choice of therapy for brucellosis a difficult task (Table 1) [57,58]. The aims of brucellosis treatment include: to shorten duration of symptoms, to prevent relapse and complications such as endocarditis, sacroiliitis and abortion [58].

Reference	Solera (MA*) 61	Skalsky (MA) 58	del Pozo (MA) 57	WHO** / FAO***[58,59]	Ioannina (IR) 59
Year of publication	1994	2008	2012	1986	2007
Studies/trials included	6	30	17	-	-
First Line Therapy	Streptomycin and Doxycycline	Doxycycline – 6 wks* + Rifampicin – 6 wks Gentamicin – 2 wks	Doxycycline – 6 wks +streptomycin – 2 wks OR Doxycycline – 6 wks +Gentamicin – 1 wk	Doxycycline – 6 wks and Rifampicin – 6 wks	Doxycycline – 6 wks and Streptomycin: 2-3 wks
Alternative Therapy	-	Doxycycline – 6 wks Gentamicin – 2 wks	Doxycycline + Rifampicin Or Ofloxacin + Rifampicin Or Doxycycline + cotrimoxazole	Doxycycline – 6 wks + Streptomycin: 2-3 wks	Doxycycline – 6 wks + Rifampicin – 6 wks
Second Line Therapy	Rifampicin and Doxycycline	Doxycycline – 6 wks + Streptomycin – 2 wks	-	-	Doxycycline – 6 wks Gentamicin – 1 wk
Optional Therapy	-	Cotrimoxazole + Doxycycline – 6 wks OR Cotrimoxazole + Rifampicin – 6 weeks	Monotherapy in patients with low risk of relapse	Cotrimoxazole	Cotrimoxazole – 6 wks or Ofloxacin – 6 wks Or Ciprofloxacin – 6 wks
Not Recommended	-	-Monotherapy -Treatment for less than 30 days -Quinolone-based treatment	Triple Therapy	-	Azithromycin Or Meropenem

* MA : metaanalysis ** WHO : World Health Organization * IR : international recommendation ** FAO : Food and Agriculture Organization * WKS : weeks

Table 1: Main Treatment Schedules for Human Brucellosis.

In 1986, WHO and FAO (food and agriculture organization) recommended the following treatment for uncomplicated case of brucellosis in adults: doxycycline and rifampicin for 6 weeks or tetracycline orally for 6 weeks in addition to parenteral streptomycin for 2 to 3 weeks (Table 1) [58,59]. In November 2006, a consensus meeting aimed to reaching a common specialist statement on the treatment of brucellosis was held in Ioannina in Greece under the auspices of the International Society of Chemotherapy and the Institute of Continuing Medical Education of Ioannina. The author panel suggested that optimal treatment of uncomplicated brucellosis should be based on a six-week regimen of doxycycline combined with streptomycin for 2 to 3 weeks or rifampicin for 6 weeks. Gentamicin might be considered as an acceptable alternative to streptomycin while all other regimens or combination therapies should be considered second-line (Table 1) [59].

Since the first effective treatments against brucellosis appeared, antimicrobial combinations have been preferred over monotherapies [57]. Therefore, optimal therapy of brucellosis should be a combination therapy [57,58]. The most commonly used drug combinations include: tetracycline (doxycycline) orally for 6 weeks in addition to either oral rifampicin for 6 weeks or a parenteral aminoglycosides (gentamicin for 1 week or streptomycin for 2-3 weeks) [57-61]. Monotherapy is not recommended, as it is associated with high relapse rates, except in cases at low risk of relapse. Triple therapy was recommended as first-line treatment in some studies, but it is no longer recommended except in complicated,

72]. Health education and screening programs for brucellosis in pregnant women living in endemic areas are of vital importance [73,74]. Also, there is no consensus on the treatment of childhood brucellosis [75]. Drug combinations incorporating cotrimoxazole in addition to rifampicin, gentamicin or tetracycline have been used successfully in the treatment of *Brucella* infections in children [75-78]. Treatment is given for up to 6 weeks although complicated cases may require therapy for up to 3 months [76,77].

Drug Resistance and Antibiotic Susceptibility

In order to decrease the development of drug resistance and the incidence of complications of brucellosis, it is essential to have antimicrobial therapy guided by antibiotic susceptibility testing. It is usually recommended to administer trimethoprim-sulfamethoxazole in combination with tetracycline or streptomycin as only 38% of *Brucella* strains are susceptible to co-trimoxazole [78].

Fluoroquinolones exhibit broad spectrum antibacterial activity. Their oral bioavailability, high tissue concentrations, evidence of intracellular penetration and in vitro activity against *Brucella* species make them attractive candidates for use against infections caused by intracellular bacteria such as Brucell species ciprofloxacin had good promise initially, but the following reasons excluded its use as a sole agent in first line therapy: lack of bactericidal activity against *Brucella* species, development of drug resistance during therapy and the high relapse rates encountered. The new generations of fluoroquinolones e.g. trovafloxacin, moxifloxacin, ofloxacin, grepafloxacin, gatifloxacin and sitafloxacin have shown excellent in vitro activities against *B. melitensis*. Therefore, use of these agents is warranted in the treatment of human brucellosis [79]. The absence of topoisomerase II-IV mutations in *B. melitensis* strains cannot rule out evolution of fluoroquinolone resistance due to interplay of several mechanisms [80]. Rifampicin is a potent broad-spectrum antibiotic and is an integral component of the combination therapy used in the treatment of human brucellosis [81]. The rpoB genetic mutations that confer rifampicin resistance were initially identified in the rough vaccine strain (RB51) of *B. abortus*. The absence of rpoB mutations in the clinical *B. melitensis* strains reinforces the first-choice status of rifampicin in the treatment of brucellosis and demonstrates the usefulness of molecular screening for resistant genotypes [81].

Control of Brucellosis

The main components of brucellosis control and eradication strategies are: (1) elimination of the pathogen at its animal source by hygiene measures such as careful herd management and health education, vaccination of animals in addition to testing and slaughtering of infected animals [82,83], (2) improving quality of the veterinary services and establishment of diagnostic laboratories that adopt international standards, (3) adoption of appropriate control and eradication programs [82] , (4) active and continuous surveillance and identification of animals and herds at high risk [82,84,85], (5) vaccination of young female animals is the most practical, economic and effective control program as mass vaccination has been adopted by many countries, (6) testing and slaughtering of seropositive adult animals [82,83] (7) health education and application of strict hygiene measures to personnel in direct contact with animals, (8) prompt diagnosis and appropriate treatment of infected humans to prevent chronic disease and further complications [82], (9) coordination and cooperation between public health officials, veterinary officers and various governmental sectors as expanded regional and even international cooperation is needed for optimal global control [82-84], and (10) animal control with restriction of animal transportation across open borders. Up to date, the most commonly used animal vaccines are: (1) *B. abortus* 519, (2) *B. melitensis* Rev.1 and (3) *B. abortus* RB51 [83,85].

Conclusions and Future Directions

The diagnosis of brucellosis can provisionally be made on clinical grounds but confirmation requires certain laboratory investigations. Isolation of the organism from blood cultures is the gold standard diagnostic test. The automated cultural techniques and the lysis concentration methods have improved the yield rates significantly. Several serological techniques are employed in the diagnosis of acute, chronic and relapsing brucellosis. Molecular tests have recently been utilized in the diagnosis and follow up of brucellosis patients.

Drug combinations are effective, while monotherapy may be associated with therapeutic failure and relapse. The most commonly used regimens are composed of doxycycline plus rifampicin and/or an aminoglycoside. Treatment duration depends on the: primary site of infection, duration of illness and presence or absence of complications. The new flouroquinolones, tigecycline, levamisole and IFN-γ can be incorporated into future therapeutic regimens. The main components of control programs are: animal vaccination, pasteurization of dairy products, health education of at risk populations and coordination between various governmental, regional and international organizations.

The recent advances in cultural, serological and molecular techniques have improved the diagnostic yield. Also, the recent genetic and immunological assays will have positive impacts on the diagnosis, treatment, vaccination and control of the disease. Although personalized therapy is advocated by certain authorities, global therapeutic language is urgently required. New diagnostic and therapeutic guidelines are vital to have optimal global control. For such guidelines to be acceptable and efficient, they should take social, financial and economic conditions of individual countries in addition to local experiences into consideration.

References

1. Al-Tawfiq JA, Abukhamsin A (2009) A 24-year study of the epidemiology of human brucellosis in a health-care system in Eastern Saudi Arabia. J Infect Public Health 2: 81-85.
2. Yagupsky P (1999) Detection of Brucellae in blood cultures. J Clin Microbiol 37: 3437-3442.
3. Gotuzzo E, Carrillo C, Guerra J, Llosa L (1986) An evaluation of diagnostic methods for brucellosis-the value of bone marrow culture. J Infect Dis 153: 122-125.
4. Iseri S, Bulut C, Yetkin MA, Kinikli S, Demiroz AP, et al. (2006) Comparison of the diagnostic value of blood and bone marrow cultures in brucellosis. Mikrobiyol Bul 40: 201-206.
5. Ruiz J, Lorente I, Perez J, Simarro E, Martinez-Campos L (1997) Diagnosis of brucellosis by using blood cultures. J Clin Microbiol 35: 2417-2418.
6. Kadanali A, Ozden K, Altoparlak U, Erturk A, Parlack M (2009) Bacteremic and nonbacteremic brucellosis: clinical and laboratory observations. Infection 37: 67-69.
7. Memish Z, Mah MW, Al Mahmoud S, Al Shaalan M, Khan MY (2000) Brucella bacteremia: clinical and laboratory observations in 160 patients. J Infect 40: 59-63.
8. Pappas G, Papadimitriou P (2007) Challenges in Brucella bacteremia. Int J Antimicrob Agents 30: 29-31.
9. Yagupsky P (2004) Use of the BACTEC MYCO/F LYTIC medium for detection of Brucella melitensis bacteremia. J Clin Microbiol 42: 2207-2208.
10. Aliskan H (2008) The value of culture and serological methods in the diagnosis of human brucellosis. Mikrobiyol Bul 42: 185-195.
11. Espinosa BJ, Chacaltana J, Mulder M, Franco MP, Blazes DL, et al. (2009) Comparison of culture techniques at different stages of brucellosis. Am J Trop Med Hyg 80: 625-627.
12. Melo-Cristino J, Salgado MJ (1999) Detection of Brucella bacteremia by using the VITAL automated system and Tryptose Broth culture. Clin Microbiol Infect 5: 706-709.

36. Park S-H, Lee Y-H, Chu H, Huang S-D, Huang K-J, et al. (2012) Application of the microagglutination test for serologic diagnosis of human brucellosis. Osong Public Res Perspect 3: 19-23.

37. Sargüzel FM, Kayman T, Celik I, Koc N (2011) Comparison of standard tube agglutination, Coombs' and BrucellaCapt tests in the diagnosis of brucellosis. New J Med 28: 113-115.

38. Meyer and Shaw (1984) Bergey's manual of systematic bacteriology. (1stedn) Krieg NR and Holt JG, The Williams & Wilkins Co., Baltimore, USA.

39. Lim ML, Richman LS (2004) Brucellosis. Infect Dis Clin Pract 12: 7-14.

40. Taleski V (2010) An overview of introducing various laboratory tests for diagnosis of human brucellosis in the republic of Macedonia. Maced J Med Sci 3: 239-245.

41. Clavijo E, Diaz R, Anguita A, Garcia A, Pinedo A, et al. (2003) Comparison of a Dipstick Assay for Detection of Brucella-Specific Immunoglobulin M Antibodies with Other Tests for Serodiagnosis of Human Brucellosis. Clin Diagn Lab Immunol 10: 612-615.

42. Nielsen K, Gall D (2001) Fluorescence polarization assay for the diagnosis of brucellosis: a review. J Immunoassay Immunochem 22: 183-201.

43. Casao MA, Navarro E, Solera J (2004) Evaluation of BrucellaCapt for the diagnosis of human brucellosis. J Infect 49: 102-108.

44. Sayan M, Erdenlig S, Stack S, Kilic S, Guducuoglu H, et al. (2011) A serological diagnostic survey for Brucella canis infection in Turkish patients with brucellosis-like symptoms. Jpn J Infect Dis 64: 516-519.

45. Lucero NE, Escobar GI, Ayala SM, Jacob N (2005). Diagnosis of human brucellosis caused by Brucella canis. J Med Microbiol 54: 457-461.

46. Yu WL, K. Nielsen K (2010) Review of detection of Brucella spp. by polymerase chain reaction. Croat Microbiol J 51: 306-313.

47. Navarro E, Casao MA, J. Solera J (2004) Diagnosis of human brucellosis using PCR. Expert Rev Mol Diagn 4: 115-123.

48. Nimri LF (2003) Diagnosis of recent and relapsed cases of human brucellosis by PCR assay. BMC Infect Dis 3:5.

49. Gemechu MY, Gill JPS, Arora AK, Ghatak S, Singh DK (2011) Polymerase chain reaction (PCR) assay for rapid diagnosis and its role in prevention of human brucellosis in Punjab, India. Int J Prev Med 2: 170-177.

50. Vrioni G, Gartzonika C, Kostoula A, Boboyianni C, Papadopoulou C, et al. (2004) Application of polymerase chain reaction enzyme immunoassay in peripheral whole blood and serum specimens for diagnosis of acute human brucellosis. Eur J Clin Microbiol Infect Dis 23: 194-199.

51. Debeaumont C, Falconnet PA, Maurin M (2005) Real-time PCR for detection of Brucella spp. DNA in human serum samples. Eur J Clin Microbiol Infect Dis 24: 842-845.

52. Al-Attas RA, Al-Khalifah M, Al-Qurashi AR, Badawy M, Al-Gualy N (2000) Evaluation of PCR, culture and serology for the diagnosis of acute human brucellosis. Ann Saudi Med 20: 224-228.

53. Almajid FM (2011) Lymphocyte activation test for diagnosis of sero-negative brucellosis in humans. Indian J Pathol Microbiol 54: 775-781.

54. de Jong MF, Tsolis RM (2012) Brucellosis and type IV secretion. Future Microbiol 7: 47-58.

55. Sun Y-H, Rolan HG, den Hartigh AB, Sondervan D, Tsolis RM (2005) Brucella abortus Vir B12 is expressed during infection but is not an essential component of the type IV secretion system. Infect Immun 73: 6048-6054.

56. Rolan HG, den Hartigh AB, Kahl-McDonagh M, Ficht T, Adams LG, et al. (2008) Vir B12 is a serological marker of Brucella infection in experimental and natural hosts. Clin Vaccine Immunol 15: 208-214.

57. del Pozo JSG, Solera J (2012) Systemic review and meta-analysis of randomized clinical trials in the treatment of human brucellosis. PLoS ONE 7: 32090.

58. Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, et al. (2008) Treatment of human brucellosis: systematic review and meta-analysis of randomized controlled trials. Br Med J 336: 701-704.

59. Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, et al. (2007) Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS ONE Med 4: e317.

60. Al-Tawfiq JA (2008) Therapeutic options for human brucellosis. Expert Rev Anti-infect Ther 6: 109-120.

84. Russo G, Pasquali P, Nenova R, Alexandrov T, Ralchev S, et al. (2009) Reemergence of human and animal brucellosis, Bulgaria. Emerg Infect Dis 15: 314-316.

85. Hotez PJ, Savioli L, Fenwick A (2012) Neglected tropical diseases of the Middle East and North Africa: review of their prevalence, distribution and opportunities for control. PLoS Negl Trop Dis 6: e 1475.

86. Eschenbrenner M, Wagner MA, Horn TA, Kraycer JA, Mujer CV, et al. (2002) Comparative proteome analysis of Brucella melitensis vaccine strain Rev 1 and a virulent strain 16M. J Bacteriol 184: 4962-4970.

87. al-Khalaf SA, Mohamad BT, Nicoletti P (1992) Control of brucellosis in Kuwait by vaccination of cattle, sheep and goats with Brucella abortus strain 19 or Brucella melitensis strain Rev.1. Trop Anim Health Prod 24: 45-49.

88. Trant CGMC, Lacerda TLS, Carvalho NB, Azevedo V, Rosinha GMS, et al. (2010) The Brucella abortus phosphoglycerate kinase mutant is highly attenuated and induces protection superior to that of vaccine strain 19 in immunocompromised and immunocompetent mice. Infect Immun 78: 2283-2291.

89. Grillo MJ, Manterola L, de Miguel MJ, Munoz PM, Blasco JM, et al. (2006) Increases of efficacy as vaccine against Brucella abortus infection in mice by simultaneous inoculation with avirulent smooth bvrS/bvrR and rough wbkA mutants. Vaccine 24: 2910-2916.

90. Ko J, Splitter GA (2003) Molecular host-pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. Clin Microbiol Rev 16: 65-78.

91. Oliveira SC, Giambartolomei GH, Cassataro J (2011) Confronting the barriers to develop novel vaccines against brucellosis. Expert Rev Vaccines 10: 1291-1305.

92. He Y, Xiang Z (2010) Bioinformatic analysis of Brucella vaccines and vaccine targets using VIOLIN. Immunome Res 6: S5: 1-12.

93. Siadat SD, Salmani AS, Aghasadeghi MR (2012) Brucellosis vaccines: an overview. Zoonosis, Dr Jacob Lorenzo-Moralesm, In Tech.

94. Ding JB, Mao KR, Cheng JS, Dai ZH, Jiang YW (2006) The application and research advances of Brucella vaccines. Wei Sheng Wu Xue Bao 46: 856-859.

95. Ficht TA, Kahl-McDonagh MM, Arenas-Gamboa AM, Rice-Ficht AC (2009) Brucellosis: the case of live, attenuated vaccines. Vaccine 27: D40-D43.

96. Clapp B, Skyberg JA, Yang X, Thornburg T, Walters N, et al. (2011) Protective live oral brucellosis vaccines stimulate Th1 and Th17 cell responses. Infect Immun 79: 4165-4174.

pathogens, *Candida* and *Cryptococcusis* species are the yeast pathogens, most frequently isolated fromimmunocompromised hosts in clinical practice. The *Candida* remains the most commom cause of invasiveyeast infections and *Cryptococcusisis* the second most common lethal fungal opportunist which causes symptomatic *Cryptococcosis*. *Candida albicans* is a normal flora of the human mucus, which causes vaginal candidiasis in more than 70% of healthy women atleast once in their life time. However, the yeast infection and clinical problemsassociated with immunocompromised patients are often causes severe illness or death. The pathogenic *C. neoformans* infects humans upon inhalation and causes the most common fungal meningoencephalitis in immunocompromised individualsworldwide. Acute neutrophilic meningitis is most frequently observed in *Candida* meningitis, whereas *Cryptococcusis neoformans* typically causes the chronic lymphocytic meningitis. Normally, when the fungus enters the body through the skin, mucosal membrane or by inhalation, defense cells such as the white blood cells, neutrophils and mononuclear phagocytes kill the fungi by phagocytosis. In immunocompromised patients, however, the risk of fungal infection is increased due to reduced number of phagocytes. Serious fungal infections affecting the immunocompromised people can be associated with impairment of T-lymphocytes and mononuclear phagocytes, which causes defective cell-mediated immunity. Candidiasis is most notable in patients with hematologic malignancies, hematopoietic stem cell transplant and organ transplant recipients. The patients hospitalized in critical care units supported with the use of invasive devices and broad-spectrum antibiotics have increased predispositions of *Candida* infection. Although, the incidence of *Cryptococcosis* is much lower in developed countries, it remains a leading opportunistc infection of patients with solid organ transplants, hematologic malignancies and AIDS. Human diseases resulting from the environmental exposure to the yeast blastospores of *Candida* or basidiospores of *Cryptococcusis* species are increased significantly ever since the increased onset of the HIV epidemic and other immunocompromised individuals.

Candida

Introduction

The yeast *Candida albicans* is the most prevalent opportunistic fungal pathogens of human. *Candida* can live as a harmless commensal of humans, and is carried in almost half of the population [4]. Colonization of *Candida* in distinct sites including skin, oral gastrointestinal tract and vaginal mucosal surfaces are extremely common in healthy individuals. However, in response to change in the host defense environment, itcan convert from a benign commensal to a disease-causing pathogen. In immunocompromised patients, it can cause infection of the mucosal epithelia followed by dissemination and colonization of internal organs and cause broad spectrum infections in the oral, gastrointestinal, genital tracts and systemic infections in other organs [5].The *Candida* infection is more prevalent in patients with impaired host defenses, often seen in those who have undergone anti-inflammatory and immunosupressive chemotherapies, organ transplants, cancer therapy, use of prosthetic devices, patients using broad-spectrum antibiotics and in AIDS patients [6-8]. The genus *Candida* contains more than 100 different species, but only a few of these species (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae*) have emerged as significant agents of disease in the last 20–30 years, causing 35–65% of candidemia (*Candida* species in the blood) [9]. Among the *Candida* species afflicting humans, *Candida albicans* is the most common. Most *Candida* species grow as yeast, as well as filamentous growth form. *C. albicans* is more morphologically diverse for its ability to switch between yeast and filamentous hyphal growth forms. Both of these morphological forms have been shown to be essential for the virulence [10]. The pathogenic species of *Candida* differ in their virulence, frequency and clinical manifestation. Recent studies have supported that *C. albicans*is the most (63-70%) predominant cause of invasive fungal infectionsfollowed by non-*albicans* species *C. glabrata* (44%), *C. tropicalis* (6%) and

member of the *Candida* species, and it expresses several virulence factors that contribute for the pathogenesis. The virulenceproperty of the *C. albicans* includes host recognition and adhesion to the host cells, morphogenesis, biofilm formation and secretion of hydrolytic enzymes [15]. The major virulence of the *C. albicans* is associated with its ability to switch between the yeast and hyphal morphology. During mucosal infections, the hyphal form plays an important role in causing disease by invading epithelial cells and causing tissue damage. *C. albicans* also regulates the expression of several genes and their products as virulence factors.

Adhesion: Pathogenesis of *Candida* species involves adhesion to the host epithelial and endothelial cells, and morphologic switching of yeast cells from the ellipsoid blastospore to various filamentous forms. Adherence of *Candida* cells to the host tissues is a complex multifactorial phenomenon. It utilizes several types of morphogenetically changing cell surfaces and proteins that bind to extracellular matrix proteins of mammalian cells, such as fibronectin, laminin, fibrinogen and collagen type I and IV. Adhesion of *Candida* species is mediated by some well characterized adhesion factors such as members of the agglutinin-like sequences (*ALS*) family of eight proteins (*ALS*1- *ALS*7 and *ALS*9), and several glycosylated proteins. Among eight *ALS* proteins, *ALS*1p have adhesion function to the human buccal epithelial cells, and also important for adherence of the pathogen to the oral mucosa during the early state of infection [22]. *ALS3* has shown to be important for hyphal associated adhesion during infection of oral epithelial cell *in vitro*, *in vivo* and also during vaginal infection [23]. Another important adhesion protein of *C. albicans* is Hwp1, which mediates adhesion to extracellular matrix proteins of human endothelial and epithelial cells [24]. Both *ALS3* and Hwp1 proteins have shown to express predominantly during hyphae formation [25]. Study on hwp1 knockout mutant of *C. albicans* has shown reduced adherences and mortality in murine models. Some of the *ALS* genes are upregulated in biofilm-associated cells both *in vivo* and *in vitro* conditions [24]. *Candida* related infections have been found to be associated with the biofilm formation. The biofilm-forming capacity of the *Candida* pathogen in host tissue, results in enhanced adherence. Invasive strains of *C. albicans* recovered from patients show greater adherence than non- invasive strains recovered from asymptomatic carriers. Poorly adherent strains of *C. albicans* are less virulent in animal models [25,26]. However, the fungal components which mediate adhesion is still controversial, but some findings indicate that cell wall components includes, mannan, mannanproteins or polysaccharides are responsible for the adhesion [27]. Factors that enhance *C. albicans* adherence include fungal cell surface hydrophobicity, morphology, pH, temperature, pregnancy, diabetes, oral contraceptive usage and nature of host immunity.

Morphogenesis: Morphogenesis (transition between yeast cell and filamentous growth) is one of the most important and best-studied virulence factors of *C. albicans*. The pathogen grows vegetatively at least in three morphogenic forms, which include, yeast, pseudohyphae and hyphae. The yeast form of *C. albicans* resembles the typical budding yeast *S. cerevisiae* [28]. Pseudohyphae has a morphological appearance that falls between yeast-form and hyphae-form, which contains a chain of ellipsoidal yeast cells that have a constriction at the junctions between adjacent cells. Whereas, hyphae are cylinder like structures with the side walls parallel along their entire length. The other morphologies include chlamydospores, which are thick-walled spore-like structures, appear as whiteopaque cells formed during switching. However, chlamydospores have not been observed in patient samples. The morphological diversity of *C. albicans* is thought to aid its survival, growth and dissemination in the mammalian host. Both yeast and filamentous form of *Candida* have been identified in patients, and there is a strong evidence on transition between yeast-to-filamentous growth is essential for virulence.Yeast forms are more suited for dissemination in tissues, whereas hyphae forms are required for tissue damage and invasion [29]. Several genes have been identified which regulate yeast-to-filamentous transition, that includes *PHR1, ECE1, HYR1, RBF1, CHS2* and *CHS3*. These genes are differentially expressed during morphogenesis of *C. albicans* [30]. Among these genes, gene disruption of *RBF1* demonstrated the alteration in

(*SAP1*, 2) and extensive penetration (*SAP8*) [42]. In addition, several studies have shown that production of *SAP*s is correlated with other virulence determinants such as phenotypic switching, hyphae formation and adherence to enhance the pathogenicity of *C. albicans*.

Phospholipases are the enzymes that hydrolyze ester linkages of glycophospholipids during tissue invasion. In *C. albicans*, phospholipases are classified as phospholipase A, B, C, and D. Of the four phospholipases, only phospholipase B has been shown to be required for virulence [43]. Phospholipase B deleted strain produced less enzyme activity *in vitro*, and also less virulent compared to wild-type cells in animal model of candidiasis [44]. Phospholipase B was detected and isolated from hyphal tips during tissue invasion and it demonstrated that increased level of phospholipase production in blood isolates compared to commensal isolates.These evidencessupport *C. albicans* phospolipase is a virulence factor.

Pathogenesis and host response

C. albicans yeast is the most common fungal commensal in healthy individuals, and also major opportunistic fungal pathogen causing high mortality risk in immunocompromised patients. *Candida* species colonize asymptomatically as a normal flora in around 25-50% of healthy individuals in a population at any given time. In healthy individuals, this fungus is controlled by the normal microbial flora, epithelial barriers and the innate immune system. However, when the host defense is weakened, *C. albicans* can cause infections that ranges from superficial mucosal infections to life-threatening systemic infections. The severity of *Candida* infection often depends upon the host's immune system, and as well as the difference in the virulence factors of *Candida* strains. When *C. albicans* commonly live in mucosal and skin surfaces, they grow benignly and are tolerated by the host immune system. Invasion of *C. albicans* into host system occur via two different routes such as induced endocytosis or active penetration. Induced endocytosis is a host driven activity in which pathogen adhere and bind to a host receptor on epithelial or endothelial cells to trigger fungal up-take [27]. Active penetration requires fungal turgor, normal vacuole formation, cell wall integrity and hyphal extension. Induction of host damage by the pathogen is the key characteristic of pathogenesis. Penetration of hyphae deep into host cells or between host cell is potentiated by virulence factors such as the secretion of hydrolases and hyphal extension. During invasive hyphal formation, the immune system induces a specific immune response that is mediated by macrophages. Both mucosal immunity and cell mediated immunity together inhibit the germination and proliferation of *Candida* on mucosal surface. In addition, oral epithelial cells have direct anti-*Candida* activity. Cell surface appears to provide an additional line of defense to local *Candida* invasion of mucosal surfaces [45]. Abrogation of these mucosal surface due to the use of chemotherapy or immunosuppression therapy may lead to proliferation, local invasion and blood borne dissemination. Intact cell mediated immunity is critical in contributing mucosal host defense against *Candida*. In HIV positive patients, due to impaired cell mediated immunity, oropharyngeal candidiasis is common. Vaginal candidiasis also may be recurrent debilitating infection in HIV-positive women [46]. Disruption of an intact epithelium in patients who are receiving cytotoxic chemotherapy for cancer is an important component of locally invasive candidiasis.In addition, during *Candida* infection, a large number of pro-inflamatory and immunoregulatory cytokines are genarated by epithelial cells. A number of Th1 and Th2 cytokines and hemopoietic growth factor modulate the effect of innate immune cells in response to *Candida* species. These cytokines may stimulate phagocytosis and intracellular killing of infiltrating neutophils as well as functions of $CD4^+$ and $CD8^+$ T-cells [47]. Phagocytes and macrophages play a crucial role in host defense against deeply invasive *Candida* infections. Phagocytes and pheripheral blood monocytes can recognise *Candida* blastoconidia, pseudohyphae and hyphae, and damage through oxidative and non-oxidative mechanisms. Whereas, macrophages clears circulating blastoconidia in liver and spleen. Thus, neutropenia condition in chronic granulomatous disease is the main risk factor for disseminated candidiasis.

infection varies from 7% to 93% [56]. Progressive systemic and local immunity damage can result in overload of *Candida* in oral cavity in HIV/AIDS patients. Both enhanced *Candida* virulence and impaired host immunity may leads to the conversion from asymptomatic carriage to oral candidiasis in AIDS patients. Oropharyngeal candidiasis may appear in a variety of clinical forms. The symptoms of oral thrush are burning pain, altered sense of taste, and difficulty in swallowing. Oral candidiasis lesions are subdivided into primary oral candidiasis and secondary candidiasis. In primary oral candidiasis, lesions are confined to the oral cavity with no involvement of skin or other mucosae, whereas in secondary candidiasis, lesions are present in the oral as well as extra oral area including skin. Primary oral candidiasis consists of pseudomembranous, erythematous, and hyperplastic variants [57]. Esophageal candidiasis occurs less frequently (10-20%) but it is the leading cause of esophageal disease. Esophageal candidiasis is more similar to oral thrush, and it is diagnosed when typical oral candidiasis symptoms occur but in some casesit has also been reported to occur without thrush. Esophageal candidiasis is most commonly seen in patients who have undergone cancer treatmentor immunosuppresion therapy and in AIDS patients. The most common symptoms include painful swallowing and substernal chest pain. Nausea and vomiting may also occur. Esophageal candidiasis can be diagnosed by biopsy during endoscopy [58]. The prevalence of vaginal candidiasis (27-60%) ismost common in HIV-associated clinical conditions in women, which tend to persist, and are associated with a significantly reduced CD4 count and higher HIV viral load. Vaginalcandidiasisis the first sign in a woman infected with HIV. Symptoms includes severe itching and burning of the vagina with slow leakage of a thick, white and cheese-like substance. The disseminated candidiasis is remarkably uncommon in AIDS [59]. This is likely due to relatively tolerable neutrophil function in most HIV-infected individuals. However, *Candida*emia has been reported in AIDS, it usually associated with other risk factors such as neutropenia, broad spectrum antibiotics, parenteral nutrition, abdominal surgery, cancer and corticosteroid use. Systemic invasive candidiasis is usually associated with a persistent fever and no diagnostic clinical features. However, any nodular skin eruption in neutropenic patient should be biopsied as it may represent cutaneous emboli which are occasionally seen in patients with disseminated candidiasis [60]. Diagnosis of disseminated candidiasis is made by histopathology of the organism invading tissues. In addition, the organism is frequently cultured from sputum, urine and feces. The clinical features of patients with infection caused by *C. albicans* and non-*albicans Candida* species are indistinguishable. Therefore, identification of *Candida* species in infected patients is important to understand their ability to cause infection and also in their susceptibility to antifungal agents.

Diagnosis of oral candidiasis is often made based on the nature of clinical features. Microbiological specimenshould be taken for the identification, quantification and the antifungal testing of *Candida*. Isolation methods of *Candida* from the oral cavity includes the use of a smear, a plain swab, collection of whole saliva and mucosal biopsy [61,62]. Vulvovaginal candidiasis can be diagnosed by direct microscopic examination of vaginal secretions and culture of vaginal swabs [63,64]. Quantitative estimation of fungal load can be made by culturing of oral rinse to differentiate between commensal carriage and pathogenic existence of higher loads in oral candidiasis.

Direct microscopy can be used to identify the *Candida* species based on the morphological features. Specimen prepared with Potassium hydroxide (KOH) reveals non-pigmented septate hyphae. In KOH-calcoflour fluorescent stain method, *Candida* yeast cells and hyphae shows fluoresce [65]. A smear from the lesions site can stain with gram stain or by the periodic acid Schiff methods, *Candida*l hyphae and yeasts appear dark blue and red/purple respectively. In chronic hyperplastic candidiasis, the presence of blastospores and hyphae or pseudohyphae are detected by using either the periodic acid Schiff or gomori's methenamine silver stains. Swabs of a lesional site or oral rinse techniques are relatively simple method to detect fungal growth and semiquantitative estimation. The collected samplesare subsequently inoculated on Sabouraud's dextrose agar media. After 24-48 h of incubation at 37°C, growth

acquired through inhalation of fungal spores from environmental sources. The two common human and animal pathogenic *Cryptococcusis* species are *C. neoformans* and *C. gattii*. The *C. neoformans* is more prevalent in causing meningitis in immunocompromised hosts and is distributed worldwide. Whereas, *C. gattii* infects both immunocompromised and immunocompetent hosts, and geographically restricted to tropical and subtropical regions, mostly found in eucalypts and other trees [85]. The major environmental sources of *C. neoformans* shown to be either soil contaminated with pigeon droppings (*C. neoformans* var. *Neoformans* and *C. neoformans* var. *grubii*) or eucalyptus trees and decaying wood forming hollows in living trees (*C. neoformans* var. *gattii*). *C. gattii* was reported earlier only in tropical and subtropical climates, but recently it has emerged as an outbreak in pacific northwest [86,87]. *C. gattii* was initially reported to be infected both in human and animals as an outbreak in 1999 in British Columbia, Canada. The environmental and clinical isolates of *C. neoformans* overlap in genotypic and phenotypic patterns. The two species *C. neoformans* and *C. gattii* have been seperated based on the geographical distribution, ecological niches, epidemiology, pathobiology, clinical presentation and molecular characterization.

After the AIDS epidemic in the 1980s, *Cryptococcosis*emerged as an important opportunistic pathogen infection in AIDS patients. Therefore, cryptococcal meningitis is considered as one of the AIDS defining conditions. The incidence of *Cryptococcosis* is approximately 5%-10% of all AIDS patients in the USA, Europe and Australia [74]. *Cryptococcusis* infects central nervous system, and causes one of the most frequent neurological complication in AIDS patients. Neuropathological conditions are present approximately in 70% to 90% of AIDS patients.The incidence of *Cryptococcosis* is prevelant among patients with AIDS in Africa and Southeast Asia than in the United States, whereas it appears less frequently in Europe. According to the United Nations Program on HIV/AIDS and the world Health Organization (http://www.unaids.org/), 2.7 million people are newly infected with HIV/AIDS disease worldwide,out of which, 1.8 million patients die from AIDS related causes.Centers for Disease Control and Prevention (CDC, Atlanta, USA, http://www.cdc.gov/) estimated that *C. neoformans* and *C. gattii* infections cause one million cases of cryptococcal meningitis per year among HIV/AIDS patients, resulting in nearly 625,000 deaths. Globally, the rates of *Cryptococcusis* meningitis incidence among people with HIV/AIDS are estimated to have risen to 12% per year [75]. The major burden of *Cryptococcosis* occurs in sub-Saharan Africa, where mortality is estimated to be 50% to 70%. The incidence of *Cryptococcosis* in Africa and Southeast Asia is relatively more common as an AIDS-related infection than in Europe or North America [88]. In Uganda, the incidence of cryptococcal disease was estimated to 10.3 cases per 100 persons, of those 17% of deaths among HIV-1-infected adults [89]. In South Africa, *Cryptococcusis* meningitis was found to cause 13% to 44% of all deaths among HIV-seropositive cohorts [90]. In Malawi, cryptococcal meningitis is the most common disease with 40% of cases from HIV-infected people. Where as in Thailand, *Cryptococcosis* accounted for 19% of AIDS-defining illnesses [91]. The high incidence of *Cryptococcosis* in parts of Africa and Asia may be due to high exposure of the pathogen. The high mortality is due to lack of early diagnosis, access to the treatment and ineffective distribution of high active antiretroviral therapy (HART) in the areas of high HIV prevalence. However, the incidence of *Cryptococcosis* is decreasing among AIDS patients in USA and other developed countries due to the availability of HART and the mortality is 12%. The HART regimens are capable of suppressing viremia thereby enhancing the immune system of the patients. Consequently, HART treatment caused a significant reduction in morbidity and mortality associated with incidence of cryptococcal meningitis in HIV patients in countries where early access to early diagnosis during initial stages of the infection [92].

Microbiology and life cycle

C. neoformans is an unicellular encapsulated budding yeast. The cells are round-to-oval shape, measuring 4-6 μm, surrounded by a polysaccharide capsule ranges from 1 to >30 μm when cultivated in the laboratory condition. Capsule production is demonstrated

[106]. The geographical distribution of all these four serotypes are unequal. *C. neoformans* isfound worldwide and responsible for the cryptococcal infections in immunocompromised individuals, and it has a predilection to infect the central nervous system (CNS) and to cause cryptococcal meningitis. Serotype A cause majority of the infection which is responsible for 95% of *Cryptococcosis*, whereas serotype D includes only 30-70% of infection [108]. Based on the mating type, *C. neoformans* occurs in two diomorphic forms, corresponding to the two opposite mating types a and α. Mating assays can be done by co-cultivting a and α mating type on V8 media and scoring filamentation and basidiospore formation after incubation [109]. Molecular analysis of mating type can be analysed by PCR amplifying MAT locus specific products. *C. neoformans* var. *grubii* and var. *neoformans* are distributed worldwide in different geographical regions showing differences in the strain occurrence. A study in 1996 of 356 clinical isolates obtained from different parts of the world showed that 96% of the isolates were serotype A (78% VNI and 18% VNII), 3% serotype D (VNIV) and 1% serotype AD (VNIII). However, serotype D is much more common in Europe and India [110].

Virulence and pathogenicity

The pathogenesis of *Cryptococcosis* is multifactorial and involves the combined action of many virulence factors. The virulence factors of *C. neoformans* is required not only for mammalian pathogenesis but also for environmental survival under selection pressure. The primary infection of the pathogen is usually asymptomatic and may be eradicated or dormant in the lungs granulomata. However, depending on the host defense, it may replicate in the human host and dessiminate extrapulmonary sites including brain. Recently, virulence factors of *Cryptococcusis* has gained considerable attentions, and molecular analysis have been used to determine virulence factors. *C. neoformans* and *C. gattii* appeared to share many of the same virulence factors to increase their ability to invade and survive in a host. The major virulence factors are the prominent large capsule, ability to grow at 37°C and laccase and melanin to combat oxidative damageby the hosts [111].

The capsule: The *Cryptococcusis* capsule is made up of polysaccharides, proteins and pigments. Interestingly, *Cryptococcusis* is the only eukaryotic pathogenic fungus that produces a polysaccharide which serves as the major virulence factor. The polysaccharide capsule is a remarkable structure that gives distinct appearance to a *C. neoformans* cell. Morphologically, the polysaccharide capsule can be visualized under microscope with the help of India ink or with immunofluorescence. The capsule is composed of the glucuronoxylomannan (90–95%) polysaccharides and glucuronoxylomannogalactan (5–10%) together with mannoproteins (1%) [112]. Mannoprotein is an immunodominant peptide which is recognised by antigen-specific T cell, that is involved in the cell mediated immunity [113] and cytokine production [114]. However, glucuronoxylomannan is recognized by the Toll-like receptor 2 and 4 on the innate immune cells, such as macrophages and dendritic cell. Both of these capsular components are critical factors in cryptococcal immunity for the activation of macrophages and fungal clearance. The capsule is typically 4–6 mm in thick but can grow as large as 30 mm. Capsular mutants are much more readily phagocytosized than their parental strains [115]. The capsular polysaccharide components are shed during replication, and which can be recovered from the culture supernatant and also from serum of the infected patients [116,117].

Growth at 37°C: Temperature is an important environmental signal for *C. neoformans* growth. The ability of *Cryptococcusis* to grow at 37°C is also considered as a virulence factor. The invitro growth temperature of 39°C to 40°C significantly reduces the growth rate of *C. neofomrans* and *C. gattii* and most strains are killed within 24h. The temperature sensitive *ROM2* gene mutants of *C. neoformans* have impaired growth at 37°C and were correlated with low virulence [118]. Mouse model studies have demonstrated that thecalcineurin A1mutants did not grow at 37°C and iron regulating *EIR1* mutants are sensitive at 37°C and

damage and provide nutrient to the pathogen toestablish in the host. The degradation of the host components can aid pathogen to escape from host phagosomal compartments and protect from the host immune response [138]. *C. neoformans* produces and release D-mannitol both *in vitro* and *in vivo* systems and it protects the pathogen against oxidative killing by scavenging distal reactive oxygen intermediates. A UV induced mannitol mutant that produces very less (10%) mannitol compared to wild type and showed less virulence in the mice model [139,140].

Pathogenicity: *Cryptococcusis* infection is acquired through inhalation of small yeast cells or basidiospores. The primary pulmonary infection is normally asymptomatic and may be eradicated or enter into dormant stage within the granuomata. However, depending on the pathogen inoculum, isolate virulence and host factors, the organism may disseminate to extrapulmonary sites, with a particular predilection for the brain. The primary initial defense against cryptococcal invasions is the macrophages and complement-mediated phagocytosis [141]. The other important host factors for defense against *Cryptococcusis* infection include CD4+ and CD8+ T cells, as well as cytokines such as tumor necrosis factor-a, interferon-c and interleukin-18 [142,143]. In immunocompormised patients, however, the absence of an intact cell-mediated response results in ineffective ingestion of the organism leading to dissemination and increased cryptococcal burden [144-146]. The capsular glucuronoxylomannan has antiphagocytic property. However, exopolysaccharides of the capsule is thought to act as a virulence factor by suppressing the immune response and inhibiting leukocyte migration. *C. neoformans* possess membrane-bound phenoloxidase enzymes which are able to convert phenolic compound into melanin. The ability to synthesize melanin from catecholamines that are present in the brain tissue in large concentrations make the organism to invade the CNS [147].

Cryptococcusis infection in immunocompromised host

The increase in *Cryptococcusis* infection is due to the use of antineoplastic and immunosuppressive agents, organ transplants, broad-spectrum antibiotics, more aggressive surgery, cancer, prosthetic device etc., which results in a larger population with significant immune system defects. *Cryptococcusis* enter the host through inhalation and can cause asymptomatic colonization to severe pneumonia. In immunocompromised persons, however, particularly HIV-infected persons with CD4<100, the infection can reactivate and spread throughout the body. The organism may cause pulmonary infection in paticularly AIDS patients, and impaired T cell mediated immunity patients [148]. In additon, it can also infect various organs of the body including skin, eye and mouth, but eventually it reach the lungs and central nervous systems (brain) [149]. The most HIV-related cases of *Cryptococcosis* (>90%) are caused by *Cryptococcusis* var. *grubii* (serotype A), while *Cryptococcusis* var. *neoformans* (serotype D) is responsible for few cases especially in Europe, and a small percentage of *C. gattii* infection (serotypes B and C). Although *C. neoformans* and *C. gattii* have been divided into separate species, most clinical laboratories will not identify *Cryptococcusis* to the species level. Meningitis is the most frequent manifestation of *Cryptococcosis* of the central nervous system. The blood-borne *C. neoformans* cells, which exist freely in the blood or within the phagocytic cells make contact with brain endothelial cells and then transmigrate into the brain parenchyma to cause meningoencephalitis [150]. Infection of the subarachnoid space is accompanied by involvement of the brain parenchyma, and causes meningoencephalitis in 40%-86% of patients [151]. *C. neoformans* infection has been reported in a diabetic renal transplant recipient with cellulitis, organ transplants and cancer patients especially those with chronic leukemia and lymphoma are at high risk of having cryptococcal meningitis. *Cryptococcosis* was also reported in 0.3% to 2% of liver transplant recipients, 2.9% of kidney transplant recipients and persons amongst thosereceiving corticosteroid therapy, particularly people with diabetes mellitus.

sequences, among which *ITS, URA5, CAP59* and *M13* [167]. Study on molecular diagnosis of *Cryptococcusis* demonstrated that PCR showed highest sensitivity (92.9%) compatred to culture (85.5%) and india ink test (76.8%).

The incidence of cryptococcal infection was drahmatically increased due to AIDS epidemic in the year 1980s until early 1990. Development of more effective antiretroviral therapy and prophylactic treatment regiments, the *Cryptococcosis* has been declined in Europe and USA since mid-1990 [168]. The cryptococcal infection, however, is still documented as one of the major common life-threatening opportunistic fungal infections in immunocompromised patients, in particular HIV infected patients. Use of antiretroviral treatment has dramatically improved the long-term prognosis of patients with HIV-associated cryptococcal disease. However, the incidence and acute mortality of HIV-associated cryptococcal meningitis remain high. The cryptococcal infection in the central nervous system is more prevalent in AIDS patients and was reported to be 46%. When cryptococcal symptoms occurs, patients should seek medical care immediately. The treatment of *Cryptococcusis* infection depends on the patient's overall conditions and the extent of infection. The goal of treatment is to eliminate the fungi, some patients may require surgery to remove a fungal mass or some patients may require appropriate medication. Immunocompromised patients are treated with amphotericin B alone or combined with flucytosine. Antifungal treatment is used for brain and severe lung infections. The treatment usually extended until the spinal fluid test negative in patients with brain infection, and lung lesions should show decrease in size in response to antifungal therapy.

Conclusion

In the past few decades, advancement of medical procedures in cancer therapy, transplantology and antibiotic treatment has led to increasing frequency of invasive fungal infection and mortality rate.Infection with *Candida* and *Cryptococcusis* yeasts depends on the host susceptibility and host-pathogen interactions. In addition to host immune system, virulence property of the pathogen also influence on the disease outcome. Better understanding of clinical manifestation and diagnosis of *Candida* and *Cryptococcusis* yeasts in immunocompromised host will lead to the discovery of effective prevention and treatment strategies.

References

1. Michael A Pfaller, Peter G Pappas, John R Wingard (2006) Invasive fungal pathogens: Current epidemiological trends. Clinical Infectious Diseases 43: 3-14.
2. Jane Kriengkauykiat, James I Ito, and Sanjeet S Dadwal (2011) Epidemiology and treatment approaches in management of invasive fungal infections. Clinical Epidemiology 3: 175-191.
3. Monica A Slavin, Liz McNeil Grist (2012) Invasive Fungal Infections: A Continuing Challenge Report from the 17th International Symposium on Infections in the Immunocompromised Host, Genoa, Italy, 24-27 July 2012. Current Fungal Infection Reports 7: 83-88.
4. Senna PM, da Silva WJ, Cury AA (2012) Denture disinfection by microwave energy: Influence of *Candida* albicans biofilm. Gerodontology 29: 186-191.
5. Koh AY, Köhler JR, Coggshall KT, Van Rooijen N, Pier GB (2008) Mucosal damage and neutropenia are required for *Candida* albicans dissemination. PLoS Pathog 4: 35.
6. Nguyen MH Yu VL (1995) Meningitis caused by *Candida* species: An emerging problem in neurosurgical patients. Clinical Infectious Diseases 21: 323-327.
7. Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen RA, et al. (2003) A prospective observational study of candidemia: Epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis 37: 634-643.
8. Kourkoumpetis T, Manolakaki D, Velmahos G, Chang Y, Alam HB, et al. (2010) *Candida* infection and colonization among non-trauma emergency surgery patients. Virulence 1: 359-366

33. Noffz CS, Liedschulte V, Lengeler K Ernst JF (2008) Functional mapping of the *Candida* albicans Efg1 regulator. Eukaryot Cell 7: 881-893.

34. Emira N, Mejdi S, Dorra K, Amina B, Eulogio V (2011) Comparison of the Adhesion ability of *Candida* albicans strains to Biotic and Abiotic surfaces. African Journal of Biotechnology 10: 977-985.

35. Douglas LJ (2003) *Candida* biofilms and their role in infection. Trends Microbiol 11: 30-36.

36. Richard ML, Nobile CJ, Bruno VM, Mitchell AP (2005) *Candida* albicans biofilm-defective mutants. Eukaryot Cell 4: 1493-1502.

37. Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM, et al. (2012) A recently evolved transcriptional network controls biofilm development in *Candida* albicans. Cell 148: 126-138.

38. Fox EP, Nobile CJ (2012) A sticky situation: Untangling the transcriptional network controlling biofilm development in *Candida* albicans. Transcription 3 [Epub ahead of print].

39. Al-Fattani MA, Douglas LJ (2006) Biofilm matrix of *Candida* albicans and *Candida* tropicalis: Chemical composition and role in drug resistance. J Med Microbiol 55: 999-1008.

40. Mayer FL, Wilson D, Hube B (2013) *Candida* albicans pathogenicity mechanisms. Virulence 4: 119-128.

41. Naglik JR, Challacombe SJ, Hube B (2003) *Candida* albicans secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev 67: 400-428.

42. Jackson BE, Wilhelmus KR, Hube B (2007) The role of secreted aspartyl proteinases in *Candida* albicans keratitis. Invest Ophthalmol Vis Sci 48: 3559-3565.

43. Ghannoum MA (2000) Potential role of phospholipases in virulence and fungal pathogenesis. Clin Microbiol Rev 13: 122-143.

44. Leidich SD, Ibrahim AS, Fu Y, Koul A, Jessup C, et al. (1998) Cloning and disruption of caPLB1, a phospholipase B gene involved in the pathogenicity of *Candida* albicans. J Biol Chem 273: 26078-26086.

45. Richardson M, Rautemaa R (2009) How the host fights against *Candida* infections. Frontiers in bioscience (Scholar edition) 1: 246-257.

46. Vazquez JA (2010) Optimal management of oropharyngeal and esophageal candidiasis in patients living with HIV infection. HIV/AIDS - Research and Palliative Care 2: 89-101.

47. Dongari-Bagtzoglou A, Fidel Jr PL (2005) The host cytokine responses and protective immunity in oropharyngeal candidiasis. J Dent Res 84: 966-977.

48. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, et al. (2006) The epidemiology of fungal infections in patients with hematologic malignancies: The SEIFEM-2004 study. Haematologica 91: 1068-1075.

49. Duncan MD, Wilkes DS (2005) Transplant-related immunosuppression: A review of immunosuppression and pulmonary infections. Proc Am Thorac Soc 2: 449-455.

50. Ascioglu S, Rex JH, De Pauw B, Bennett JE, Bille J, et al. (2002) Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. Clin Infect Dis 34: 7-14.

51. Baradkar VP, Kumar S (2009) Species identification of *Candida* isolates obtained from oral lesions of HIV infected patients. Indian J Dermatol 54: 385-386.

52. Abu-Elteen KH, Hamad MA, Salah SA (2006) Prevalence of oral *Candida* infections in diabetic patients. Bahrain Medical Bulletin 28: 12-17.

53. Goswami D, Goswami R, Banerjee U, Dadhwal V, Miglani S, et al. (2006) Pattern of *Candida* species isolated from patients with diabetes mellitus and vulvovaginal candidiasis and their response to single dose oral fluconazole therapy. J Infect 52: 111-117.

54. Daniluk T, Tokajuk G, Stokowska W, Fiedoruk K, Sciepuk M, et al. (2006) Occurrence rate of oral *Candida* albicans in denture wearer patients. Adv Med Sci 51: 77-80.

55. Badiee P, Alborzi A, Davarpanah MA, Shakiba E (2010) Distributions and antifungal susceptibility of *Candida* species from mucosal sites in HIV positive patients. Arch Iran Med 13: 282-287.

56. Bodhade AS, Ganvir SM, Hazarey VK (2011) Oral manifestations of HIV infection and their correlation with CD4 count. J Oral Sci 53: 203-211.

78. Ngamskulrungroj P, Chang Y, Sionov E, Kwon-Chunga KJ (2012) The primary target organ of *Cryptococcusis* gattii is different from that of *Cryptococcusis* neoformans in a murine model. MBio 3: e00103-e00112.

79. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, Freedberg KA (2003) Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. Clin Infect Dis 36: 652-662.

80. Espié E, Pinoges L, Balkan S, Chanchhaya N, Molfino L, et al. (2010) Cryptococcal meningitis in HIV-infected patients: A longitudinal study in Cambodia. Trop Med Int Health 15: 1375-1381.

81. Zhu LP, Shi YZ, Weng XH, Müller FMC (2002) Case reports. Pulmonary cryptococcosis associated with cryptococcal meningitis in non-AIDS patients. Mycoses 45: 111-117.

82. Asselman V, Thienemann F, Pepper DJ, Boulle A, Wilkinson RJ, et al. (2010) Central nervous system disorders after starting antiretroviral therapy in South Africa. AIDS 24: 2871-2876.

83. Singh R, Kaur M, Arora D (2011) Neurological complications in late-stage hospitalized patients with HIV disease. Ann of Indian Acad Neurol 14: 172-177.

84. Littman ML, Borok R (1968) Relation of the pigeon to cryptococcosis: Natural carrier state, heat resistance and survival of *Cryptococcusis* neoformans. Mycopathol Mycol Appl 35: 329-345.

85. Byrnes Iii EJ, Marr KA (2011) The outbreak of *Cryptococcusis* gattii in western North America: Epidemiology and clinical issues. Curr Infect Dis Rep 13: 256-261.

86. Chowdhary A, Rhandhawa HS, Prakash A, Meis JF (2012) Environmental prevalence of *Cryptococcusis* neoformans and *Cryptococcusis* gattii in India: An update. Crit Rev Microbiol 38: 1-16.

87. Ellis DH, Pfeiffer TJ (1990) Natural habitat of *Cryptococcusis* neoformans var. gattii. J Clin Microbiol 28: 1642-1644.

88. Zhou Y, Li G (2010) Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Chinese Journal of Infection and Chemotherapy 10: 161-166.

89. French N, Gray K, Watera C, Nakiyingi J, Lugada E, et al. (2002) Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS 16: 1031-1038.

90. Corbett EL, Churchyard GJ, Charalambos S, Samb B, Moloi V, et al. (2002) Morbidity and mortality in South African gold miners: Impact of untreated disease due to human immunodeficiency virus. Clin Infect Dis 34: 1251-1258.

91. Chariyalertsak S, Sirisanthana T, Saengwonloey O, Nelson KE (2001) Clinical presentation and risk behaviors of patients with acquired immunodeficiency syndrome in Thailand, 1994-1998: Regional variation and temporal trends. Clin Infect Dis 32: 955-962.

92. Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, et al. (2012) Antiretroviral treatment of adult HIV infection: 2012 Recommendations of the International Antiviral Society-USA panel. JAMA 308: 387-402.

93. Wan R, Wang M, Zhao S, Li G, Zhou Y, et al. (2011) A new cost-effective staining method for rapid identification of *Cryptococcusis*. African Journal of Microbiology Research 5: 1420-1427.

94. Fries BC, Taborda CP, Serfass E, Casadevall A (2001) Phenotypic switching of *Cryptococcusis* neoformans occurs in vivo and influences the outcome of infection. J Clin Invest 108: 1639-1648.

95. Velagapudi R, Hsueh YP, Geunes-Boyer S, Wright JR, Heitman J (2009) Spores as infectious propagules of *Cryptococcusis* neoformans. Infect Immun 77: 4345-4355.

96. Gross NT, Nessa K, Camner P, Chinchilla M, Jarstrand C (1997) Interaction between *Cryptococcusis* neoformans and alveolar macrophages. J Med Vet Mycol 35: 263-269.

97. Nessa K, Gross NT, Jarstrand C, Johansson A, Camner P, et al. (1997) In vivo interaction between alveolar macrophages and *Cryptococcusis* neoformans. Mycopathologia 139: 1-7.

98. Tucker SC, Casadevall A (2002) Replication of *Cryptococcusis* neoformans in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc Natl Acad Sci USA 99: 3165-3170.

99. Bemis DA, Krahwinkel DJ, Bowman LA, Mondon P, Kwon-Chung KJ (2000) Temperature-sensitive strain of *Cryptococcusis* neoformans producing hyphal elements in a feline nasal granuloma. J Clin Microbiol 38: 926-928.

100. Lin X, Hull CM, Heitman J (2005) Sexual reproduction between partners of the same mating type in *Cryptococcusis* neoformans. Nature 434: 1017-1021.

121. Zhu X, Gibbons J, Garcia-Rivera J, Casadevall A, Williamson PR (2001) Laccase of *Cryptococcusis* neoformans is a cell wall-associated virulence factor. Infect Immun 69: 5589-5596.

122. Salas SD, Bennett JE, Kwon-Chung KJ, Perfect JR, Williamson PR (1996) Effect of the laccase gene, CNLAC1, on virulence of *Cryptococcusis* neoformans. Journal of Experimental Medicine 184: 377-386.

123. Liu L, Tewari RP, Williamson PR (1999) Laccase protects *Cryptococcusis* neoformans from antifungal activity of alveolar macrophages. Infect Immun 67: 6034-6039.

124. Garcia-Rivera J, Eisenman HC, Nosanchuk JD, Aisen P, Zaragoza O, et al. (2005) Comparative analysis of *Cryptococcusis* neoformans acid-resistant particles generated from pigmented cells grown in different laccase substrates. Fungal Genet Biol 42: 989-998.

125. Eisenman HC, Chow SK, Tsé KK, McClelland EE, Casadevall A (2011) The effect of L-DOPA on *Cryptococcusis* neoformans growth and gene expression. Virulence 2: 329-336.

126. Jacobson ES (2000) Pathogenic roles for fungal melanins. Clin Microbiol Rev 13: 708-717.

127. Williamson PR (1997) Laccase and melanin in the pathogenesis of *Cryptococcusis* neoformans. Front Biosci 2: 99-107.

128. Wang Y, Casadevall A (1994) Growth of *Cryptococcusis* neoformans in presence of L-Dopa decreases its susceptibility to amphotericin B. Antimicrob Agents Chemother 38: 2648-2650.

129. Kwon-Chung KJ, Edman JC, Wickes BL (1992) Genetic association of mating types and virulence in *Cryptococcusis* neoformans. Infect Immun 60: 602-605.

130. Chen SCA, Wright LC, Santangelo RT, Muller M, Moran VR, et al. (1997) Identification of extracellular phospholipase B, lysophospholipase, and acyltransferase produced by *Cryptococcusis* neoformans. Infect Immun 65: 405-411.

131. Wright LC, Santangelo RM, Ganendren R, Payne J, Djordjevic JT, et al. (2007) Cryptococcal lipid metabolism: Phospholipase B1 is implicated in transcellular metabolism of macrophage-derived lipids. Eukaryot Cell 6: 37-47.

132. Chen SC, Muller M, Jin Zhong Z, Wright LC, Sorrell TC (1997) Phospholipase activity in *Cryptococcusis* neoformans: A new virulence factor? J Infect Dis 175: 414-420.

133. Cox GM, McDade HC, Chen SCA, Tucker SC, Gottfredsson M, et al. (2001) Extracellular phospholipase activity is a virulence factor for *Cryptococcusis* neoformans. Mol Microbiol 39: 166-175.

134. Cox GM, Mukherjee J, Cole GT, Casadevall A, Perfect JR (2000) Urease as a virulence factor in experimental cryptococcosis. Infect Immun 68: 443-448.

135. Varma A, Wu S, Guo N, Liao W, Lu G, et al. (2006) Identification of a novel gene, URE2, that functionally complements a urease-negative clinical strain of *Cryptococcusis* neoformans. Microbiology 152: 3723-3731.

136. Ruma-Haynes P, Brownlee AG, Sorrell TC (2000) A rapid method for detecting extracellular proteinase activity in *Cryptococcusis* neoformans and a survey of 63 isolates. J Med Microbiol 49: 733-737.

137. Clemons KV, Azzi R, Stevens DA (1996) Experimental systemic cryptococcosis in SCID mice. J Med Vet Mycol 34: 331-335.

138. Del Poeta M (2004) Role of phagocytosis in the virulence of *Cryptococcusis* neoformans. Eukaryot Cell 3: 1067-1075.

139. Chaturvedi V, Wong B, Newman SL (1996) Oxidative killing of *Cryptococcusis* neoformans by human neutrophils: Evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. J Immunol 156: 3836-3840.

140. Chaturvedi V, Flynn T, Niehaus WG, Wong B (1996) Stress tolerance and pathogenic potential of a mannitol mutant of *Cryptococcusis* neoformans. Microbiology 142: 937-943.

141. Kwon-Chung KJ, Kozel TR, Edman JC, Polacheck I, Ellis D, et al. (1992) Recent advances in biology and immunology of *Cryptococcusis* neoformans. J Med Vet Mycol 30: 133-142.

142. Huffnagle GB, Lipscomb MF (1998) Cells and cytokines in pulmonary cryptococcosis. Res Immunol 149: 387-396.

166. Veron V, Simon S, Blanchet D, Aznar C (2009) Real-time polymerase chain reaction detection of *Cryptococcusis* neoformans and *Cryptococcusis* gattii in human samples. Diagn Microbiol Infect Dis 65: 69-72.

167. Sidrim JJC, Costa AKF, Cordeiro RA, Brilhante RSN, Moura FEA, et al. (2010) Molecular methods for the diagnosis and characterization of *Cryptococcusis*: A review. Can J Microbiol 56: 445-458.

168. Mirza SA, Phelan M, Rimland D, Graviss E, Hamill R, et al. (2003) The changing epidemiology of cryptococcosis: An update from population-based active surveillance in 2 large metropolitan areas, 1992-2000. Clin Infect Dis 36: 789-794.

Dimorph and Filamentous Fungi

The fungi are more evolutionarily advanced forms of microorganisms, as compared to the prokaryotes (such as bacteria).Fungi are commonly divided into two distinct morphological forms: yeasts and hyphae (or filamentous). Yeasts are unicellular fungi which reproduce asexually by blastoconidia formation (budding) or fission. Pure forms of yeasts fall outside the scope of this chapter.

Hyphae are multi-cellular fungi which reproduce asexually and/or sexually. Such fungi are more commonly referred to as ilamentous fungi or simply as moulds. Filamentous fungi are composed of very fine threads (hyphae). Hyphae grow at the tip and divide repeatedly along their length creating long and branching chains. Most filamentous fungi grow in a polar fashion (by extension into one direction) by elongation at the tip (apex) of the hypha [4]. The hyphae continue to grow and intertwine until they form a network of threads called a mycelium. Digestive enzymes are secreted from the hyphal tip. These enzymes break down the organic matter found in the soil into smaller molecules which are used by the fungus as a nutrient source. Some of the hyphal branches grow into the air and spores form on these aerial branches [5]. Fungal spores are either unicellular or multicellular, which develop into a number of different phases of the complex life cycles of the fungi. Fungi are often classified according to their spore-producing structures, for example, spores produced by an ascus are characteristic of ascomycetes.

Dimorphic fungi are fungi which can exist as mould/hyphal/filamentous form or as yeast [6]. Examples include *Penicillium marneffei*, *Coccidioides immitis, Paracoccidioides brasiliensis, Candida albicans, Ustilago maydis, Blastomyces dermatitidis, Histoplasma capsulatum*, and *Sporothrix schenckii*. Dimorphism is a rare phenomenon and very few fungi exhibit dimorphism. Most fungi occur in the hyphae form as branching, threadlike tubular filaments.

Many fungi produce biologically active compounds, several of which are toxic to animals or plants and are therefore called mycotoxins. This can lead to human disease (mycotoxicosis). The symptoms of a mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual [7].

Characterisation of Fungi

Accurate identification of an infecting fungal species is the key to selecting the appropriate anti-fungal treatment. For example, many *Candida* species, including *C. albicans*, *C. parapsilosis* and *C. tropicalis*, are reliably susceptible to the antifungal compound fluconazole, whereas others, such as *C. glabrata* and *C. krusei*, display reduced susceptibility or resistance to fluconazole [8]. Therefore, characterising the fungus is important for targeting the specific treatment.

Identification of fungal isolates from clinical environments using standard identification procedures requires experienced and skilled laboratory staff. When infections are assumed, accurate fungal identification is needed if the contamination source has to be determined and tracked.

Some identification systems are available for the identification of fungi, including biochemical systems such as the API Candida, Omnilog and Vitek [9]. There has also been some advancement with genotypic techniques. For filamentous fungal identification, however, this needs more expensive methods such as PCR-based internal transcribed spacer regions (ITS) sequencing by molecular methods. Advances have also been made with newer techniques, such as beta-D-glucandetection (using a (1–3)-β-D-Glucanassays, based on the *Limulus* amebocytelysate test) for the detection of fungal infections.

In contrast to such expensive tests, conventional culture and staining methods can

fungal structures, and cotton blue which stains the chitin in the fungal cell walls [14]. For the removal of a portion of the fungus for examination from agar, a method like the adhesive tape technique is routinely used [15].

Where a confirmed culture of a mould (including *Aspergillus* species, *Fusarium* species, zygomycetes, *Scedosporium* species) or *C. neoformans* from sputum is isolated, the likelihood of a fungal infection is high [16]. Fungi can also be detected from a positive culture or cytology/direct microscopy for moulds from sinus aspirate; specific antigens from blood; identification from sterile body fluids; urine samples; blood culture; or from pulmonary abnormality [17]. Where a fungal infection is suspected, skin samples, scrapings, nail clippings and hair debris can additionally be taken.

Infection Control Measures

There are three models of anti-fungal control. These are therapeutic administration to treat clinical infection using anti-fungal drugs; and prophylactic and pre-emptive administration to prevent or abort a clinical infection [18].

In terms of prophylactic approaches, the emphasis is upon prevention of infection. Infections of immunocompromised hosts can arise from several settings. These include: the general environment (termed 'community based'); from within clinical and hospital settings; and from contaminated medications.

Many of the disease causing microorganisms which cause infections in immunocompromised hosts are spread via hospitals, such as through hospital air-systems, especially where the air has not passed through a HEPA (high efficiency particulate air) filter [19]; and via transmission by healthcare workers. Hand hygiene is a primary part of preventing multidrug-resistant organism (MDRO) transmission. Facilities should ensure that healthcare personnel are trained in correct hand hygiene technique, using alcohol based hand sanitizers (such as 70% iso-propyl alcohol) [20]. Effective hand sanitisation should apply to hospital visitors as well as to healthcare workers. Other effective measures include observing strict isolation procedures, observing aseptic technique, and the careful cleaning, disinfection and monitoring of respirators, catheters, and other instruments.

Patients in acute care settings who are colonised or infected with pathogenic microorganisms should be placed under special precautions (which may include isolation). It also stands that systems should be in place to identify patients with a history of colonisation or infection at the time of admission so that they can additionally be placed under special precautions if required.

Controls should also be applied to medical devices. The use of devices (such as central venous catheters, endotracheal tubes, urinary catheters) can put patients at risk from device-associated infections, especially if they are not handled or disinfected correctly [21]. Control can be achieved through a robust cleaning and disinfection strategy, as well as by minimising device use. Such measures can decrease the incidence of associated infections.

Antimicrobial stewardship is another primary part of MDRO control. MDROs are disease-causing microorganisms that are resistant to a range of different chemicals. Facilities should ensure that antimicrobials are used for appropriate indications and duration and that the narrowest spectrum antimicrobial that is appropriate for the specific clinical scenario is used [22]. A further measure is the education of healthcare workers and staff in relation to cross-contamination [23]. Control measures should also be applied to the cleaning and disinfection of hospital wards and to maintaining hospital water systems in a sanitary state so that the bio burden levels are low [24].

morbidity and mortality. Risk factors for invasive fungal infection include malignancy, haematopoietic stem cell or solid organ transplantation, neutropenia, chemotherapy, corticosteroid use, acquired immunodeficiency and broad spectrum antimicrobial use. Arguably, the number of at risk patients for invasive mycotic infection has increased since the early 2000s, as more patients have undergone chemotherapy and transplantation and received a growing array of immunosuppressive agents [26].

The three main types of fungal infections, and those that pose the greatest risk to immunocompromised hosts, are fungal meningitis, fungal keratitis and onychomycosis.

These are discussed below.

a) Fungal meningtis

Fungal meningitis can develop after a fungus spreads through the bloodstream from somewhere else within the body. The condition develops as a result of the fungus being introduced directly into the central nervous system, or from an infected body site infection next to the central nervous system. One of the most common causes of fungal meningitis for people with weak immune systems is *Cryptococcus*.

Fungal meningitis can also occur after taking medications that weaken the immune system. Examples of these medications include steroids (such as prednisone), medications given after organ transplantation, or anti-TNF (anti-tumor necrosis factor) medications, which are sometimes given for treatment of rheumatoid arthritis or other autoimmune conditions.

Different types of fungi are transmitted in several ways. *Cryptococcus* is considered to be acquired through inhaling soil contaminated with bird droppings; similarly *Histoplasma* is found in environments with heavy contamination of bird or bat droppings. In contrast, *Blastomyces* is thought to exist in soil rich in decaying organic matter; whereas *Coccidioides* is found in the soil of endemic areas (for example: South western US and parts of Central and South America). When these environments are disturbed, the fungal spores can be inhaled. Aside from these environmental sources, *Candida* is usually acquired in a hospital setting.

Signs and symptoms of fungal meningitis may include the following:

- Fever,
- Headache,
- Stiff neck,
- Nausea and vomiting,
- Photophobia (sensitivity to light),
- Altered mental status.

Fungal meningitis can be treated with long courses of high dose anti-fungal medications. These are usually given through an intravenous feed in a hospital. The length of treatment depends on the status of the immune system and the type of fungus that caused the infection. For people with immune systems that do not function well because of other conditions, like AIDS, diabetes, or cancer, treatment is often longer.

b) Fungal keratitis

Keratitis is an inflammation of the cornea and it is often caused by an infection. Bacteria, viruses, amoeba, and fungi can all cause keratitis. Fungal keratitis is an inflammation of the cornea. The types of fungi that have been known to cause fungal keratitis include: *Fusarium* spp., *Aspergillus* spp., and *Candida spp* [27].

Pathogenic Dimoprhic and Filamentous Fungi

There are several species of fungi that are known, and common, pathogens to human hosts [37]. These can be divided into three groups:

a) Opportunistic infections

Opportunistic infections include ecryptococcosis and aspergillosis. These infections are problematic for people with weakened immune systems, such as cancer patients, transplant recipients, and people with HIV/AIDS.

b) Hospital-associated infections

Hospital acquired infections include candidemia, which is the leading cause of bloodstream infections. An associated risk is that advancements and changes in healthcare practices can, as this chapter has discussed, provide opportunities for new and drug-resistant fungi to emerge in hospital settings [38].

c) Community-acquired infections

Infections within the general populace include coccidioidomycosis, blastomycosis, and histoplasmosis. These diseases are caused by fungi that are abundant in the environment, such as soil, on plants, or in compost heaps. Climate change may affect these fungi, given that small changes in temperature or moisture can affect fungal growth, leading to an increased level of incidents.

Primary fungal infections

The main species of fungi which are most commonly associated with diseases in immunocompromised hosts are:

a) *Aspergillus*

Aspergillus is a common fungus that can be found in indoor and outdoor environments. *Aspergillus* thrives on a variety of substrates such as corn, decaying vegetation and soil. These fungi are also common contaminants in air. Most people breathe in *Aspergillus* spores every day without being affected.

Aspergillosis is a disease caused by fungi within this genus and it usually occurs in people with lung diseases (such as cystic fibrosis) or weakened immune systems [39]. The spectrum of illness includes allergic reactions, lung infections, and infections in other organs [40]. In humans, the major forms of disease are:

- Allergic broncho pulmonary aspergillosis or ABPA, which affects patients with respiratory diseases such as asthma, cystic fibrosis, and sinusitis [41].
- Acute invasive aspergillosis, a form that grows into surrounding tissue, more common in those with weakened immune systems such as AIDS or chemotherapy patients.
- Disseminated invasive aspergillosis, an infection spread widely through the body.
- Aspergilloma, a “fungus ball” that can form within cavities such as the lung [42].

The clinical manifestation and severity of the disease depends upon the immunologic state of the patient [43].There are three clinical types of pulmonary aspergillosis:

1. Allergic hypersensitivity to the organism. Symptoms may vary from mild respiratory distress to alveolar fibrosis.
2. Aggressive tissue invasion. Aspergillosis is primarily a pulmonary disease, but the aspergilli may disseminate to any organ. They may cause endocarditis, osteomyelitis, otomycosis and cutaneous lesions.
3. Fungus ball where the ‘lesion’ (actually a colony of mould growing in the cavity) is

- Gastrointestinal. Heartburn, bloating, diarrhea or constipation.
- Respiratory allergy. Rhinitis, sneezing and/or wheezing.
- Central nervous system. Anxiety, depression, memory deficits and/or loss of ability to concentrate.
- Menstrual abnormalities. Severe premenstrual tension and/or menstrual irregularities.
- Other Systemic Symptoms. Fatigue, headache and/or irritability.

d) *Cladosporium*

Cladosporium is a common mould found outdoors, on soil and plants, and indoors, on wet surfaces, including wallpaper and carpet. Many species are cosmopolitan fungi isolated from soil, plant debris and leaf surfaces. *Cladosporium* is very frequently isolated from air, especially during seasons in which humidity is elevated.

The growth rate of *Cladosporium* colonies is moderate on Potato Dextrose Agar at 25°C and the texture is velvety to powdery. The colony colour ranges from olivaceous green to black, from the front and black from the reverse. Most of the *Cladosporium* spp. do not grow at temperatures above 35°C [53]. Examples include *Cladosporium cladosporioides, Cladosporium sphaerospermum* and *Cladosporium herbarum*.

The most common infections caused by *Cladosporium* are skin and toenail infections, but the fungus can cause sinus and lung infections and eye (corneal) ulcers [54]. *Cladosporium* can also cause allergies and asthma attacks. Although *Cladosporium* rarely causes infections for people, *Cladosporium* is till recognised as a human pathogen [55].

e) *Coccidioides*

Coccidioidomycosis is the infection caused by the dimorphic fungus *Coccidio idesimmitis* [56]. *Coccidioides* is a fungus found in the soil of dry, low rainfall areas. Coccidioidomycosis, also known as Valley Fever, is a common cause of pneumonia in endemic areas. At least 30% – 60% of people who live in an endemic region are exposed to the fungus at some point during their lives. In most people the infection is short lasting and not life threatening, however for people who develop severe infections or chronic pneumonia, medical treatment is necessary. Certain groups of people are at higher risk of developing severe disease [57].

f) *Cryptococcus*

Cryptococcus is a fungus (a heterothallic basidiomycete) that is found in the soil and produces spores that can be inhaled. People can become infected with Cryptococcus early in life but be unaware of the infection. If a person's immune system is weakened (for example, by HIV), Cryptococcus can cause a life-threatening infection called cryptococcal meningitis [58], an infection of the meninges (the tissue covering the brain)

There are over thirty different species of Cryptococcus, but two species – *Cryptococcus neoformans* and *Cryptococcus gattii* – cause nearly all cryptococcal infections in humans and animals. Of the two, *C. neoformans*is the most prevalent (*C. neoformans* var. *grubii* (serotype A) accounts for the vast majority of the disease worldwide). The fungus is found in soil throughout the world. People at risk can become infected after inhaling microscopic, airborne fungal spores. Sometimes these spores cause symptoms of a lung infection, but other times there are no symptoms at all. In people with weakened immune systems, the fungus can spread to other parts of the body and cause serious disease [59].

Cryptococcus is detected using a blood test for the cryptococcal antigen. There are primarily two types of commercial tests, latex agglutination and ELISA systems [60].

handling contaminated plant material, when the fungus enters the skin through a small cut or scrape. Pulmonary and disseminated forms of infection, although uncommon, can occur. Most cases of sporotrichosis are localised to the skin and subcutaneous tissues [69].

Conclusion

This chapter has examined the primary mycotic diseases (arising from dimorphs and filamentous fungi) that pose a significant risk to the immunocompromised host. What is clear from the list of different microorganisms is that many of them are found in the general environment on the human body and that they do not; in general, pose a risk to those with healthy immune systems. Such microorganisms become a risk when the immune system is weakened (due to primary or secondary reasons). Thus the immunocompromised person is especially vulnerable to opportunistic infections.

As well as outlining the different infectious agents, the chapter has discussed some of the microbiological and diagnostic attributes required for the identification of the disease within the infected person and for the characterisation of the organism through microbiological identification methods. Understanding the type of fungus is important for considering the treatment. In relation to treatments the chapter has outlined some of the common anti-fungal medicines.

References

1. Raguin G (1997) Prevention of bacterial infections in immunocompromised hosts, excluding HIV infection and mycobacterium infections. Ann Med Interne (Paris) 148: 218-225.
2. Rosen FS, Cooper MD, Wedgwood RJ (1995) The primary immunodeficiencies. N Engl J Med 333: 431–440.
3. Vijayakumar R, Sandle T, Manoharan C (2012) A review of fungal contamination in pharmaceutical products and phenotypic identification of contaminants by conventional methods. European Journal of Parenteral and Pharmaceutical Sciences 17: 4-19.
4. Shoji JY, Arioka M, Kitamoto K (2006) Possible involvement of pleiomorphicvacuolar networks in nutrient recycling in filamentous fungi. Autophagy 2: 226–227.
5. Fischer R, Zekert N, Takeshita N (2008) Polarized growth in fungi—interplay between the cytoskeleton, positional markers and membrane domains. Mol Microbiol 68: 813–826.
6. Chandler JM, Treece ER, Trenary HR, Brennemen JL, Flickner TJ, et al. (2008) Protein profiling of the dimorphic, pathogenic fungus, Penicillium marneffei. Proteome Sci 6: 17.
7. Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16: 497–516.
8. Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20: 133–163.
9. Palmieri MJ, Carito SL, Meyer J (1988) Comparison of rapid NFT and API 20E with conventional methods for identification of gram-negative nonfermentative bacilli from pharmaceutical and cosmetics. Appl Environ Microbiol 54: 2838–3241.
10. McFaddin, Jean F (1985) Media for isolation-cultivation-identification maintenance of medical bacteria. Williams & Wilkins.
11. Beever RE, Bollard EG (1970) The nature of the stimulation of fungal growth by potato extract. J Gen Microbiol 60: 273–279.
12. Koneman EW (1997) Color Atlas and Textbook of Diagnostic Microbiology, (5thedn) PA: JB Lippincott Company, Philadelphia, USA.
13. American Society for Microbiology (1980) Cumitech 11: Practical Methods for Culture and Identification of Fungi in the Clinical Microbiology Laboratory. Washington, DC, USA.
14. Leck A (1999) Preparation of lactophenol cotton blue slide mounts. Community Eye Health 12.

37. Revankar SG, Sutton DA (2010) Melanized fungi in human disease. Clin Microbiol Rev 23: 884-928.

38. McCann S, Byrne JL, Rovira M, Shaw P, Ribaud P, et al (2004) Outbreaks of infectious diseases in stem cell transplant units: a silent cause of death for patients and transplant programmes. Bone Marrow Transplant 33: 519–529.

39. Cimon B, Carre`re J, Chazalette JP, Ginies JL, Chabasse D, et al. (2000) Bronchopulmonary mycoses in cystic fibrosis. Results of a five-year longitudinal study. Journal de Mycologie Médicale 10: 128-135.

40. Barnes PD, Marr KA (2006) Aspergillosis: spectrum of disease, diagnosis, and treatment. Infect Dis Clin North Am 20: 545–561.

41. Bozkurt MK, Ozcelik T, Saydam L, Kutluay L (2008) A case of isolated aspergillosis of the maxillary sinus. Kulak Burun Bogaz Ihtis Derg 18: 53-55.

42. Przyjemski CJ, Mattii R (1980) The Formation of Pulmonary Mycetomata. Cancer 46: 1701–1704.

43. Bennett JE (1995) *Aspergillus* species, In Mandell GL, Bennett JE, and Dolin R (eds), Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, (4thedn) Churchill Livingstone, New York: 2306-2310.

44. Perfect JR, Cox GM, Lee JY, Kauffmen CA, de Repentigny L, et al. (2001) The impact of culture isolation of *Aspergillus* species: A hospital-based survey of aspergillosis. Clin Infect Dis 33: 1824–1833.

45. Lalitha P, Vijayakumar R, Prajna NV, Srinivasan M (2008) Atlas of Fungal Corneal Ulcer – Clinical Features & Laboratory Identification Methods. Jaypee Publishers, India.

46. Raper KB, Fennell DI (1965) The Genus *Aspergillus*. Williams & Wilkins, Baltimore, USA.

47. Walsh TJ, Groll AH (2001) Overview: non-fumigatus species of *Aspergillus*: perspectives on emerging pathogens in immunocompromised hosts. Curr Opin Investig Drugs 2: 1366–1367.

48. Fujimura M, Ishiura Y, Kasahara K, Amemiya T, Myou S, et al. (1998) Necrotizing bronchial aspergillosis as a cause of hemoptysis in sarcoidosis. Am J Med Sci. 315: 56-58.

49. Dworkin MS, Duckro AN, Proia L, Semel JD, Huhn G (2005) The epidemiology of blastomycosis in Illinois and factors associated with death. Clin Infect Dis 41: 107-111.

50. Bodey GP (1988) The emergence of fungi as major hospital pathogens. J Hosp Infect 11: 411-426.

51. Fridkin SK (2005) The changing face of fungal infections in health care settings. Clin Infect Dis 41: 1455–1460.

52. Blonz ER (1986) Is there an epidemic of chronic candidiasis in our midst. JAMA 256: 3138-3139.

53. Sutton DA, Fothergill AW, Rinaldi MG (1998) Guide to Clinically Significant Fungi. (1stedn) Williams & Wilkins, Baltimore, USA.

54. Bouziane H, Latge JP, Fitting C, Mecheri S, Lelong M, et al. (2005) Comparison of the allergenic potency of spores and mycelium of Cladosporium. Allergol Immunopathol (Madr) 33: 125-130.

55. Yano S, Koyabashi K, Kato K (2003) Intrabronchial lesion due to Cladosporiumsphaerospermum in a healthy, non-asthmatic woman. Mycoses 46: 348-350.

56. Drutz DJ, A Catanzaro (1978) Coccidioidomycosis. Part I. Am Rev Respir Dis. 117: 559-85.

57. Chiller TM, Galgiani JN, Stevens DA (2003) Coccidioidomycosis. Infect Dis Clin North Am 17: 41–57.

58. Al-Doory Y (2010) A bibliography of cryptococcosis. Mycopathol Mycol Appl 45: 1-60.

59. Albert-Braun S, Venema F, Bausch J, Hunfeld KP, Schafer V (2005) Cryptococcus neoformansperitionitis in a patient with alcoholic cirrhosis: case report and review of the literature. Infection 33: 282-288.

60. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, et al. (2009) Screening for cryptococcalantigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis 48: 856-862.

61. Bronson DM, Desai DR, Barsky S, Foley SM (1983) An epidemic of infection with Trichophytontonsurans revealed in a 20-year survey of fungal infections I Chicago. J Am Acad Dermatol 8: 322-330.

62. Padhye AA, Summerbell RC (2005) The dermatophytes. In: Merz WG, Hay RJ, eds. Topley and Wilson's Microbiology and Microbial Infections: Medical Mycology, 10th edn. London: Hodder Arnold; 2005; 220–243.

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Chapter: XIII

Microsporidia Infections in Immunocompromised Hosts

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Introduction

Microsporidia are unicellular eukaryotes, an obligatory intracellular parasite, which for many years were considered protozoan, but recently have been reclassified as belonging to the kingdom Fungi [1,2]. There are approximately 1,300 named species of Microsporidia, most infect invertebrates and fish. Eight genera have been described in human hosts: *Enterocytozoon, Encephalitozoon, Pleistophora, Trachipleistophora, Vittaforma, Brachiola, Nosema* and *Microsporidium*, and more than 14 microsporidian species are implicated in human pathology [3].

The first human case of microsporidial infection was a case of disseminated *Encephalitozoon* infection in a 9-year-old Japanese boy, who suffered from recurrent fever, headache, vomiting, and spastic convulsions, reported by Matsubayashi et al. in 1959 [4]. Few cases of microsporidiosis have been described until 1985, when the new species *Enterocytozoon bieneusi* was described in a Haitian acquired immunodeficiency syndrome - AIDS patient from France [5,6], and so many cases of intestinal infection in HIV-positive were reported as similar biopsy [7]. Today we know that microsporidia are ubiquitous pathogens that can be observed in insects, fish and mammals, including man [8].

Life Cycle and Morphology

Microsporidia are eukaryotic, unicellular organisms belonging to the phylum Microspora. All microsporidia are obligate, spore-forming, intracellular parasites that invade vertebrates and invertebrates cells [8]. The life cycle of microsporidia includes three distinct phases: the spores responsible for transmission of infection, the proliferation of vegetative forms of intracellular calls schizogony or merogony, and finally sporogony or the formation of spores [9,10].

The mature and infectious spores of the microsporidia species that infect mammals are relatively small, measuring 1.0–3.0 µm x 1.5–4.0 µm (Figure 1) [1]. The spores contain a long and convoluted tubular extrusion apparatus (polar tubule), which distinguishes them from other organisms and has a crucial role in invasion of host cell [11]. Microsporidian spores are relatively resistant in the environment and are surrounded by an outer electron-dense

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and a thick wall, making the spores mature. The spores spread through the tissues of the host by infecting new cells and continuing the cycle [9,14].

Immune Response Against Microsporidia

Cellular immunity is critical for the survival of the hosts infected with *E. cuniculi* or another microsporidian. Mice experimentally infected with *E. cuniculi* show few clinical signs of disease [15]. After inoculation of *E. cuniculi*, many mammals have chronic infection, associated with high antibody titer and a continuous and persistent inflammation, for example persistent encephalitis and nephritis in rabbits [16] or congenital disease in foxes [17]. However, there are more susceptible mouse strains and more resistant to infection by *E. cuniculi*, which has been evidenced by the high percentage of infected macrophages after the intraperitoneal inoculation, suggesting a genetic basis for resistance innate [15]. Because it is an opportunistic agent, the immunodeficient mice such as athymic and SCID are the ones who develop the deadly disease after experimental inoculation of *E. cuniculi* [18,19], generally manifested in the form disseminate and characterized by ascites and the presence of spores in all organ systems.

Macrophages are critical in linking innate and acquired immunity. Macrophages, in particular located in the gut quickly recognize the microsporidia spores ingested with food or water through multiple classes of receptors, including pattern recognition receptors (PRR) located on its surface. Further doubts exist regarding these receptors, it is thought to be the possibility of Toll-like receptor 2 (TLR2) and TLR4, which activate nuclear factor Kappa B and increase secretion of chemokines, which recruit macrophages from monocytes. Additionally, the recognition of microsporidia by macrophages can also result in the production of a large number of other mediators of defense, including cytokines and reactive oxygen and nitrogen [20,21].

T cells are important for protection against infection by *E. cuniculi* in the normal host [20,21]. The role of individual subtypes of T cells during infection by *E. cuniculi* has been described in recent years. The phenotypic analysis of spleen cells from infected animals showed an increase in the population of CD8+ T cells starting from day 10 post-infection (PI). This increase in CD8+ T lymphocytes continued until day 17 PI, when it was seen an increase of more than 3 times in this subtype of cells compared to non-infected control animals. Further analysis with activation markers suggested that CD8+ T cells are activated on day 3 PI *E. cuniculi*. In contrast, no significant increase in CD4+ T cells during the course of infection [21].

In most cases, CD8+ T cells are activated by IL-2 produced by CD4+ T lymphocytes [22]. However, in certain viral infections *in vivo* was observed response of CD8+ T cells in the absence of CD4+ T cells [23]. As mentioned previously [24], the absence of CD4+ T cells does not affect the result of infection by *E. cuniculi* in knockout animals. Thus, infection by *E. cuniculi* is a good example of intracellular parasitic infection, wherein the CD8+ T cells can be induced in the absence of CD4+ T cells.

The cytokines IFN-γ and IL-12 are important for protective immunity against a number of intracellular infections viral, bacterial and parasitic diseases [25,26]. Studies with *Encephalitozoon intestinalis* showed that mice lacking the gene for IFN-γ are unable to control the infection [27]. Based on observations in vitro, it was suggested that IFN-γ also plays an important role in protective immunity against infection with *E. cuniculi* [28,29]. However, the importance of IFN-γ in natural infection by *E. cuniculi in vivo* has not been fully elucidated [30]. Mice infected with *E. cuniculi* and treated with antibodies against IFN-γ or IL-12 showed increased mortality. The use of gene knockout mice to Th1 confirms the importance of IFN-γ or IL-12 in the immune response against *E. cuniculi*.

Encephalitozoon	Development within parasitophorous vacuoles. Spores measure 2.0–2.5 × 1.0–1.5 µm with five to seven turns of the polar tubule	*Encephalitozoon intestinalis*-Enteritis, cholangitis, cholecystitis, peritonitis, nephritis, bronchitis, rhinitis, sinusitis, keratoconjonctivitis, disseminated infection *Encephalitozoon hellem*-Keratoconjonctivitis, rhinitis, sinusitis, pneumonia, bronchitis, nephritis, uretritis, cystitis, prostatic abscess, urinary tract infection *Encephalitozoon cuniculi*-Encephalitis, hepatitis, cholecystitis, enteritis, nephritis, rhinitis, sinusitis, keratoconjonctivitis, disseminated infection
Microsporidium	*Microsporidium africanum*-Spores measure 4.5 × 1.5 µm with 15–16 turns of the polar tubule and no developmental stages of the parasite were seen. *Microsporidium ceylonensis*-Spores measure 1.5 × 3.5 µm, no meronts or sporonts were seen.	*Microsporidium africanum* and *Microsporidium ceylonensis*-Cornea ulcer
Nosema	Spores measured 3.7 × 1.0 µm	*Nosema ocularum*–Keratoconjonctivitis
Pleistophora	The parasites develop within a vesicle, bounded by a thick parasite-formed coat named sporophorous vesicle. Spores measured 2.0–2.8 × 3.0–4.0 µm and had 10–12 turns of the polar tubule	*Pleistophora ronneafie*-Myositis
Trachipleistophora	*Trachipleistophora hominis*-Spores measured 4 × 2.4 µm *Trachipleistophora anthropophthera*-Spores measured 3.7 × 2.0 µm	*Trachipleistophora hominis*-Myositis, keratoconjonctivitis, sinusitis, rhinitis *Trachipleistophora anthropophthera*-Keratitis, myositis, encephalitis, disseminated infection
Vittaforma	Development direct contact with the cell cytoplasm. Spores measured 3.7 × 1.0 µm and had 6 turns of the polar tubule	*Vittaforma cornea*-Keratitis, nephritis

Table 1: Clinical manifestations and biological characteristics of the main species of human microsporidia.

In immunocompetent individuals, infection by *E. bieneusi* cause self-limiting diarrhea lasting about a month (Table 1). The microsporidiosis by *E. bieneusi* in healthy individuals and immunocompent is described in travelers and geriatric persons. Asymptomatic infections in children and child-care workers were reported [8,10].

Three pathogenic species of *Encephalitozoon* that infect humans, *Encephalitozoon cuniculi* and *Encephalitozoon hellem*, are morphologically similar by light and electron microscopy, and can only be distinguished by antigenic, biochemical, or nucleic acid analysis.

Encephalitozoon infections in immunodeficient humans and animals result in necrosis and lymphocytic infiltration of affected organs, particularly of small intestine, liver, kidney, nasal cavities, sinuses, and pancreas. Several cases of *Encephalitozoon* infection were reported to occur in patients with and without AIDS prior to 1991 [36,37]. The infections by species of *Encephalitozoon* develop in the digestive tract or respiratory tract, respectively, by ingestion and inhalation of spores. *Encephalitozoon* infections are generally widespread, but in the case of *E. intestinalis* can be started by an intestinal infection. *Encephalitozoon intestinalis* as *E. bieneusi* are responsible for most of microsporidia infections, affecting the intestine; however there is a tendency to spread with clinical syndromes that included sinusitis, keratoconjunctivitis, encephalitis, tracheobronchitis, interstitial nephritis, hepatitis, or myositis (Table 1). Disseminated infections with all three *Encephalitozoon* spp. have been recognized in several severely immunosuppressed HIV-infected patients.

Disseminated infections with other microsporidian species (*N. connori*, *V. corneae*, *T. hominis*, *T. anthropophthera*) have been reported only as single case reports [10].

Before the AIDS pandemic, ocular microsporidiosis was considered rare. Today ocular localization is the second most common localization in humans after gastrointestinal infections [38,39]. Ocular microsporidiosis produces keratoconjunctivitis or deep keratitis

RNA or the internal transcribed spacer (ITS). Species identification has been accomplished by use of species-specific primers or pan-microsporidian primers are used (i.e. primers that amplify more than one microsporidian species), species identification can be determined by subjecting the amplicons to nested PCR with a second set of specific primers, restriction enzyme digestion to generate a restriction fragment length polymorphism (RFLP) pattern, nucleotide sequencing for BLAST analysis, southern analysis using species-specific probes, or heteroduplex mobility shift analysis. Quantitative PCR is a specific highly sensitive method with a detection threshold less than 40 spores/mL in stool suspension [45].

Epidemiology

According to geographical location and the diagnostic method used, the prevalence of disease among HIV-positive patients ranges between 1.7% and 50% [1,3,9]. In the 90s, before the use of effective antiretroviral therapy in HIV-positive patients with diarrhea were identified prevalence rates of 30 and 44% by intestinal biopsy [50,51]. Since the administration of antiretroviral therapy has enabled HIV-infected patients to restore their immune status, the number of cases of microsporidia infections has dropped off dramatically in developed countries, for example, was observed in 3.9% of HIV-positive patients with diarrhea in 2003 [52]. But other immunodeficiency may also modify the host-parasite balance. They have various origins: pathological, other than AIDS (cytomegalovirus infections, cancers such as leukaemia); post-therapeutic (massive and prolonged corticotherapy, immunosuppressive treatments against transplant rejection); congenital deficiency in immunoglobulins, or even gerontological [31,53-55].

Microsporidia have been considered to be ubiquitous organisms found throughout the environment, and potential reservoirs of microsporidia species that can be transmitted to humans include other infected humans, animals, food and water (Figure 3). Zoonotic transmission of microsporidia also is likely since a wide range of animals are infected with species and genotypes of microsporidia that infect humans, and among the risk factors associated with microsporidiosis in HIV-infected individuals were association with animals and eating undercooked meat [56]. The zoonotic transmission has also been reinforced by the description one case of seroconversion in a 10-year-old child in close contact with a dog infected with *Encephalitozoon cuniculi* [57].

The pathways of microsporidia infections, modes, or routes of transmission, and the knowledge of the epidemiology are still uncertain. Because microsporidial spores are released into the environment via stool, urine, and respiratory secretions, possible sources of infection may be persons or animals infected with microsporidia [1,3].

Waterborne transmission is one of the main risk factors for intestinal diseases causing an important morbidity and mortality worldwide. The presence of human pathogens in surface water may suggest the presence of living environmental reservoirs, such as domestic and wild animals. Among the latter, aquatic birds may play an important role in the transmission of different pathogens [58]. Recent studies suggest the involvement of water in the epidemiology of human microsporidiosis [59].

Microsporidia are recognized category B biodefense agents on the National Institutes of Health list, and the transmission of microsporidian spores is seriously considered by American agencies concerned with the quality of drinking water [59]. We evaluated the presence of microsporidia in stool samples from wildlife animals, captured in an area of deforestation for the construction of two water reservoirs in the state of São Paulo (Brazil) [60]. Microsporidian spores were seen in the stools of 12 animals - 6 small rodents, 3 marsupials and 3 hairy-legged vampire bats. This was the first description of microsporidiosis in wildlife animals in Brazil and emphasizes the importance of these animals, particularly

5. Modigliani R, Bories C, Le Charpentier Y, Salmeron M, Messing B, et al. (1985) Diarrhoea and malabsorption in acquired immune deficiency syndrome: a study of four cases with special emphasis on opportunistic protozoan infestations. Gut 26: 179-187.

6. Desportes I, Le Charpentier Y, Galian A, Bernard F, Cochand Priollet B, et al. (1985) Occurrence of a new microsporidan: *Enterocytozoon bieneusi* n.g., n. sp., in the enterocytes of a human patient with AIDS. J Protozool 32: 250-254.

7. Orenstein JM (1991) Microsporidiosis in the acquired immunodeficiency syndrome. J Parasitol 77: 843-864.

8. Thellier M, BretonJ (2008) *Enterocytozoon bieneusi* in human and animals, focus on laboratory identification and molecular epidemiology. Parasite 15: 349-358.

9. Mathis A, Ndiritu W, Thompson RCA, Didier ES, Müller-Doblies U, et al. (2000) Genetic heterogeneity and host specificity of the microsporidian *Enterocytozoon bieneusi*. Acta Parasitol 45: 190.

10. Franzen C, Müller A (2001) Microsporidiosis: human diseases and diagnosis. Microbes Infect 3: 389-400.

11. Desportes-Livage I (2000) Biology of microsporidia. Contrib Microbiol 6: 140-165.

12. Canning EU, Vavra J (2000) The Illustrated Guide to the Protozoa. (2ndedn) Society of Protozoologists, Lawrence, Kansas, USA.

13. Keeling PJ, Fast NM (2002) Microsporidia: biology and evolution of highly reduced intracellular parasites. Annu Rev Microbiol 56: 93-116.

14. Weiser J (2005) Microsporidia and the society for invertebrate pathology: A personal point of view. J Invertebr Pathol 89: 12-18.

15. Niederkorn JY, Shadduck JA, Schmidt EC (1981) Susceptibility of selected inbred strains of mice to *Encephalitozoon cuniculi*. J Infect Dis. 144: 249-253.

16. Shadduck JA, Pakes SP (1971) *Encephalitozoon*osis (nosematosis) and toxoplasmosis. Am J Pathol 64: 657-671.

17. Botha WS, Van Dellen AF, Stewart CG (1979) Canine *Encephalitozoon*osis in South Africa. J S Afr Vet Assoc 50: 135-144.

18. Schmidt EC, Shadduck JA (1983) Murine *Encephalitozoon*osis model for studying the host-parasite relationship of a chronic infection. Infect Immun 40: 936-942.

19. Koudela B, Vítovec J, Kucerová Z, Ditrich O, Trávnícek J (1993) The severe combined immunodeficient mouse as a model for *Encephalitozoon cuniculi* microsporidiosis. Folia Parasitol 40: 279-286.

20. Texier C, Vidau C, Vigues B, Alaoui HE, Delbac F (2010) Microsporidia: a model for minimal parasite-host interactions.Cur Op Microbiol 13: 443-449.

21. Khan IA, Moretto M, Weiss LM (2001) Immune response to *Encephalitozoon cuniculi* infection. Microbes Infec 3: 401-405.

22. Smith KA (1988) Interleukin-2: inception, impact, and implications. Science 240: 1169 - 1176.

23. Binder D, Kundig TM (1991) Antiviral protection by CD8+ versus CD4+ T cells. CD8+ T cells correlating with cytotoxic activity in vitro are more efficient in antivaccinia virus protection than CD4-dependent IL. J Immunol 146: 4301-4307.

24. Khan IA, Schwartzman JD, Kasper LH, Moretto M (1999) CD8+ CTLs are essential for protective immunity against *Encephalitozoon cuniculi* infection. J Immunol 162: 6086-6091.

25. Suzuki Y, Conley FK, Remington JS (1989) Importance of endogenous IFN-gamma for prevention of toxoplasmic encephalitis in mice. J Immunol 143: 2045-2050.

26. Yang J, Mitsuyama M (1997) An essential role for endogenous interferon-gamma in the generation of protective T cells against *Mycobacterium bovis* BCG in mice. Immunol 91: 529-535.

27. Achbarou A, Ombrouck C, Gneragbe T, Charlotte F, Rénia L (1996) Experimental model for human intestinal microsporidiosis in interferon gamma receptor knockout mice infected by *Encephalitozoon intestinalis*. Parasite Immunol 18: 387-392.

28. Didier ES (1995) Reactive nitrogen intermediates implicated in the inhibition of *Encephalitozoon cuniculi* (phylum microspora) replication in murine peritoneal macrophages. Parasite Immunol 17: 405-412.

54. Muñoz P, Valerio M, Puga D, Bouza E (2010). Parasitic infections in solid organ transplant recipients. Infect Dis Clin N Am 24: 461-495.

55. Rabodonirina M, Cotte L, Radenne S, Besada E, Trepo C (2003). Microsporidiosis and transplantation: a retrospective study of 23 cases. J Eukaryot Microbiol 50: 583.

56. Hutin YJ, Sombardier MN, Liguory O, Sarfati C, Derouin F, et al. (1998). Risk factors for intestinal microsporidiosis in patients with human immunodeficiency virus infection: a case-control study. J Infect Dis 178: 904-907.

57. Mc Innes EF, Stewart CG (1991) The pathology of subclinical infection of *Encephalitozoon cuniculi* in canine dams producing pups with overt *Encephalitozoon*osis. J S Afr Vet Assoc 62: 51-54.

58. Slodkowicz-Kowalska A, Gracyk TK, Tamang L, Girouard, AS, Majewska AC (2006) Asymptomatic *Enterocytozoon bieneusi* microsporidiosis in captive mammals. Parasitol Res 100: 505-509.

59. Nwachcuku N, Gerba CP (2004) Emerging waterborne pathogens: can we kill them all? Curr Opin Biotechnol 15: 175-180.

60. Lallo MA, Pereira A, Favorito SE et al. (2009) Ocorrência de *Giardia*, *Cryptosporidium* e microsporídios em animais silvestres em área de desmatamento no Estado de São Paulo, Brasil. Cien Rural 39: 1465-1470.

61. Costa SF, Weiss LM (2000) Drug treatment of microsporidiosis. Drug Resist Updat 3: 384-399.

62. Canning EU, Lom J (1986) The microsporidia of vertebrades. Academic Press, London, UK.

63. Didier PJ, Phillips JN, Kuebler DJ, Nasr M, Brindley PJ, et al. (2006) Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives in vitro and *in vivo*. Antimicrob Agents Chemother 50: 2146-2155.