16

Fungal Infections in Patients of Paediatric Age

Giuseppina Caggiano
and Maria Teresa Montagna
Department of Biomedical Science and Human Oncology, Hygiene Section, University of Bari "Aldo Moro", Bari, Italy

1. Introduction

In recent decades, fungi have emerged as important causes of human infection, due primarily to the increased numbers of patients subjected to severe immunosuppression, so the demand for information on the pathogenic role of these microorganisms and the diseases they cause is growing.

Despite the development of more active, less toxic antifungal agents and the use of antifungal prophylaxis, the mycoses (especially those invasive) continue to be a serious infective complication in several patients' outcomes, resulting in high mortality rates (Lehrnbecher et al., 2010). Both paediatric and adult patients are exposed to fungal infections, even if important differences of epidemiology, diagnostic approaches and therapeutic management have to be considered. To date, globally, these infections have been well studied in different populations of adult patients while thorough epidemiological analyses in paediatric patients, including immunocompromised subjects, e.g. children with haematological malignancies and preterm neonates, are fairly sparse.

1.1 General characteristics of fungi

Fungi are ubiquitous organisms that live as environment saprophytes or as commensal microorganisms of humans and animals.

They morphologically are distinguished in yeasts (unicellular organisms) and moulds (multicellular organisms). Most fungi are found in morphological organization as yeasts or moulds but some species, known as dimorphic fungi, can be found as yeasts or moulds depending on the temperature and substrate on which they develop. They grow in environment as moulds and in humans as yeasts.

Of the more than 100,000 species of fungi identified to date, only few species can infect man and of them only a few are sufficiently virulent to infect a healthy host.

On the basis of their pathogenic role, fungi can be divided into:

- *primary pathogens* (dimorphic fungi, such as *Histoplasma, Blastomyces, Coccidioides*) which can also infect immune-competent subjects. They are microorganisms endemic to America, Africa and Asia.
- *opportunistic pathogens* (yeasts and moulds, such as *Candida* spp., *Cryptococcus* spp, *Aspergillus* spp, *Zygomycetes* and *Fusarium* spp) which can cause damage to the host if the immune system is compromised.

According to the site of infection and degree of tissue involvement, fungi can cause superficial, cutaneous, subcutaneous and deep mycoses (Kern & Blenvins, 1997).

The superficial mycoses are limited to the stratum corneum and essentially elicit no inflammation response; they are caused specially by fungi dermatophytes that produce keratinase and live on human keratin (skin, hair and nails). The cutaneous infections involve the integument and its appendages. The sub-cutaneous mycoses affect the subcutaneous tissues usually at the point of traumatic inoculation of the etiological agent. The deep infections involve systems and organs stimulating high immune response. The Table 1 shows the classification of most fungal infections.

Pattern of infection	Fungal infection	Etiological agent
Superficial and cutaneous	Pityriasis versicolor	*Malassetia furfur*
	Tinea nigra	*Exophiala werneckii*
	White piedra	*Trichosporon beigelii*
	Black piedra	*Piedraia hortae*
	Dermatophytosis	*Microsporum spp* *Trichophyton spp* *Epydermophyton floccosum*
	Candidiasis of skin, nail, mucosa	*Candida spp*
Sub- cutaneous	Chromoblastomycosis	*Fonsecaea pedrosoi* *Phialophora verrucosa etc.*
	Mycetoma	*Madurella mycetomatis* *Acremonium, Pseudallescheria, Exophiala, Curvularia, Fusarium, Aspergillus, etc.*
	Sporotrichosis	*Sporothrix schenckii*
Deep by dimorphic fungi	Blastomycosis	*Blastomyces dermatitidis*
	Coccidioidomycosis	*Coccidioides immitis*
	Histoplasmosis	*Histoplasma capsulatum*
	Paracoccidioidomycosis	*Paracoccidioides brasiliensis*
Deep by opportunistic pathogen fungi	Aspergillosis	*Aspergillus spp*
	Sistemic Candidiasis	*Candida spp*
	Cryptococcosis	*Cryptococcus neoformans/gattii*
	Zygomycoses	*Rhizopus, Absidia, Mucor etc.*

Table 1. Classification of most fungal infections and their principal etiological agents.

The deep mycoses are those of highest concern for the life-threatening consequences. They can be identified in:

Invasive Mycoses: deep fungal infection with predominant deep tissue invasion in the absence of blood dissemination.

Disseminated Mycoses: deep fungal infection involving multiple organs or systems that do not touch each other and without being transported by blood or lymph.

Systemic Mycoses: deep fungal infection confined to a single organ or system achieved by blood.

Infecting fungi may be either exogenous or endogenous so, according to the route of acquisition, a mycoses may be designated as exogenous or endogenous in origin.

The moulds have their natural habitat in the environment so they always originate exogenous infections caused mainly by the inhalation of air widespread conidia . This kind of infections are often correlated to environmental contamination especially during construction works. Given the ubiquitous nature of fungal spores, in particular of *Aspergillus* spp, in the external environment, numerous sources have been identified also in hospitals: unfiltered air; conditioned air systems with poorly maintenance; persistent construction works. Therefore, the presence of *Aspergillus* spp in the hospital setting is the major extrinsic risk factor for the occurrence of nosocomial invasive aspergillosis mainly among neutropenic patients.

On the contrary, the yeasts can be endogen and exogenous. The endogen yeasts colonize the mucosa and in particular conditions (immunocompromise, mucosite post-chemotherapy) can invade the organism and cause the disease. The exogenous yeasts are commensal of skin, they can invade the organism *via percutaneum*, such as parenteral nutrition or intravascular catheter contamined especially by the hands of health care workers. Many of these invasive mycoses are difficult to diagnose early, yet the patient's outcome depends, other than restoration of host defences, on fast and correct etiological diagnosis and so on early and proper antifungal treatment.

2. Paediatric patients at risk of fungal infection

At the beginning century, fungal infections were quite rare, but in the last years the paediatric and adult populations at risk of mycoses continue to expand so also the spectrum of opportunistic fungal pathogens continue to increase.

Even if both children and adults are exposed to these significant infections, important differences between the two populations are documented as predisposing factors and epidemiology.

The main paediatric patients at risk of invasive fungal infections are pre-term neonates, children with congenital immune deficiencies, with acquired immune deficiencies associated with HIV infection, cancer and patients in treatment with corticosteroids or who have chronic destructive lung diseases.

Generally, functional defects of phagocytes cells predispose to invasive infections by opportunistic fungi (*Candida* spp, *Aspergillus* spp, *Fusarium* spp), while alterations of T lymphocytes function are correlated to muco-cutaneous candidiasis and invasive infection by *Cryptococcus neoformans*. In addition, a large variety of non-immunological factors are

recognized as predisposing to fungal infection, such as the presence of intravascular catheter, *Candida* colonization, use of broad-spectrum antibiotics, parenteral nutrition and abdominal surgery (Table 2).

Predisposing conditions, patterns of infection, etiological agents and therapeutic treatment are different among diverse paediatric populations.

RISK FACTORS	
Immunological	Non-immunological
Functional defects of phagocytes cells	Intravascular catheter
Alterations of T lymphocytes function	*Candida* colonization
	Use of broad-spectrum antibiotics
	Parenteral nutrition
	Abdominal surgery

Table 2. Classification of some immunological and non-immunological risk factors for invasive fungal infections.

3. Neonate patients

At birth, neonates can be colonized by *Candida* spp at the level of muco-cutaneous surfaces, so also healthy babies can have fungal oral thrush and diaper rash. The thrush is a typical *Candida* oral infection by the mouths of babies. It is caused generally by *Candida albicans*, and more rarely by *Candida glabrata* or *Candida tropicalis*. The infection can develop suddenly and appears as white plaques on mucosa of oral cave (including palate, tongue). In general, candidal trush does not cause fever and the predisposing factors are antibiotics therapy, corticosteroids (especially if inhaled). This infection can be treated with topical antifungal drugs, such as nystatin, miconazole, Gentian violet or amphotericin B. Gentian violet is a dye with antifungal activity that can be used for breastfeeding thrush, but its use is not recommended in large quantities because it can cause mouth and throat ulcerations in nursing babies.

Yeast diaper rash is a reaction of the skin that can appear around anus, on the thighs, genital creases and the infected area is usually red and elevated. It is estimated that 15-20% of diaper rash are due to yeast and in particular to *C. albicans*.

On the contrary of health babies, in pre-term and in critically ill neonates *Candida* spp is often the cause of life-threatening invasive infections. Generally, the incidence of invasive candidiasis is higher in the paediatric population than adult and with the higher risk in neonates. In critically ill neonates, *Candida* spp is the third most common agent of late-onset infections, with an incidence ranging from 2.6-10% among very low birth weight (1001-1500 g) babies and from 5.5-20% among extremely low birth weight (<1000 g) infants (Benjamin et al., 2006; Chapman et al., 2007; Cotten et al., 2006; Levy et al., 2006). The crude mortality associated with these infections ranges from 15-30% and an attributable mortality of 6-22% despite appropriate therapy (Benjamin et al., 2006; Zautis et al.,2007) but these values can increase to 60% (Castagnola & Buratti, 2009). The high mortality rate can be related to the difficulty in making an early diagnosis. In fact, the reduced sensitivity of diagnostic tests, non-specific clinical signs and inadequate or delayed treatments can condition the outcome of patients (Brecht et al., 2009; Stronati & Decembrino, 2006).

Whereas in paediatric patients the risk factors may differ according to the underlying disease and the consequent specific immunodeficiency, in neonates the primary risk factors are prematurity and colonization. In particular *Candida* colonization, originating from the endogenous flora inhabiting the gastrointestinal tract, is considered a prerequisite for the development of invasive candidiasis. Manzoni et al. (Manzoni, et al., 2006) showed that *Candida* colonization in multiple body sites is an important predictor of progression to invasive mycoses, emphasizing the need of systematic surveillance cultures in the preterm infants. It is important to underline that the preterm infants are commonly infected by *C. albicans* and *C. parapsilosis*, the children aged younger than 1 year are infected most often by *C. parapsilosis*, while in the adolescents the incidence of *C. glabrata* exceeds that of *C. parapsilosis* (Sai et al, 2011). Besides, *C. albicans* and *C. parapsilosis* are by a long time recognized as causative agent of infections related to the intravascular catheters and drains that can be contaminated by the hands of health care workers (Velasco et al. 2011, Weems et al., 1986). The relatedness between the strains isolated from the patients and those isolated from the hands of health care workers, strongly suggests person to person transmission and supports the widely held view that the hands of personnel are the route of transmission of some *Candida* spp, in particular *C. parapsilosis*. Also *Malassetia* spp, a commensal lipophilic yeast that colonizes the human skin can cause systemic infection in premature babies. The transmission of infection is liable to contamination of vascular catheters by the hands of healthcare workers or contamination of intravenous solution (Chang et al., 2006; Oliveri et al., 2011).

Recently Montagna et al. (Montagna et al., 2010) evaluated the epidemiology of invasive fungal infections among infants admitted to Neonatal Intensive Care in southern Italy by the multicenter surveillance "Aurora Project". They observed that overall incidence was 1.3% and crude mortality was 23.8%. Infants weighing 1500 g (4.3%) showed a significantly higher incidence than those 2500 g (0.2%). *C. parapsilosis* (61.9%) was the most frequent isolated species. The outcomes for neonates differ markedly from those in older patients. Even if the mortality is generally lower in neonates, they frequently have serious complications as meningoencephalitis.

The neonatal patients rarely show the infections caused by filamentous fungi, such as *Aspegillus* and *Zygomycetes*, however they can cause skin infection after necrotizing skin lesions and gastrointestinal tract infection after mucositis induced by enterocolitis. The filamentous fungi infections are mainly correlated to contaminated water and ventilation systems (Abdul Salam et al., 2010; Robertson et al., 1997). The neonates admitted to intensive care units may be predisposed to aspergillosis because of their immature phagocytic capacity, the frequent administration of corticosteroids and prolonged hospitalization. In a study carried out by Groll et al. (Groll et al., 1998a) on the cases of aspergillosis documented in babies < 3 months of age, 32% had disseminated aspergillosis, 25% had primary cutaneus aspergillosis and 23% had invasive pulmonary aspergillosis. At least 41% of the patients had received corticosteroid therapy before diagnosis and only one patient had been neutropenic, while the prematurity was the major underlying condition (43%), only 14% had proven chronic granulomatous disease. Among different species of *Aspergillus* responsible for invasive aspergillosis, *A. fumigatus* was the species most frequently recovered in babies, followed by *A. flavus* , *A. terreus* and *A. niger*.

Regarding neonatal zygomycosis, poor data are available. Roileds et al. (Roileds et al., 2009) analyzed the documented cases in literature. The prematurity was a major underlying factor among neonatal cases, the most common manifestations of zygomycosis were gastrointestinal (54%) and cutaneous (36%) and the overall mortality was 64%.

3.1 Children patients

Although healthy children have strong natural immunity against fungal infections, some superficial mycoses such as *Tinea* frequently occur. *Tinea,* caused by dermatophyte fungi such as *Trichophyton, Epydermophyton, Microsporum,* is a skin infection that transmit by direct skin-to-skin contact with an infected person, or by contact with contaminated surface such as floors in shower and locker rooms (Andrews et al., 2008). The infection can involve the body in particular trunk, arms and leg, known as *Tinea corporis*; while *Tinea capitis* is a scalp infection, it is frequent among children of age 3–9 years that live in overcrowded areas; *Tinea pedis* is an infection of foot known as *the athletes' foot*, it is acquired by walking barefoot on contaminated surface (including carpet, floors in shower) (Jain et al., 2010).

In children and adolescents with cancer the most important risk factors for fungal infections are the intravenous catheter, the mucositis induced by chemotherapy, broad spectrum antibiotics, and the therapeutic use of corticosteroids, especially in patients with acute leukaemia. *Candida* spp and *Aspergillus* spp are the most common etiological agents. In the neutropenic children, in particular in children with leukaemia or bone marrow transplantation, the frequency of the invasive candidiasis (candidemia, disseminated candidiasis etc.) is 8-10% with a crude mortality until to 100% in patients with persistent neutropenia or after hematopoietic stem cell transplantation (HSCT) (Castagnola et al. 2008; Finco et al. 2011; Klingspor et al., 1997).

Even if *Candida albicans* is recognized as the most common agent of such infections, other species are progressively increasing, such as *Candida glabrata, Candida parapsilosis, Candida krusei* and *Candida tropicalis*. In many locations in USA, in Asia and Latin America, *C. glabrata*, after *C. albicans*, is the second most frequent species, while in Europe *C. parapsilosis* is the most prevalent. *Candida parapsilosis* is a commensal of human skin and often causes exogenous systemic infection related to i.v. catheter use (Barchiesi et al., 2004; Velasco et al. 2011). *Candida parapsilosis,* as well as *C. albicans*, can form a biofilm on the plastic surface of the catheter so it becomes a continuative source of infection. In this regard the Infectious Diseases Society of America (IDSA) guidelines recommend removing the catheter, if at all possible (Pappas et al., 2009). In general the catheter removal is associated with shorter duration of candidemia and reduced mortality in neonates, adults and neutropenic patients, although the management of intravascular catheters in these subjects with candidemia is more complicated than with others. As it is observed in adult patients, the therapy with corticosteroids is one of the most frequent features that increase the risk of fungal infection in children immunocompromised for solid organ transplantation, engraftment after bone marrow transplantation or for immunological disorders (Fonseca et al., 2006).

Other than *Candida* spp infections, in immunocompromised children *Aspergillus* spp infections are also documented. *Aspergillus* spp can cause invasive, saprophytic or allergic diseases. The saprophytic affliction includes *Aspergillus* spp otomycosis and pulmonary

aspergilloma; the allergic conditions include allergic sinusitis and allergic bronchopulmonary aspergillosis (APBA). APBA is a hypersensitivity disease of the lungs associated with inflammatory destruction of airways in response to *Aspergillus* spp. It can increase for clinical stages of asthma managed with corticosteroid for a long time. It can be defined through primary diagnostic criteria (episodic bronchial obstruction, asthma, peripheral eosinophilia, elevated serum IgE concentrations, central bronchiectasis) and secondary diagnostic criteria (repeated detection of *Aspergillus* spp in sputum samples, Arthus reaction -late skin reactivity- to *Aspergillus* antigen) (Walsh et al., 2008). ABPA also affects a significant proportion of cystic fibrosis patients because of prolonged colonization with *Aspergillus* spp, this fact has been associated with accelerated deterioration of lung function.

The invasive aspergillosis (IA) represents an important cause of morbidity and mortality in children with haematological malignancies or those undergoing bone marrow transplantation. In fact its frequency is estimated to be 4.5-10% with crude mortality of 40-94% (Steinback, 2005a). The Antimicrobial Availability Task Force (AATF) of the Infectious Diseases Society of America has identified *Aspergillus* spp among the particularly problematic pathogens for which an early treatment is urgently needed, together with other microorganisms, such as *Acinetobacter baumannii*, ESBL–producing *Enterobacteriaceae,* vancomycin-resistant *Enterococcus faecium* (VRE), *Pseudomonas aeruginosa,* and *methicillin resistant Staphylococcus aureus (*MRSA) (Talbot et al., 2006). IA is typical of neutropenic patients and it is usually absent in children in treatment for solid tumours. The most frequent species are *A. fumigatus*, *A. flavus* and *A. terreus.* The infection occurs by inhalation of conidia, of about 2-5 microns in size, small enough to be inhaled and deposited in the lungs of immunocompromised patients, in particular, of subjects exposed to prolonged and severe neutropenia. To date, it is not yet well defined what is the concentration of conidia that exposes the patient to risk of infection, especially for child patients. The concentration of spores in the air depends on several factors: weather, winds, seasonal factors, type of vegetation. So because of the ubiquitous spread of *Aspergillus* spp, environmental control measures, especially in the presence of construction works, are necessary to prevent infections in hospitals. In this regard, to avoid these fungal infections, some prevention measures are needed, such as:

- high efficiency filters (High-Efficiency Particulate Air: HEPA) (Cornet et al., 1999);
- horizontal laminar flow systems (Laminar Air Flow: LAF) (Barnes & Rogers, 1989), although the protection conferred by the LAF systems is still debated;
- hospital rooms with positive pressure (Humphreys, 2004).

In according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (De Paw et al, 2008) the diagnosis of invasive fungal infection can be classified as proven, probable and possible. It is considered *proven* when a histopathological documentation of infection and a positive result of culture of a specimen from a normally sterile site are available. It is defined *probable* when there are existing host factors, clinical manifestations (symptoms, signs and radiological features) and microbiological evidence; it is defined *possible* when there are only existing host factors and clinical manifestations. Early initiation of antifungal therapy in patients with strongly suspected invasive aspergillosis is required while a diagnostic evaluation is conducted (Cornely et al., 2007; Greene et al., 2007). With regard to aspergillosis, in addition to children

with haematological malignancy, children with primary immunodeficiency, chronic granulomatous disease and cystic fibrosis, are at high risk of IA (Steinbach, 2005a).

4. Laboratory diagnosis of fungal infection

One of the most difficult challenges of medical mycology is still the early initiation of an effective antifungal therapy to improve the outcome of patients, especially if affected by deep mycoses, but this fact is strongly related to a fast and proper etiological diagnosis.

Despite the scientific progress and newer available diagnostic tools, the fungal infection diagnosis is still complex. Actually, the microscopic and cultural investigations remain the gold standard of mycological diagnosis, even if it is difficult to obtain appropriate specimens, the cultures have long duration and often the results are negative. In fact, in neonates with candidemia, the blood cultures have difficulty in providing positive results because only a small quantity of blood can be taken. Besides, blood cultures result positive for *Candida* spp only in 24-60% of cases and the fungal strains grow slowly (Connell et al., 2007; Montagna et al., 2009), this fact is incompatible with an early diagnosis.

Besides the serological assays results are difficult to interpret because, for example, the circulating antibodies to *Candida* spp may occur in healthy subjects for the colonization of mucosal surfaces and their production in the immunocompromised patients can vary according to immune status (Ellepola & Morrison, 2005).

Although, in neonatal patients the detection of mannan antigen, it is an antigen of membrane of *Candida* spp can show a sensitivity and specificity of 94.4% and 94.2% respectively, repetitive sampling is required because of the transient nature of mannan antigen. Besides, the literature data report a very low sensitivity of the *Candida* antigen test in patients with *C. parapsilosis* infection (Oliveri et al., 2008; Montagna et al., 2011a). In recent years, other important serum markers have been studied, such as the 1→3-β-D-glucan (BDG), so it has been included among the relevant diagnostic criteria by the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (De Pauw et al., 2008). BDG is a component of the cell wall of a wide variety of fungi except for *zigomycetes* and *Cryptococcus* spp (Mancini et al., 2010; Odabasi et al., 2006). This test has been studied in adult patients, while in paediatric population only a few reports in particular describe the clinical relevance of BDG in preterm infants or onco-haematological paediatrics, yet this test seems to be very promising especially for the diagnosis of invasive candidiasis (Mularoni et al., 2010). However, it is important to consider that glucan is ubiquitous in the environment, so some medical sources of BDG can lead to a false positive result in the absence of deep mycoses: dialysis membranes and filters made from cellulose, cotton gauze employed during surgery, specific fractionated blood products, such as serum albumin and immunoglobulins, are reported to contain BDG, as well as exposure to some antitumor polysaccharides and certain parenteral antimicrobials (Chandrasekar, 2010; Marty et al., 2006). Montagna et al. (Montagna et al., 2011a) evaluated the performance of the BDG, individually and in comparison with the *Candida* mannan antigen, in preterm infants and onco-haematological paediatric patients with *Candida* BSI already proven by positive culture. The serum levels of BDG resulted positive on the same day as one positive blood culture in all examined patients, while *Candida* mannan antigen was negative in a child with *C. albicans* BSI and in all patients with *C. parapsilosis* fungemia.

Generally, in adults patients, the diagnosis of invasive pulmonary aspergillosis avails of radiology and galactomannan assay, on the contrary in the paediatric population the subjects with invasive pulmonary aspergillosis do not exhibit a "halo" sign and the galactomannan test specificity and sensitivity are very low. Galactomannan is a polysaccharide found within the cell wall of *Aspergillus* spp. It is released into the extracellular fluids (serum, urine, bronchoalveolar lavage, cerebrospinal fluid and other specimens) during hyphal growth and cell wall turnover. The detection of galactomannan in serum have been approved by the US Food and Drug Administration and have been included in the consensus criteria for diagnosis of invasive fungal infections (De Pauw et al., 2008). In a study, overall positive predictive value of galactomannan was calculated to be 92.1% for adult HSCT recipients and 15.4% for children (Herbrecht et al., 2002). The specificity of this test may be compromised during therapy with some antibiotics, such as piperacillin-tazobactam, amoxicillin or amoxicillin with clavulanic acid, because these drugs contain the galactomannan so lead to false positive results. Besides, often the neonates are colonized in the intestinal tract by *Bifidobacterium bifidum* that may interfere with the galactomannan test and load the false-positive test, as well as the food or water containing galactomannan (Mennink-Kersten et al., 2004).

The table 3 shows the advantage and disadvantage of major diagnostic methods.

METHODS	ADVANTAGES	DISADVANTAGES
Cultural investigations	Golden standard Accurate Specific	Fungi strains grow slowly Needs of invasive techniques to obtain appropriate specimen Results may be falsely negative
Galactomannan test	High specificity Non-invasive	Possible false-positives/ Possible false-negatives
1→3-β-D-glucan test	Non-invasive High negative-predictive value for most fungi	Possible false-positives
Candida mannan antigen	Non-invasive Sensitivity Specificity	Transient nature Very low sensitivity in C. parapsilosis infection Possible false-positives
Polymerase chain reaction	Identification genus /species High specificity	Not standardized Not commercially available

Table 3. Advantages and disadvantages of major diagnostic methods.

Another possible marker of invasive fungal infections is the Procalcitonin (PCT), a 116-amino acid protein synthesized in the C cells of the thyroid gland. It is virtually undetectable in healthy subjects (<0.5 ng/mL), but its concentration may increase up to 1000 ng/mL in patients with systemic bacterial infections or septic shock. Some authors have considered the PCT value as a potential biological marker of fungal sepsis (Christofilopoulou et al., 2002; Charles et al., 2009; Martini et al., 2010), but to date its role in diagnosis of these diseases has

not yet been elucidated. In particular, the newborns may exhibit elevated PCT levels because of a physiological increase in the first days of life perhaps caused by birth trauma or host response to the initial establishment of the normal intestinal bacterial flora, but 3–4 days after birth the PCT levels revert to normal (Montagna, et al. 2011b; Turner et al., 2006).

Several molecular methods have also been described for the diagnosis of opportunistic mycoses. However, they have not been standardized and have only been used in experimental cases, in the study of phylogenesis or for epidemiological purposes to investigate an epidemic cluster (Wise et al., 2007). The low application of this method in routine investigations can be explained with the very high sensibility of PCR methods (up to 10 fg of nucleic acid, equivalent to 1-10 cells), so it is difficult to distinguish the fungal colonization or contamination from the real infection.

5. Antifungal therapy

Whilst several antibiotics are available, the number of antifungal drugs is relatively low. The slow progress in discovering new drugs could be elucidated by the fact that fungi are eukaryotic cells like mammalian cells, so the antifungal agents that have as target the biosynthesis of protein, RNA or DNA, result in being toxic for humans, especially at the level of liver and kidney. Nevertheless, over recent decades the number of antifungal agents has increased and new molecules with new mechanisms of action have been discovered, such as Echinocandins that act on the cell wall of the fungi. Although, today we have several therapeutic options, the treatment of deep mycoses in the paediatric population is still limited (Steinbach, 2005b; Zautis, 2010).

To date four major categories of antifungal agents are available in clinical use: Polyenes, Azoles, Fluoro-pyrimidines and Echinocandins. Some of these molecules have existed for a long time; others have been introduced into clinical practice only recently (i.e. caspofungin , posaconazole or voriconazole) (Table 4).

The Polyenes are fungicidal drugs with a broad spectrum of activity against the most common yeasts and moulds, even if *Aspergillus terreus*, *Aspergillus versicolor*, *Aspergillus lentulus,* some strains of *Aspergillus flavus*, *Scedosporium* spp and *C. lusitaniae* result in being resistant. These drugs integrate with ergosterol of membranes and form the channels trans-membrane that, increasing the permeability, cause leakage of cytoplasmic contents and cell death. This class includes Nystatin and Amphotericin B (AmB).

Nystatin is used to treat cutaneous, vaginal, mucosal infections caused by *Candida* spp and it may be given orally as well as applied topically. In the UK its licence for treating neonatal oral thrush is restricted to those over the age of one month.

Amphotericin B is available other than in deoxycholate formulation (D-AmB), in three lipid formulations (LFAmBs) approved for use in humans: AmB lipid complex (ABLC), colloidal dispersion (ABCD), and in liposome (L-AmB). The lipid formulations are licensed for patients with invasive mycoses refractory or intolerant of D-AmB, in fact the infusion related reactions and nephrotoxicity often limit therapy with D-AmB. However, D-AmB at dosage of 1 mg/kg daily is recommended for neonates with disseminated candidiasis. If the urinary tract is not involved, L-AmB can be used at the dosage of 3–5 mg/kg daily. Even if not actually licensed, LFAmBs are frequently used as the first-line therapy for treatment of invasive aspergillosis, invasive candidiasis and zygomicosis (Cornely et al., 2007; Kuse et al., 2007).

CLASS AND COMPOUND	MECHANISM OF ACTION	CLINICAL USE
POLYENES	Integrate with ergosterol of membrane and form the channels trans-membrane causing leakage of cytoplasmic contents and cell death	
Amphotericin B		Invasive aspergillosis, invasive candidiasis and zygomycosis
Nystatin		Nongenital muco-cutaneous candidiasis, oro-pharyngeal candidiasis. In the UK the licence for treating neonatal oral thrush is restricted to those over the age of one month.
AZOLES	Interact with cytochrome P-450, during the biosynthesis of ergosterol at the step of synthesis by lanosterol to ergosterol, causing ergosterol depletion and accumulation of sterols in the membrane	
Econazole		inea, pityriasis versicolor
Ketoconazole		Superficial infections such as athlete's foot, ringworm, candidiasis
Fluconazole		Secondary treatment of invasive candidiasis; prophylaxis in neonates whose birth weight is <1000g.
Itraconazole		Invasive aspergillosis in patients who are refractory to standard therapy and in patients with ABPA
Miconazole		Superficial infections (athlete's foot, ringworm), oral or vaginal thrush, lip disorder angular cheilitis. It is used in treatment of neonatal oral thrush. It is an alternative to nystatin for babies of age under one month.

CLASS AND COMPOUND	MECHANISM OF ACTION	CLINICAL USE
Posaconazole		Recommended for prevention of invasive aspergillosis in neutropenic patients with haematological disease; active also against Zygomycetes
....Voriconazole		Primary treatment of invasive aspergillosis; prevention of invasive aspergillosis in neutropenic patients
FLUORO-PYRIMIDINES	Inhibits the biosynthesis of both RNA and DNA	
5-Flucytosine		Rarely administered as a single drug, usually given in combination with AmB for patients with invasive diseases, such as *Candida* endocarditis or meningitis.
ECHINOCANDINS	Inhibit beta 1, 3 D-glucan, block the formation of cell wall of fungi	
Anidulafungin		In neonatal candidiasis should be used with caution and limited to situations in which resistance or toxicity precludes the use of Fluconazole or AmB
Caspofungin		In neonatal candidiasis should be used with caution and limited to situations in which resistance or toxicity precludes the use of Fluconazole or AmB
Micafungina		Approved as first-line therapy against invasive and oropharyngeal candidiasis in the newborn and older children

Table 4. Mechanisms of action of some antifungal agents and their utility in clinical practice.

The Azoles have fungistatic activity and take effect against major yeasts and filamentous fungi. They inhibit the enzymes necessary to convert lanosterol to ergosterol, causing ergosterol depletion and accumulation of sterols in the membrane. The disruption of the structure and many functions of membrane lead to inhibition of fungal growth.

This class includes a major number of molecules. Some drugs are used for the treatment of superficial and cutaneous infections such as Miconazole that is mainly used externally for the treatment of superficial infections (athlete's foot, ringworm) and for treatment of oral or

vaginal thrush, for the lip disorder angular cheilitis. It is used in treatment of neonatal oral thrush and it can be an alternative to nystatin for babies of age under one month.

Econazole is used to treat skin tinea, pityriasis versicolor; ketoconazole is usually prescribed for topical infections such as athlete's foot, ringworm, candidiasis.

The azoles principally used in invasive mycoses are Fluconazole, Itraconazole, Posaconazole and Voriconazole.

Fluconazole is rapidly cleared in children, so the daily dose is doubled from 6 to 12 mg/kg for children of all age and neonates. Fluconazole can be used for Neonatal Candidiasis as an alternative to treatment with AmB, besides prophylaxis with Fluconazole may be considered in neonates whose birth weight is <1000 g (Manzoni et al., 2011).

Itraconazole is used for treatment of invasive aspergillosis in patients who are refractory to standard therapy. In combination with corticosteroids, it is recommended for the treatment of allergic bronchopulmonary aspergillosis while there are few data that examine the use of Itraconazole in the treatment of invasive candidiasis.

Posaconazole is recommended for prevention of invasive aspergillosis in neutropenic patients with haematological disease and is active also against zygomycetes. Posaconazole is not indicated for paediatric age.

Voriconazole is approved by FDA for the primary treatment of invasive aspergillosis, and has also been proven effective against *Fusarium* spp and *Scedosporium apiospermum*, it is the first and only drug ever specifically indicated for their treatment by the FDA. Voriconazole is licensed for children over 2 years old.

The class of Fluoro-pyrimidines only represents the 5-Fluoro cytosine, a molecule that inhibits the biosynthesis of both RNA and DNA. Very low birth weight neonates may accumulate high plasma concentrations because of poor renal function due to immaturity, thus, the use of flucytosine needs careful monitoring of serum drug levels in this group of patients. The role of flucytosine in neonates with *Candida* meningitis is questionable and is not routinely recommended (Benjamin et al., 2006). Besides, rapidly the strains can develop resistance to flucytosine, so it is rarely administered as a single agent, but usually in combination with AmB for patients with invasive diseases.

The Echinocandins are the newer antifungal drugs. 1,3 beta–D-glucan and block the formation of the cell wall of the fungi, but some strains such as *Candida guilliermondii* and *Candida parapsilosis, Zygomycetes, Cryptococcus, Trichosporon, Fusarium* and *Scedosporium* are intrinsically less sensitive or even resistant. This class includes Anidulafungin, Caspofungin and Micafungin, they are available only as parenteral preparations. Echinocandins should be used with caution in treatment of neonatal candidiasis and generally limited to situations in which resistance or toxicity precludes the use of Fluconazole or AmB. Micafungin is the only drug that has been authorized for by the EMA (European Medicines Agency) (Manzoni et al, 2011) and approved as first-line therapy against invasive and oropharyngeal candidiasis in the newborn and older children, it has recently been introduced into clinical onco-haematology Practice (Mikulska & Viscoli, 2011).

To date the resistance to the antifungal agents most commonly used in clinical practice is relatively rare, nevertheless the frequent use of antifungal drugs as prophylactic in long-

term treatments or empiric and pre-emptive therapy, might select resistant strains. In general the opportunistic fungi have variable susceptibilities to currently available antifungal drugs. For example *Candida krusei* is intrinsically resistant to Fluconazole, *Candida glabrata* is less susceptible than other *Candida* species, *Candida lusitaniae* and *Aspergillus terreus* are resistant to AmB, thus the need for prompt identification at level of genera and species and *in vitro* susceptibility testing is more and more pressing.

The Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2008a, Clinical and Laboratory Standards Institute, 2008b) has developed a reference microdilution broth method for antifungal susceptibility testing of yeasts and moulds, but it is not easy for routinely use in the clinical laboratory. Alternatively, more simplified and efficient approaches also have been developed such as YeastOne Colorimetric Antifungal plate and E-test. At regarding, it is important to underline that antifungal susceptibility testing have some limitations as an imperfect clinical correlation, so the drug susceptibility *in vitro* does not guarantee clinical success or failure.

In every case, the proper antifungal treatment and the patient's outcome depend on an accurate identification of the aetiological agent at the level of genera and species. In fact, although in recent years some fungal strains have become resistant to common drugs, generally the susceptibility of fungi to the currently available antifungal agents is predictable if the species of the infecting isolate is known (Pappas et al., 2009). However, to know the susceptibility *in vitro* and the local epidemiology of drug resistance can help the clinicians to choose the most appropriate therapy and therefore to guide the management of fungal infection.

6. Conclusion

Despite recent advances in diagnostic field and the current armamentarium of antifungal agents with widespread use of prophylaxis in high-risk groups, the mycoses (especially the deep mycoses) are yet a persistent public health problem of difficult management particularly in pediatric age. In these patients the epidemiology of fungal infections is complex and different in various ages, for example: in the children the risk factors may differ according to the specific pathology of the base and the consequent specific immunodeficiency while in neonates the primary risk factors are prematurity and colonization; some drugs are licensed only for children of age > 2 years etc.

To date these fungal infections have been well studied in different populations of adult patients while a thorough analyses of predisposing factors, diagnostic methods and therapeutic options in pediatric age have not been well described. Infact, several studies have been carried out to know the fungal infections and improve the patients' outcome, but most of them have exclude the pediatirc population. This fact has limited the knowledge of the epidemiology and management of pediatric fungal disease, so we often extrapolate from adult data, information for children and neonates. Therefore, diagnostic tests standardized for adult patients or antifungal therapies licensed for adults are often used improperly in pediatric age .The future hope of medical science is to have new tools to improve the immune system of immunocompromised patients to control the opportunistic infections. Besides, the most difficult challenge of medical mycology is still the early initiation of an effective antifungal therapy to improve the patient's outcome, so it is essential to have a fast and proper aetiological diagnosis. In addition, because studies among the paediatric populations affected

by fungal infections are few, it would be important in the future that clinical and laboratory investigations are focused in tandem on fungal infections in pediatric age to have an optimal diagnoses and appropriate antifungal treatment.

7. References

Abdul Salam, Z.H.; Karlin, R.B.; Ling, M.L. et al. (2010) The impact of portable high-efficiency particulate air filters on the incidence of invasive aspergillosis in a large acute tertiary-care hospital. *Am J Infect Control,* 38(4): 1-7

Andrews MD, Burns M. (2008). Common tinea infections in children. Am Fam Physician. 77(10):1415-1420.

Benjamin, D.K. Jr; Stoll, B.J.; Fanaroff, A.A., et al. (2006). Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and developmental outcomes at 18 to 22 months. *Pediatrics*, 117: 84-92.

Barchiesi, F.; Caggiano, G.; Falconi Di Francesco, L., et al. (2004) Outbreak of fungemia due to Candida parapsilosis in a pediatric oncology unit. *Diagn Microbiol Infect Dis,* 49(4):269-271.

Barnes, R.A.; Rogers, T.R. (1989) Control of an outbreak of nosocomial aspergillosis by laminar air-flow isolation. *J Hosp Infect*, 14: 89-94

Brecht, M.; Clerihew, L.; McGuire, W. (2009). Prevention and treatment of invasive fungal infection in very low birth weight infants. *Arch Dis Child Fetal Neonatal Ed*, 94: 65-69.

Castagnola, E.; Buratti, S. (2009). Clinical aspects of invasive candidiasis in paediatric patients. *Drugs*, 69(1):45-50.

Castagnola, E.; Faraci, M.; Moroni C et al. (2008). Invasive mycoses in children receiving hemopoietic SCT. Bone Marrow Transplant, 41 Suppl 2:107-111.

Castagnola E, Franceschi A, Natalizia AR, et al. (2007). Combined antifungal therapy for persistent central venous catheter-related candidemia in extremely low birth weight neonates. J Chemother 21:234-235.

Chandrasekar, P. (2010). Diagnostic challenges and recent advances in the early management of invasive fungal infection. *Eur. J. Haemat,* 84, 281-290.

Chang, H.J.; Miller, H.L.; Watkins, N.; et al. (1998). An epidemic of Malassezia pachydermatis in an intensive care nursery associated with colonization of health care workers' pet dogs. *N Engl J Med*, 338:706-711

Chapman, R.L. (2007). Prevention and treatment of Candida infections in neonates. *Semin Perinatol*, 31:39-46.

Charles, P.E.; Castro, C.; Ruiz-Santana, S.; et al. (2009). Serum procalcitonin levels in critically ill patients colonized with Candida spp: new clues for the early recognition of invasive candidiasis? Intensive CareMed, 35: 2146-2150.

Chiou, C.C.; Wong, T.T.; Lin, H.H.; et al. (1994). Fungal infection of ventriculoperitoneal shunts in children. *Clin Infect Dis,* 19: 1049-1053

Christofilopoulou, S.; Charvalos, E.; Petrikkos, G. (2002). Could procalcitonin be a predictive biological marker in systemic fungal infections? Study of 14 cases. Eur J Intern Med, 13(8): 493-495.

Clinical and Laboratory Standards Institute. (2008). Reference method for broth dilution antifungal susceptibility testing of yeast. Approved standard M27-A3, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Clinical and Laboratory Standard Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard—2nd edn. CLSI Document M38-A2. Wayne, PA. Clinical and Laboratory Standard Institute, 2008

Connell, T.G.; Rele, M.; Cowley, D.; et al. (2007). How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Paediatrics*, 119 (5): 891-896.

Cornely, O.A.; Maertens, J.; Bresnik, M.; et al. &; AmBiLoad Trial Study Group. (2007) Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis*, 44: 1289–1297.

Cotton, C.M.; McDonald, S.; Stoll, B.; et al. (2006). National Institute for Child Health and Human Development Neonatal Research Network. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. *Pediatrics*, 118: 717-722.

Cornet, M.; Levy, V; Fleury, L.; et al. (1999). Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against Aspergillus airborne contamination during hospital renovation. *Infect Control Hosp Epidemiol*, 20: 508-513

De Pauw, B.; Walsh, T.J.; Donnelly, J.P.; et al. (2008). Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin. Infect. Dis.*, 46, 1813–1821.

Ellepola, A.N.; Morrison, C.J. (2005). Laboratory diagnosis of invasive candidiasis. *J. Microbiol*., 43, 65-84.

Finco G, Sanna D, Evangelista M, Mura P, Musu M. (2011) Fungal infections in pediatric intensive care units. J Matern Fetal Neonatal Med. 24 Suppl 2:21-3.

Fonseca-Aten, M.; Michaels, M.G. (2006). Infections in pediatric solid organ transplant recipients. *Semin Pediatr Surg,* 15:153-161

Groll, A. H.; Jaeger, G.; Allendorf, A.; et al. (1998). Invasive Pulmonary Aspergillosis in a Critically Ill Neonate: Case Report and Review of Invasive Aspergillosis During the First 3 Months of Life. *Clin. Infect. Dis.,* 27:437–452

Greene, R.E.; Schlamm, H.T.; Oestmann, J.W. et al. (2007) Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clin Infect Dis*, 44:373–9.

Humphreys, H. (2004). Positive pressure isolation and the prevention of invasive asprgillosis. What is the evidence? *J Hosp Infect,* 56: 93-100

Herbrecht, R.; Letscher-Bru, V.; Oprea, C. et al. (2002). *Aspergillus* galattomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol,* 20: 1898-1906

Jain A, Jain S, and Rawat S. (2010) Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention .J Pharm Bioallied Sci. 2(4): 314–320.

Kaufman, D.; Fairchild, K.D. (2004). Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. *Clin Microbiol Rev*, 17:638-680.

Kern, M.E.; Blevins K.S. (1997). *Medical Mycology*: *A self-Instructional Text*. 1th edn. Philadelphia, PA: FA Davis Co;

Klingspor, L.; Stintzing, G.; Tollemar, J. (1997). Deep Candida infection in children with leukemia: clinical presentations, diagnosis and outcome. *Acta Paediatr,* 86:30-36.

Kuse, E.R.; Chetchotisakd, P.; da Cunha, C.A.; et al. & Micafungin Invasive Candidiasis Working Group (2007). *Lancet,* 369(9572):1519-1527.

Lehrnbecher, T.; Frank, C.; Engels, K.; et al. (2010). Trends in the postmortem epidemiology of invasive infections at a university hospital. *J. Infect*., 61(3), 259-265

Levy, I.; Shalit, I.; Askenazi, S. et al. (2006). Duration and outcome of persistent candidaemia in newborn infants. *Mycoses*, 49:197-201.

Malani, P.N.; Brandley, S.F.; Little, R.S.; et al. (2001).Trends in species causing fungaemia in a tertiary care medical centre over 12 years. *Mycoses,* 44: 446-449

Mancini, N.; Carletti, S.; Ghidoli, N.; et al. (2010). The era of molecular and other non-culture-based methods in diagnosis of sepsis. *Clin. Microbiol. Rev*., 23: 235-251.

Manzoni, P.; Farina, D.; Leonessa, M., et al. (2006) Risk factors for progression to invasive fungal infection in preterm neonates with fungal colonization. *Pediatrics*; 118: 2359-2364

Manzoni P, Benjamin DK, Hope W, Rizzollo S, Del Sordo P, Stronati M, Jacqz-Aigrain E, Castagnola E, Farina D. (2011). The management of Candida infections in preterm neonates and the role of micafungin. J Matern Fetal Neonatal Med.; 24 Suppl 2:24-27

Marty, F.M.; Lowry, C.M.; Lempitski, S.J.; et al. (2006). Reactivity of (1,3)-beta-d-glucan assay with commonly used intravenous antimicrobials. *Antimicrob. Agents Chemother*., 50: 3450-3453.

Martini, A.; Gottin, L.; Menestrina N. (2010). Procalcitonin levels in surgical patients at risk of candidemia. *J Infect*., 60(6): 425-430.

Mennink-Kersten, M.; Klont, R.R.; Warris, A. et al. (2004). *Bifidobacterium lipoteichoic* acid and false ELISA reactivity in *Aspergillus* antigen detection. *Lancet,* 363: 325-327.

Mikulska M, Viscoli C. (2011). Current role of echinocandins in the management of invasive aspergillosis. *Curr Infect Dis Rep*, 13(6):517-527

Montagna, M.T.; Caggiano, G.; Borghi, E.; et al. (2009). The Role of the Laboratory in the Diagnosis of Invasive Candidiasis. *Drugs,* 69 (1): 59-63

Montagna, M.T.; Lovero G.; De Giglio O.; et al. and "Aurora Project Group" (2010). Invasive fungal infections in Neonatal Intensive Care Units of Southern Italy: a multicentre regional active surveillance (AURORA Project). *J Prev Med Hyg.* 51: 125-130

Montagna, M.T.; Coretti, C.; Lovero, G. et al (2011) Diagnostic Performance of 1,3-beta-D-Glucan in Neonatal and Paediatric Patients with Candidemia. *Int. J. Mol. Sci.,* 12 (9): 5871-5878

Montagna, M.T.; Coretti, C.; Caggiano, G. (2011) Procalcitonin: a possible marker of invasive fungal infection in high risk patients? *J Prev Med Hyg*. 52(1): 38-39.

Mularoni, A.; Furfaro, E.; Faraci, M.; et al. (2010). High Levels of beta-D-glucan in immunocompromised children with proven invasive fungal disease. *Clin. Vaccine Immunol*., 17: 882-883.

Oliveri, S.; Trovato, L.; Betta, P.; et al. (2008). Experience with the Platelia Candida ELISA for the diagnosis of invasive candidosis in neonatal patients. *Clin. Microbiol. Infect,*14: 391-393

Oliveri, S., Trovato, L., Betta, P., et al. (2011). Malassezia furfur fungaemia in a neonatal patient detected by lysis-centrifugation blood culture method: first case reported in Italy. *Mycoses*, 54, 638-640

Odabasi, Z.; Paetznick, V.L.; Rodriguez, J.R.; Chen, E.; et al. (2006). Differences in beta-glucan levels in culture supernatants of a variety of fungi. *Med. Mycol.,* 44, 267-272.

Robertson, A.F.; Joshi, V.V.; Ellison, D. et al. (1997). Zygomycoses in neonates. *Pediatr Infect Dis J*., 21 16: 812-815

Pappas, P.G.; Kauffman, C.A.; Andes D.; et al. (2009). Clinical Practice Guidelines for the Management of Candidiasis: 2009. Update by the Infectious Diseases Society of America. *Clin Infect Dis,* 48: 503-535

Roilides, E.; Zaoutis, T.E.; Walsh, T.J. (2009). Invasive zygomycosis in neonates and children. *Clin Microbiol Infect.*, 15 (5):50-54.

Sai, S.; Holland, L.M.; McGee, C.F., et al. (2011). Evolution of mating within the *Candida parapsilosis* species group. *Eukaryot Cell*. 10(4):578-587.

Steinbach, W.J. (2005). Pediatric aspergillosis: disease and treatment differences in children. *Pediatr. Infect. Dis. J*., 24(4), 358-364.

Steinbach, W.J. (2005). Antifungal agents in children. *Pediatr Clin North Am*, 52: 895–915

Stronati, M.; Decembrino, L. (2006). Neonatal invasive candidiasis. *Minerva Pediatr*, 58: 537-549.

Talbot, G.H.; Bradley, J., Edwards, J.E. Jr, et al. (2006). Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis*., 42(5): 657-668.

Turner, D.; Hammerman, C.; Rudensky, B.; et al. (2006). The role of procalcitonin as a predictor of nosocomial sepsis in preterm infants. *Acta Paediatr.,* 95(12): 1571-1576.

Velasco, E., Portugal, R.D. (2011). Factors prompting early central venous catheter removal from cancer patients with candidaemia. *Scand J Infect Dis*. 43(1): 27-31.

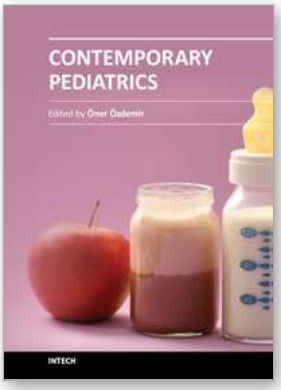
Weems, J.J. Jr; Chamberland, M.E.; Waerd, J.; et al. (1987). *Candida parapsilosis* fungemia associated with parenteral nutrition and contaminated blood pressure transducers. *J Clin Microbiol,* 25: 1029-1032.

Wise, M.G.; Healy, M.; Reece, K.; et al. (2007). Species identification and strain differentiation of clinical Candida isolates using the Diversi Lab system of automated repetitive sequence based PCR. *J Med Microbiol*, 56: 778-787.

Walsh, T.J.; Anaissie, J.E.; Denning, D.W., et al. Guidelines of the Infectious Diseases Society of America . *Clin Infect Dis*, 46, 327-370.

Zaoutis T. Candidemia in children. (2010) *Curr Med Res Opin*. 26(7):1761-8.

Zaoutis, T.E; Walsh, T.J. (2007). Antifungal therapy for neonatal candidiasis. *Curr Opin. Infect. Dis*. 20 (6), 592-597.



Contemporary Pediatrics
Edited by Dr. Öner Özdemir

ISBN 978-953-51-0154-3
Hard cover, 434 pages
Publisher InTech
Published online 21, March, 2012
Published in print edition March, 2012

Book Contemporary Pediatrics with its 17 chapters will help get us and patients enlightened with the new developments on the contemporary pediatric issues. In this book volume, beyond classical themes, a different approach was made to current pediatric issues and topics. This volume, as understood from its title, describes nutritional infant health and some interesting topics from pediatric subspecialties such as cardiology, hemato-oncology and infectious diseases.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821