STUDY ON MULTI-DRUG RESISTANT STRAINS OF CANDIDA SPECIES FROM COMMUNITY ACQUIRED INFECTIONS

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

Master of Philosophy in Biotechnology

By ANIS AHMAD

Under the Supervision of DR. ASAD ULLAH KHAN (Supervisor)

INTERDISCIPLINARY BIOTECHNOLOGY UNIT ALIGARH MUSLIM UNIVERSITY ALIGARH (INDIA) 2007
STUDY ON MULTI-DRUG RESISTANT STRAINS OF CANDIDA SPECIES FROM COMMUNITY ACQUIRED INFECTIONS

Date: ________________________________

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ALIGARH (INDIA)
2007
DECLARATION

I hereby declare that the work embodied in the dissertation entitled “STUDY ON MULTI-DRUG RESISTANT STRAINS OF CANDIDA SPECIES FROM COMMUNITY ACQUIRED INFECTIONS” had been carried out by me.

Anis Ahmad
I certify that the work embodied in the dissertation entitled "Study on Multi-drug Resistant Strains of Candida Species from Community Acquired Infections" is an original work, unless otherwise stated, carried out by Anis Ahmad under my supervision and is suitable for submission in partial fulfillment for the award of M.Phil degree in Biotechnology.

Dr. Asad Ullah Khan
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Recent reports suggest that candidemia caused by antifungal resistant strains is increasing in certain women and neonates populations. We evaluated the annual incidence of neonatal candidemia and the frequency of disease caused by different species of Candida among neonates in NICU (Neonatal intensive care unit) and gynecology OPD, J.N.M.C and FSHRC, Aligarh.

The present study was conducted for a period of 1 year and 4 months from Nov. 2005 to March 2007 on samples isolated from Jawaharlal Nehru Medical College (JNMC) and Hospital, AMU and Feroz Specialist Hospital & Research Center (FSHRC), Aligarh. A total of 1470 vaginal yeast cultures were collected from pregnant ladies in the labor room and from Gynecology OPD of JNMC and FSHRC. Out of 1470 samples 600 samples were showing signs and symptoms of the disease. Of these 600 samples, only 175 samples were positive for Candida. From these 175 positive samples 200 Candida isolates were obtained.

A total of 1825 samples were collected from NICU, JNMC, AMU Aligarh. Out of 1825 samples 500 samples were showing signs and symptoms of the disease. Out of these 500 samples only 138 samples were positive for Candida. From these 138 positive samples 150 Candida isolates were obtained. These isolates were subjected to undergo morphological and biochemical identification and characterization was further done. Clinical data such as age, sex, site of infection predisposing factors, and history of exposure to antifungal and clinical out come of the patients are noted and shown in tables.

Morphological test done by Lacto phenol Cotton Blue (LPCB), KOH Preparation, Gram Staining, Corn Meal Agar (CMA), Germ Tube Test (GTT) and CHROM Candida Agar. Carbohydrate fermentation and carbohydrate assimilation test were used for biochemical characterization of the isolates. Antifungal susceptibility testing by Disc Diffusion method.

In women C. albicans was 64.5%, C. glabrata 19%, C. parapsilosis 7%, C. tropicalis 5%, C. krusie 3% and C. kyfer 1.5%. While in neonates, C. albicans 52.67%, C. tropicalis 18%, C. parapsilosis 12.67%, C. glabrata 10%, C. krusie 4%,
and C. guilliermondii 2.67%. In gynecology unit C. glabrata was 2nd most common species While in neonates 2nd most species was C. tropicalis. Blood is the most prevalent site of infection in neonates while in women vagina was the most prevalent site of Candida infection. In women least antifungal resistance was observed in Amphotericin-B and Clotrimazole (5.70% and 10.67% respectively).

In patients attending gynecology OPD it is observed that the C. albicans is the most common pathogenic fungi followed by C. glabrata. Hormone replacement therapy and use of antibiotics and contraceptive are major risk factors for Candida infections in women. VLBWC (very low birth weight) neonates or SGA (small for gestational age) are higher risk of Candida as compared to LGAC (large for gestational age) neonates. Preterms are having more Candida infection as compared to terms neonates due to their low immunity and often are having problems in gaining weight.

Our study indicates that transmission of Candida from mother to neonates is not the major cause of Candida infection in neonates but non-perinatal nosocomial transmission of Candida species is the predominant mode of acquisition by neonates in NICU, at AMU. Mother may be colonized with multiple strains of Candida simultaneously, colonizing Candida strains can cause invasive disease in neonates; and molecular biology based techniques are necessary to determine epidemiologic relatedness of maternal and infant Candida isolates and to facilitate mode of transmission.
Acknowledgement
ACKNOWLEDGEMENTS

I bow in reverence to the almighty god whose benign benediction gave me the required zeal for the completion of this work.

I wish to express my sincere gratitude to my revered and learned supervision, Dr. Asad Ullah Khan, for his illuminating, scholarly guidance and creative supervision right from its inception to its culmination in the present work. He has always been inexhaustible source of inspiration and guidance to me. Without his unceasing encouragement and cooperation this work would not have been completed.

I am extremely, grateful to Prof. M. Saleemuddin, co-ordinator, Interdisciplinary Biotechnology Unit, for providing all the necessary facilities and help. I also express my thanks to other faculty members Dr. Rizwan Hasan Khan, Dr. M. Owais and Dr. Hina Yonus for their help and cooperation.

I would like to thanks Prof. Masood Ahmad, Deptt. of Biochemistry, AMU, Aligarh for encouraging me time to time.

I would like to express my sincere gratitude Dr. Zaki Arshad, Dr. Nasreen (FSHRC), Dr. Munazir Khan (NICU, JNMC, AMU) for providing me samples.

I am obliged to Dr. S.S. Guarav, Head Department of Biotechnology, CCS University, Meerut for helping me in sample collection.

I am getting short of words to express my gratitude for Mr. Akram Wali and Ms. Barira Isam who were a hand stretch away when ever I needed them.

I would like to thanks Mr. Shahper N. Khan for his selfless help rendered as and when required.

I sincerely acknowledge the help and cooperation which I received from all my colleagues Mr. Saeeduzaffar, Mr. Shazi, Ms. Rosina, Mr. Mairaj.
Mr. Farz, Mr. Ejaz, Mr. Ejaj, Ms. Ankita, Mr. Javed, Ms. Nishat, Mr. Azmat and Ms. Sana, Ms. Munazza, Mr. Qamar Zia.

I am obliged to my seniors Mr. Afif, Mr. Arif, Mr. Varun, Mr. Maroof, Ms. Rubab, Ms. Abgeena, Mr. Basir, Ms. Sadaf, Mr. Priyankar, Ms. Hafeez, Ms. Sahar, A.I. Malik, Ms. Farah, Dr. Rukhshana.

I am extremely grateful to Dr. Masood Alam Khan, Dr. Faisal providing me proper guidance from time to time.

I would like to thank Mrs. Umri-e-Nishan for providing me all facilities during sine die.

Thanks are also due to members to distribution information sub-centre Mr. S. Faisal Maqbool, Mr. Aqtedar Husain and Ms. Parveen Salahuddin.

I also extended my thanks to non-teaching staff Mr. Amir, Mr. Lal Mohammad, Mr. Ramesh, Mr. Isham, Mr. Nasir, Mr. Mashkoor, Mr. Karam Veer and Mr. Rajender.

Last but not the least my deep sense of gratitude to my family standing beside life when iron wall always.

ANIS AHMAD
Dedicated
To
My family & Mentors
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Chapter-1
Introduction
Fungi are eukaryotic organisms that reproduce both in sexual and asexual fusion. They have also been called molds, yeast and mushrooms (Nader-Djalal et al. 1998; Odds 1988). In 1954 Candida was officially accepted as the genus name for Monilis albicans (Winner et al 1964). Candida is yeast and the most common cause of opportunistic mycoses worldwide. As well as being a pathogen and a colonizer, it is found in the environment, particularly on leaves, flowers, water, and soil. While most of the Candida sp. is mitosporic, some have known teleomorphic state and produce sexual sporesycoses worldwide. (Bodey 1966). The most common organism implicated in fungal infections is the ubiquitous Candida, which is found in the human digestive tract, mouth, and genital region (Eggimann et al 2003). Under normal circumstances, levels of Candida are controlled by beneficial bacteria. However, if the bacteria-fungus balance is upset, by the use of antibiotics for example, or if the immune system is compromised, an overgrowth of Candida can occur, resulting in infection (Braunwald 2001). Candida is a genus of yeasts. Clinically, the most significant member of the genus is Candida albicans, which can cause numerous infections (called candidiasis or thrush) in humans and other animals, especially in immunocompromised patients. Various Candida species are members of gut flora in animals, including C. albicans (Ryan et al 2004). Grown in the laboratory, Candida appears as large, round, white or cream (albicans is from Latin meaning 'whitish') colonies on agar plates. (www.DoctorFungus.org).

Last decade has seen the sustained medical importance of opportunistic infections due to different Candida species mainly due to the worldwide increase in the number of immunocompromised patients, who are highly susceptible to opportunistic infections (Enfert et al 2007). Meanwhile, the genome sequence of several Candida species has been completed, enabling the detailed investigation of some aspects of their biology with the aid of post-genomic approaches. The basic knowledge gained from these investigations of pathogenic Candida and related yeasts, can translate into innovations in the development of novel antifungal therapies, original approaches for targeted immuno-interventions, or highly sensitive diagnosis of fungal infections (Enfert et al 2007).
Fungal overgrowth is encouraged by certain pH levels and the availability of sugar (glucose) (McGinnis et al 1996; Buddington et al 1996; Howard et al 1995). Candida are also responsible for a number of life-threatening opportunistic infections in AIDS patients and other immuno-compromised people - including patients treated in intensive care units (ICUs), cancer patients receiving chemotherapy, and organ transplant patients (Enfert et al 2007).

People with the right conditions for fungal infection, such as a high sugar diet, are at higher risk. Also, Candida infections can be spread to vulnerable people with depressed immune systems who are in the hospital, where the fungus is commonly found on the hands of caregivers and where indwelling catheters can allow an infection to take hold (McGinnis et al 1996).

**Pathogenicity and clinical significance**

Infections caused by Candida spp. are in general referred to as Candidiasis. The clinical spectrum of candidiasis is extremely diverse. Almost any organ or system in the body can be affected. Candidiasis may be superficial and local or deep-seated and disseminated. Disseminated infections arise from hematogenous spread from the primarily infected locus. (Bielsa et al 1987; Bodey et al 1992; Bodey 1966). The colonies of Candida spp. are cream colored to yellowish, grow rapidly and mature in 3 days. The texture of the colony may be pasty, smooth, glistening or dry, wrinkled and dull, depending on the species (Larone 1995).

The microscopic features of Candida sp. also show species-related variations. All species produce blastoconidia singly or in small clusters. Blastoconidia may be round or elongate. Most species produce pseudohyphae which may be long, branched or curved. True hyphae and chlamydospores are produced by strains of some Candida sp. (Abi-Said 1997).

Although they are the members of the same genus, the various species do have some degree of unique behavior with respect to their colony texture, microscopic morphology on Cornmeal Tween 80 Agar at 25°C (Dalmau method) and fermentation or assimilation profiles in biochemical tests (Larone 1995).
Histopathologic features

*Candida* sp. should be differentiated from other clinically encountered yeasts, such as *Blastoschizomyces*, *Cryptococcus*, *Geotrichum*, *Malassezia*, *Rhodotorula*, *Saccharomyces*, and *Trichosporon*. Morphology on Cornmeal Tween 80 Agar, capsule production, urease activity, ability to grow in presence of cycloheximide, growth pattern in Sabouraud broth, and fermentation assimilation profiles help in differentiation of *Candida* from other yeasts. Well-developed pseudohyphae and one-celled blastoconidia characterize the common species of *Candida*. *Candida* differs from *Cryptococcus* by having well-developed pseudohyphae. The lack of arthroconidia is the major microscopic feature which differentiates *Candida* from *Trichosporon* and *Geotrichum*, the two genera that produce abundant arthroconidia (Larone 1995).

*Candida* species and features

The genus *Candida* includes around 154 species. Among these, six are most frequently found in human infections. Among these *Candida albicans* is the most abundant and significant species, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, and *Candida lusitaniae* are also isolated as causative agents of *Candidiasis*. Importantly, there has been a recent increase in infections due to non-albicans *Candida* sp., such as *Candida glabrata* and *Candida krusei* (Abi-Said 1997; Aisner et al 1976; Arié et al 1996). Patients receiving fluconazole prophylaxis are particularly at risk of developing infections due to fluconazole-resistant *Candida krusei* and *Candida glabrata* strains (Barchiesi et al 1993).

Nevertheless, the diversity of *Candida* sp. that are encountered in infections is expanding and the emergence of other species that were rarely in play in the past but now a days their infections are increasing (Baily et al 1997; Blinkhorn et al 1989; Bonomo et al 1996; Borg-von Zepelin et al 1993).
**Table. 1 Species commonly causing invasive Candidiasis**

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>50%</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>15-30%</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>15-30%</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>15-30%</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>~1%</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>~1%</td>
</tr>
</tbody>
</table>

**Candida albicans**

*Candida albicans* is a diploid asexual fungus (a form of yeast), and a causal agent of opportunistic oral and vaginal infections in humans (Ryan et al 2004; Enfert et al 2007). Systemic fungal infections (fungemias) have emerged as important causes of morbidity and mortality in immunocompromised patients (*e.g.*, AIDS, cancer chemotherapy, organ or bone marrow transplantation). In addition, hospital-related infections in patients not previously considered at risk (*e.g.* patients on an intensive care unit) have become a cause of major health concern.

*C. albicans* is among the gut flora, the many organisms that live in the human mouth and gastrointestinal tract. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis. Candidiasis is often observed in immunocompromised individuals such as HIV-positive patients. Candidiasis also may occur in the blood and in the genital tract. Candidiasis, also known as "thrush", is a common condition that is usually easily cured in people who are not immunocompromised. To infect host tissue, the usual unicellular yeast-like form of *Candida albicans* reacts to environment and switches into an invasive, multicellular filamentous form (Ryan et al 2004).
This species is the most commonly-isolated yeast in human disease. It has been implicated in both superficial and systemic disease. Recent reports of infections include corneal (Ritterband et al 2006), nail (Lange et al 2006), ear (Martin et al 2005), endocarditis (Levy et al 2006) and bloodstream (Viviani et al 2006). Risk factors for infections with \textit{C. albicans} include age of 65 years or above, immunosuppression prior to steroid use, leukocytosis, intensive care unit stays, or presence of intravascular or urinary catheters. For those patients who have undergone cancer chemotherapy and who often appear less critically ill, infections are most likely to be caused by \textit{Candida} species other than \textit{C. albicans} (Cheng et al 2005). Although this species continues to be the most common species isolated in bloodstream infections, reports show that the incidence is decreasing and the resistance is rare in neonatal populations (Fridkin et al 2006). \textit{Candida albicans} is also a predominate species in fungal biofilms on medical devises (He et al 2006; Adler et al 2006).

Genome

One of the most interesting features of the \textit{C. albicans} genome is the occurrence of numeric and structural chromosomal rearrangements as means of generating genetic diversity, named chromosome length polymorphisms (contraction/expansion of repeats), reciprocal translocations, chromosome deletions and trisomy of individual chromosomes. These karyotypic alterations lead to changes in the phenotype, which is an adaptation strategy of this fungus. These mechanisms will be better understood with the complete analysis of the \textit{C. albicans} genome.

The \textit{Candida albicans} genome for strain SC5314 was sequenced at the Stanford DNA Sequencing and Technology Center (Jones et al 2004; Braun 2005). The genome of the WO1 strain was sequenced by the Broad Institute of MIT and Harvard (Candida albicans at NCBI Taxonomy browser 2006 ). The sequencing of the \textit{C. albicans} genome and subsequently of the genomes of several other medically relevant \textit{Candida} species has profoundly and irreversibly changed the way \textit{Candida} species are now investigated and understood (Enfert et al 2007). The \textit{C. albicans}
genome sequencing effort was launched in October 1996. Successive releases of the sequencing data and genome assemblies have marked the last 10 years, culminating with the release of the diploid assembly 19 that provided a haploid version of the genome along with data on allelic regions in the genome (Enfert et al. 2007). A refined assembly 20 with the eight assembled *C. albicans* chromosomes has been released in the summer of 2006. Importantly, the availability of sequencing data prior to the completion of the genome sequence has made it possible to start *C. albicans* post-genomics early on. In this regard, genome databases have been made available to the research community providing different forms of genome annotation. These have been merged in a community-based annotation hosted by the Candida Genome Database.

The availability of the genome sequence has paved the way for the implementation of post-genomic approaches to the study of *C. albicans*: macroarrays and then microarrays have been developed and used to study the *C. albicans* transcriptome; proteomics has also been developed and complements transcriptional analyses; furthermore, systematic approaches are becoming available to study the contribution of each *C. albicans* gene in different contexts. Other *Candida* genome sequences have been, or are being, determined: *C. glabrata, C. dubliniensis, C. parapsilosis, C. guilliermondii, C. lusitaniae,* and *C. tropicalis.* These species will soon enter the post-genomic era as well and provide interesting comparative data. The genome sequences obtained for the different *Candida* species along with those of non-pathogenic hemiascomycetes provide a wealth of knowledge on the evolutionary processes that have shaped the hemiascomycete group as well as those that may have contributed to the success of different *Candida* species as pathogens (Enfert et al. 2007).

The genome of *C. albicans* is highly dynamic and this variability has been used advantageously for molecular epidemiological studies of *C. albicans* and population studies in this species. A remarkable discovery that has arisen from the genome sequence is the presence of a parasexual cycle in *C. albicans.* This parasexual cycle is under the control of mating-type loci and switching between
white and opaque phenotypes. Investigating the role that the mating process plays in the dynamics of the *C. albicans* population or in other aspects of *C. albicans* biology and pathogenicity will undoubtedly represent an important focus for future research (Enfert et al. 2007).

**Dimorphism**

In a process that superficially resembles dimorphism, *C. albicans* undergoes a process called phenotypic switching, in which different cellular morphologies are generated spontaneously. One of the classically studied strains that undergoes phenotypic switching is WO-1, which consists of two phases - one that grows as smooth white colonies and one that is rod-like and grows as flat gray colonies. The other strain known to undergo switching is 3153A; this strain produces at least seven different colony morphologies. In both the WO-1 and 3153A strains, the different phases convert spontaneously to the other(s) at a low frequency. The switching is reversible, and colony type can be inherited from one generation to another. While several genes that are expressed differently in different colony morphologies have been identified, some recent efforts have focused on what might be controlling these changes. Further, whether there is a potential molecular link between dimorphism and phenotypic switching is a tantalizing question.

In the 3153A strain, a gene called SIR2 (for silent information regulator) has been found that seems to be important for phenotypic switching. SIR2 was originally found in *Saccharomyces cerevisiae* (brewer's yeast), where it is involved in chromosomal silencing — a form of transcriptional regulation in which regions of the genome are reversibly inactivated by changes in chromatin structure (chromatin is the complex of DNA and proteins that make chromosomes). In yeast, genes involved in the control of mating type are found in these silent regions, and SIR2 represses their expression by maintaining a silent-competent chromatin structure in this region. The discovery of a *C. albicans* SIR2 that is implicated in phenotypic switching suggests that it too has silent regions controlled by SIR2, in which the phenotype-specific genes may perhaps reside.
Another potential regulatory molecule is Efg1p, a transcription factor found in the WO-1 strain that regulates dimorphism, and more recently has been suggested to help regulate phenotypic switching. Efg1p is expressed only in the white and not in the gray cell-type, and overexpression of Efg1p in the gray form causes a rapid conversion to the white form.

So far there are few data that says that dimorphism and phenotypic switching use common molecular components. However, it is not inconceivable that phenotypic switching may occur in response to some change in the environment as well as being a spontaneous event. How SIR2 itself is regulated in *Saccharomyces cerevisiae* may yet provide clues as to the switching mechanisms of *C. albicans*.

**Macrosopic morphology**

Colonies on Sabouraud dextrose agar at 25°C are white to cream, soft, and smooth to wrinkled. This isolate grows at 42°C and on media containing cycloheximide.

**Microscopic morphology**

On cornmeal following 72 hours incubation at 25°C, abundant branched pseudohyphae and true hyphae with blastoconidia are present. The blastoconidia are formed in grape-like clusters along the length of the hyphae. Terminal chlamydoconidia may be formed with extended incubation (Sutton et al 1998).

*Candida glabrata*

*Candida glabrata* is known for its decreased susceptibility to azoles (Magill SS 2006) but good susceptibility patterns to candins (Ernst et al 2002; Olson et al 2005). Despite the decreased susceptibility to azoles, fluconazole prophylaxis has not contributed to an increased incidence of bloodstream infection caused by *C. glabrata* (Lin et al 2005). This species do, however, contribute to mortality in approximately 21% of pediatric patients with bloodstream infections (Fridkin et al 2006). Although this species may be recovered from virtually all infection sites, recent reports of infection include corneal (Al-Assiri et al 2006), endocarditis (Lye et al 2005) vaginitis (De Vos et al 2005) and oral (Redding 2004).
**Macroscopic morphology**

Colonies on Sabouraud dextrose agar at 25°C are white, smooth and glistening. This isolate grows at 42°C but fails to grow on media containing cycloheximide.

**Microscopic morphology**

On cornmeal following 72 hours incubation at 25°C, only blastoconidia are observed. Yeast cells are quite small measuring only 2.5-4.0 x 3.0-6.0 μm as compared to *C. albicans* which measures 3.5-6.0 x 4.0-8.0 μm (Kurtzman et al 2000).

**Candida dubliniensis**

This isolate is germ tube positive which accounts for its historic misidentification as *C. albicans*. Initially thought to be associated only with HIV disease, several reports have been published since its description implicating it in non-HIV associated infection as well (Carr et al 2005; Faggi et al 2005; Miron et al 2005). Molecular analysis show that *C. dubliniensis* is distinct from *C. albicans* by 13-15 nucleotides in the ribosomal RNA gene sequences (Sullivan et al 1995). Early reports purported that *C. dubliniensis* was responsible for fluconazole-resistant thrush but susceptibility studies reveal that it's categorical distribution is similar to *C. albicans* with isolates ranging from susceptible to resistant.

**Macroscopic morphology**

Colonies on Sabouraud dextrose agar at 25°C are white to cream, soft, and smooth to wrinkled. This isolate has poor to no growth at 42°C. Colonies grown on CHROMagar are dark green as opposed to the light blue-green exhibited by *C. albicans*.

**Microscopic morphology**

On cornmeal following 72 hours incubation at 25°C, abundant branched pseudohyphae and true hyphae with blastoconidia are present. Many strains produce an abundance of chlamydoconidia resulting in excess of 25-30 being observed in
each microscopic field. Chlamydoconidial arrangement includes single, pairs, chains, and clusters.

**Candida guilliermondii**

**Macrosopic morphology**

*Candida guilliermondii* colonies are flat, moist, smooth, and cream to yellow in color on Sabouraud dextrose agar. It does not grow on the surface when inoculated into Sabouraud broth (Larone 1995; Sutton et al 1998).

**Microscopic morphology**

On cornmeal tween 80 agar and at 25°C after 72 h, it produces clusters of small blastospores along the pseudohyphae and particularly at septal points. Pseudohyphae are short and few in number (Larone 1995; Sutton et al 1998).

**Candida kefyr**

This species has been reported as an emerging pathogen (Corpus et al 2004). This species may have been previously reported in the literature by the obsolete name of *Candida pseudotropicalis*. Reported infections include burn wounds (Gupta et al 2004) blood (Pfaller et al 2004), and vaginal (Erdem et al 2003).

**Macrosopic morphology**

Colonies on Sabouraud dextrose agar at 25°C are cream to brown, glossy, and smooth. This isolate has grows at 37°C and on media containing cycloheximide.

**Microscopic morphology**

On cornmeal following 72 hours incubation at 25°C, highly-branched pseudohyphae are found. This species is not distinguishable from several other species on CHROMagar Candida (Hospenthal et al 2006).

**Candida lusitaniae**

*Candida lusitaniae* is known for amphotericin B resistance. The first report of fatal infection occurred in a neutropenic patient where the initial isolate was
susceptible to amphotericin B but subsequent isolates had developed amphotericin B resistance (Pappagianis et al 1979). It is well-documented that this species is able to develop resistance, but some strains display resistance prior to amphotericin B therapy (Merz 1984). Although amphotericin B resistance is a concern, reports exist where amphotericin B has been used to successfully treat fungemia in severely immunocompromised patients (Blinkhorn et al 1989).

**Macroscopic morphology**

Colonies on Sabouraud dextrose agar at 25°C are white to cream, glistening, and smooth but may be rugose. Colonies may be fringed with pseudohyphae. This isolate grows both at 42°C and on media containing cycloheximide.

**Microscopic morphology**

On cornmeal following 72 hours incubation at 25°C, abundant branched pseudohyphae may be present. Some strains have rudimentary to no pseudohyphae.

---

*Candida parapsilosis*

**Macroscopic morphology**

*Candida parapsilosis* colonies are white, creamy, shiny, and smooth or wrinkled on Sabouraud dextrose agar. It does not grow on the surface when inoculated into Sabouraud broth (Larone 1995; Sutton 1998).

**Microscopic morphology**

On cornmeal tween 80 agar and at 25°C after 72 h, it produces blastospores which are located along the pseudohyphae. Typically, the pseudohyphae may be curved and large mycelial-(hyphal) elements which are called "giant cells" may be observed (Larone 1995; Sutton 1998).
**Candida tropicalis**

**Macroscopic morphology**

On Sabouraud dextrose agar, *Candida tropicalis* colonies are cream-colored with a slightly mycelial border. It may produce a thin surface film and bubbles when grown in Sabouraud broth (Larone 1995; Sutton 1998).

**Microscopic morphology**

On cornmeal tween 80 agar and at 25°C after 72 h, it produces oval blastospores which are located along the long pseudohyphae. The blastospores may appear singly or in clusters. The pseudohyphae branch abundantly. *Candida tropicalis* may also produce true hyphae (Larone 1995; Sutton 1998).

**Other species of Candida**

Apart from the *Candida* sp. discussed separately in other pages, there are others less frequently involved in infections. Major characteristics of these other *Candida* sp. are summarized in the Table below.
Table 2: Microscopic and macroscopic features of different Candida spp.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MACROSCOPIC MORPHOLOGY*</th>
<th>MACROSCOPIC MORPHOLOGY**</th>
<th>SPECIAL NOTES</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida ciferrii</td>
<td>White to cream in color, powdery</td>
<td>Pseudohyphae, true hyphae, branching chains of blastospores</td>
<td></td>
<td>Sutton et al 1998</td>
</tr>
<tr>
<td>Candida famata</td>
<td>White to cream in color, smooth</td>
<td>Small blastospores, no pseudohyphae</td>
<td></td>
<td>Barchiesi et al 1999; Carrillo-Munoz et al 1999; Quindos et al 1994</td>
</tr>
<tr>
<td>Candida lambica</td>
<td>White to cream in color, smooth</td>
<td>Pseudohyphae, branching chains of elongate blastospores</td>
<td></td>
<td>Sutton et al 1998</td>
</tr>
<tr>
<td>Candida lipolytica</td>
<td>White in color, dry, cerebriform or creamy</td>
<td>Branching true hyphae, rare, short, and elongate branching blastospores</td>
<td>Nosocomial bloodstream pathogen</td>
<td>Larone 1995; Shin et al 2000; Sutton et al 1998</td>
</tr>
<tr>
<td>Candida norvegensis</td>
<td>White to yellowish, may have pinkish to beige color from reverse, has a specific odor</td>
<td>Small blastospores, rare pseudohyphae</td>
<td>May be resistant to fluconazole</td>
<td>Ahearn et al 1984; Hood et al 1996</td>
</tr>
<tr>
<td>Candida viswanathii</td>
<td>No typical morphology defined</td>
<td>No typical morphology defined</td>
<td>May be resistant to nystatin</td>
<td>Carrillo-Munoz, et al 1999 Collier et al 1998; Elie et al 1998; Mannarelli et al 1998</td>
</tr>
<tr>
<td>Candida zeylanoides</td>
<td>White to cream in color, smooth, dull</td>
<td>Pseudohyphae, blastospores forming feather-like appearance along the pseudohyphae</td>
<td>Causative agent of fungemia, arthritis</td>
<td>Bisbe et al 1987; Larone 1995; Sutton et al 1998</td>
</tr>
</tbody>
</table>

* Colony colour on Sabroud Dextrose Agar; ** Colony colour on Cornmeal Agar
**Candida infections**

Candidiasis is mostly an endogenous infection, arising from overgrowth of the fungus inhabiting in the normal flora. However, it may occasionally be acquired from exogenous sources (such as catheters or prosthetic devices) (Band et al 1979) or by person-to-person transmission (such as oral candidiasis in neonates of mothers with vaginal candidiasis or endophthalmitis following corneal transplantation from an infected donor) (Behrens-Baumann et al 1991).

Fungal infections have increased in medical and surgical intensive care units with associated mortality and morbidity (Beck-Saque et al 1993; Jarvis 1995). Intensive care patients are susceptible to fungal infections during their stay in the hospital. Candida albicans has become a major nosocomial pathogen in the hospitals. It is the fourth leading species found in positive blood cultures in U.S. hospitals (Benerjee 1991). The growing list of resistant pathogens, nosocomial transmission and lack of an effective antifungal drug with a lower toxicity, are the potential problems associated with systemic fungal diseases. Epidemiological studies have shown that Candida infections may be transmitted from patient to patient and from health care provider to patients (Bauer et al 1990).

*Candida* species are the leading cause of nosocomial infections (Beck-Saque et al 1993; Jarvis 1995). Candidemia is frequently associated with the signs and symptoms (Rex et al 2000; Pappas et al 2004). A recent epidemiological study of sepsis revealed that the annual number of cases of sepsis caused by fungal organisms increased by 20.7% between 1979 and 2000 (Guyer et al 1995). This is likely due to an increased prevalence of susceptible hosts, who receive intensive care therapies, immunosuppressive therapies associated with transplantation, and broad-spectrum antibiotics (Fanaroff et al 1998; Makhoul et al 2001).

Candidal infections are an emerging problem in hospital medical practice. *Candida* infections differ markedly between hospitalized children and hospitalized adults (Pappas et al 2003; Wey et al 1988; Rentz et al 1998; Gudlaugsson et al 2003). Previously *C. albicans* was responsible for nearly 80% of candidemia in
many hospitals. Recently shift has occurred in the distribution of infections has changed, non albicans Candida species are being increasingly detected (Pfaller et al 1998a; Nguyen et al 1996; Martin et al 1992).

The clinical prevalence of candidiasis has changed (Rippon 1988). Invasive fungal infections (IFI) are increasing by less common Candida species (Rattan 1999; Chakrabarti et al 1999; Hadley et al 2002).

1. Vaginal candidiasis (Vulvovaginal Candidiasis)

Vaginitis is the most frequent gynecologic diagnosis encountered by physicians who provide primary care to women (Kent 1991; McCue 1989; Carr et al 1998; Sobel 1999b; Haefner 1999). Accurate diagnosis can be elusive; complicating treatment (Eschenbach et al 1989; Schaaf et al 1990; Sobel 1997; Berg et al 1984). Furthermore, the availability of over the counter medications increases the likelihood of inappropriate or partial treatment of vaginitis (Horowitz et al 1992).

Epidemiology

The prevalence and causes of vaginitis are uncertain, in part because the condition is so often self-diagnosed and self-treated. In addition, vaginitis is frequently asymptomatic or has more than one cause. Most experts believe that up to 90 percent of vaginitis cases are secondary to bacterial vaginosis, vulvovaginal candidiasis and trichomoniiasis (Sobel 1997; 1999b). Noninfectious causes include vaginal atrophy, allergies and chemical irritation.

Vaginal candidiasis is primarily an infection of premenoposal woman. It suggests that gonadal hormones may play a major role in the pathogenesis of the infection (Reed 1992). In post menopausal woman vaginal carriage of Candida is expected to be much lower because of estrogen deficiency (Sobel 1997).

Vulvovaginal candidiasis is the second most common cause of vaginitis in the United States and the most common cause in Europe (Kent 1991). An estimated
75 percent of women have vulvovaginal candidiasis at some time in life, and approximately 5 percent of women have recurrent episodes (Monif 1985; Foxman 1990; Sobel 1993; Geiger et al 1996). *Candida albicans* is the infecting agent in 80 to 90 percent of patients (Sobel 1997; Horowitz 1992). Recently, the frequency of non-albicans species (e.g., *Candida glabrata*) has increased, possibly secondary to greater use of over-the-counter antifungal products (Horowitz 1992)

Risk factors for uncomplicated vulvovaginal candidiasis have been difficult to determine (Sobel et al 1998a). Studies have shown that the risk of this infection is increased in women who use oral contraceptive pills, a diaphragm and spermicide, or an IUD (Barbone et al 1990; Spinillo et al 1995; Hooton et al 1994). Other risk factors include young age at first intercourse, intercourse more than four times per month and receptive oral sex. (Foxman 1990; Geiger et al 1996; Sobel et al 1998a; Skinner 1996; Eckert et al 1998) The risk of vulvovaginal candidiasis is also increased in some women who have diabetes, are pregnant or are taking antibiotics. (Foxman 1990; Sobel et al 1998a; Spinillo et al 1999)

Complications of vulvovaginal candidiasis are rare. Chorio-amnionitis in pregnancy and vulvar vestibulitis syndrome have been reported (Cotch et al 1998; Pagano 1999). Establishing *Candida* species as the cause of vaginitis can be difficult because as many as 50 percent of asymptomatic women have candidal organisms as part of their endogenous vaginal flora (Sobel 1993). Candidal organisms are not transmitted sexually, and episodes of vulvovaginal candidiasis do not appear to be related to the number of sexual partners (Foxman 1990; Geiger et al 1996; Sobel et al 1998a). Treating the male partner is unnecessary unless he is uncircumcised or has inflammation of the glans of the penis (MMWR Morb Mortal Wkly Rep 1998).

*Candida* vaginitis is a common problem attributable to over growth of *Candida* species; it is estimated that 75% of all women will experience an episode in their lifetime (Sobel 1997; 1999b). By the age of 25 years, nearly one-half of all college-age women will have at least 1 episode of *Candida* vaginitis (Geiger et al 1995). *Candida albicans* accounts for 80%–95% of all episodes of *Candida* vaginitis worldwide (Sobel 1997; 1999b). Like other topical *Candida* infections, *Candida*
vaginitis is treated effectively with azole-based antifungal drugs. However, such therapy can be complicated by the emergence of drug-resistant yeasts (Sobel 1998a, 1998b; 1999a). Prolonged exposure to fluconazole can shift the predominant vaginal yeast flora from *C. albicans* to more intrinsically azole-resistant species, as has been described for immunosuppressed women (Sobel 1998b; Vazquez et al 1999). In the 1990s, there was a significant increase in the prevalence of drug-resistant fungal infections due to *Candida* species in patients hospitalized for mucosal or systemic diseases (Nguyen et al 1996; Pfaffer et al 1998b, 1999). The widespread application of fluconazole or related azole antifungals is postulated to promote selection of resistant subpopulations by shifting colonization to more naturally resistant species, such as *Candida krusei* or *Candida glabrata* (Alexander et al 1997; Pfaffer 1995; Rex et al 1995a). Alternatively, azole-resistant subspecies have arisen in vivo and in vitro that show changes in the target enzyme lanosterol 14-α demethylase, in expression of multidrug efflux pumps, or in both (Marr et al 1998; Sanglard et al 1995; 1998). Numerous effective topical vaginal antimycotic agents are available that provide high cure rates with favorable therapeutic indexes. Given the growing correlation between azole antifungal exposure and emergence of drug-resistant *Candida* species, there is a concern that this problem may be exacerbated in healthy women by use of over-the-counter (OTC) products, which became available in the early to mid-1990s for self-treatment of vaginitis (Sobel 1998c; 1999a). Most notably, because many of the OTC drugs are azole based, it is likely that frequent or prolonged use of these products has the potential to select for widespread drug resistance. In fact, cross-resistance between OTC drugs (miconazole, clotrimazole, and tioconazole) and fluconazole has been observed in clinical isolates of *C. glabrata* and *C. albicans* (Arias et al 1996; Cross et al 2000). To better address this concern, a multicenter exposure to OTC azole antifungal drugs is not associated with widespread colonization of drug-resistant *Candida* species in the vaginal flora of largely healthy college-age women. In contrast to oropharyngeal candidiasis in patients with AIDS, azole resistance has only been reported in 1 case of vaginitis caused by *C. albicans* (Sobel et al 1996). In vitro resistance remains rare among isolates from women with vaginitis due to *C. albicans*, even among isolates from HIV-positive women, in whom there is a tendency for such infections to occur in the
oral cavity. The same is true of patients with recurrent vulvovaginal candidiasis in which vaginal isolates remain susceptible to azole-based antifungal drugs and do not show increased resistance to any drug despite long-term exposure to azoles (Lynch et al 1996). The widespread use of azoles has led to an increase in the prevalence of fluconazole-resistant non-\textit{C. albicans} species, especially \textit{C. glabrata} (Sobel 1998b; Vazquez et al 1999; Nguyen et al 1996; Cross et al 2000; Hitchcock et al 1993; Xu et al 1999). The presence of asymptomatic women colonized with azole resistant strains has important implications for therapeutic management of future vaginitis episodes in women. Perhaps of more concern is the possibility that a primary drug resistant colonizing strain could predominate at later time if the women become immunosuppressed. Furthermore, the spread of these primary drug-resistant colonizers by sexual transmission is a distinct possibility (Barchiesi et al 1995).

If candidiasis is caused by the host's original commensal strain or strains, knowledge of the yeast microflora of high-risk patients before the manifestation of candidiasis (e.g., susceptibility to different antifungal drugs) could lead to improved strategies for prophylaxis and treatment (Xu et al 1999).

Another concern is the transmission of drug-resistant commensal colonizing strains to family members. Muller (Muller et al 1999) reported that transmission of azole-resistant strains from symptomatic HIV-infected patients with oropharyngeal candidiasis to asymptomatic family members is possible and may represent a previously underappreciated risk for families that include members infected with HIV. The acquisition of an azole-resistant strain of \textit{C. albicans} by an asymptomatic patient has important clinical implications, especially in high-risk populations where presentation of oropharyngeal candidiasis may result in therapy refractory to azoles. The selection of non-\textit{C. albicans} species, especially \textit{C. glabrata}, in HIV-positive women receiving fluconazole therapy is well established, although \textit{C. albicans} is still the predominant pathogen, at 185 percent of all isolates from HIV-seronegative women (Sobel 1999b).
Symptoms and diagnosis of Vulvovaginal Candidiasis

Women with vulvovaginal candidiasis frequently complain of pruritus, vaginal irritation and dysuria. Vulvovaginal itching generally is not a normal finding in healthy women; if this symptom is present, other dermatologic conditions (e.g., lichen sclerosis and, rarely, vulvar cancer) should also be considered, especially in the absence of candidal infection (Haefner 1999; Reilly et al 1991).

In vulvovaginal candidiasis, the discharge is usually white and thick, with no odor and a normal pH. Women with candidiasis can have vulvar and vaginal erythema and, occasionally, scaling and fissures of vulvar tissue (Sobel 1997; ACOG technical bulletin 1996). Microscopic examinations of wet-mount and KOH preparations are positive in 50 to 70 percent of patients with candidal infections (ACOG technical bulletin 1996). In patients with a negative microscopic examination but symptoms compatible with yeast vaginitis, a Gram stain or a culture using Nickerson's medium or Sabouraud's dextrose agar may be helpful (Haefner 1999; Sobel 1998a). Other tests, such as the Pap smear or the latex agglutination test, provide no advantage over microscopic techniques (Monif 1985; Sobel 1993a: 1998a).

Causes of Recurrence

Although Candida albicans is the pathogen identified in most patients with vulvovaginal candidiasis, other possible pathogens include Candida tropicalis and Candida glabrata. Increasingly, Candida species other than C. albicans have been found to cause yeast vaginitis (i.e., 9.9 percent of cases in 1988 and 17.2 percent of cases in 1995) (Spinillo et al 1997a). In fact, recurrent infections may be caused by the resistance of non C. albicans species to antifungal agents.

In vitro studies have shown that imidazole antifungal agents such as miconazole and clotrimazole are not as effective against non C. albicans fungi. C. tropicalis and C. glabrata are 10 times less sensitive to miconazole than is C. albicans (Horowitz 1991). Imidazoles are still the first-line treatment for C. albicans infections. A 1993 in vitro study examined more than 250 strains of C. albicans and...
found that no strain was resistant to ketoconazole, itraconazole and clotrimazole (Fong et al 1993.)

Noncompliance with a treatment regimen may result in persistent infection that is mislabeled as a recurrence. For example, a patient may not complete the entire course of antifungal therapy, especially if an inconvenient topical treatment has been prescribed. A recurrence may also represent an inadequately treated infection. Between 15 and 20 percent of women with negative cultures after treatment have positive cultures within three months (Horowitz et al 1992). If an infection recurs at least three months after the previous episode, it is more likely to be caused by a different *C. albicans* strain (O'Connor et al 1986).

Antibiotics are often implicated as a cause of recurrent vulvovaginal candidiasis. Frequent antibiotic use decreases protective vaginal flora and allows colonization by *Candida* species (Reed 1992). The risk of a yeast infection increases with the duration of antibiotic use, but no specific antibiotic has been shown to be more likely to cause yeast infections (Spinillo et al 1999).

Diabetes mellitus is often considered a predisposing factor for recurrent vulvovaginal candidiasis. Hyperglycemia enhances the ability of *C. albicans* to bind to vaginal epithelial cells (Bohannon 1998). However, unless other symptoms are suggestive of diabetes, patients with recurrent vulvovaginal candidiasis are rarely found to be diabetic (Reed 1992).

Contraceptive methods may also promote recurrences of vulvovaginal candidiasis. Use of spermicidal jellies and creams increases susceptibility to infection by altering the vaginal flora and increasing the adhesion of Candida organisms. Women who take oral contraceptive pills have a higher rate of vulvovaginal candidiasis (Sobel 1998a). According to one theory, Candida cells have estrogen and progesterone receptors that, when stimulated, increase fungal proliferation (Reed 1992).

Women who are prone to recurrent vulvovaginal candidiasis may have deficient cell-mediated immunity. Similarly, persons with acquired immunodeficiency syndrome are susceptible to systemic candidal infection. Some studies suggest that 40 to 70 percent of women with recurrent vulvovaginal
candidiasis have some specific anergy resulting in a subnormal T-lymphocyte response to *Candida* (Sobel 1992a). One study found that Lewis A and B blood group antigens on the vaginal epithelium are protective against candidal infection (Hilton et al 1995).

Mechanical factors may also be important. Perspiration associated with tightly fitted clothes or poorly ventilated underwear increases local temperature and moisture. Mechanical irritation of the vulvovaginal area by clothing or with sexual intercourse may also predispose already colonized areas to infection. One study demonstrated a positive relationship between the monthly frequency of sexual intercourse and the incidence of recurrent vulvovaginal candidiasis (Spinillo et al 1993).

Dietary habits have been suggested as causes of recurrent vulvovaginal candidiasis. However, most studies do not support a role for dietary factors in the etiology of recurrences, and adherence to strict diets has not been beneficial (Reed 1992).

The role of sexual transmission is controversial. One study found identical Candida strains in the sexual partners of 48 percent of women with recurrent infections. (O'Connor et al 1986) A randomized, controlled trial evaluated the effect that treating male sexual partners with oral ketoconazole had on the recurrence rates for vulvovaginal candidiasis (Fong 1992 a). The recurrence rates in the treated and untreated partner groups were found to be similar at six months and one year. Topical antifungal therapy has been ineffective in male sexual partners, probably because of the presence of reservoirs not reached by this treatment. In summary, no clinical trial has found that the treatment of male sexual partners prevents recurrences of vulvovaginal candidiasis in women.

Some investigators have advocated the elimination of Candida from the gastrointestinal tract. The rationale is that reinfection from an intestinal reservoir contributes to vaginal recurrences. However, studies have not found an association between recurrent vulvovaginal candidiasis and the presence of intestinal Candida (Horowitz et al 1992).
Treatment

If a patient has infrequent recurrences, the simplest and most cost-effective regimen involves self-diagnosis and the early initiation of topical therapy. A prospective randomized, open, crossover study in 23 women with proven recurrent vulvovaginal candidiasis examined the efficacy and cost benefit of monthly prophylaxis compared with empiric self-treatment at the onset of symptoms (Fong 1994). Patients were randomized to receive one 500-mg dose of clotrimazole intravaginally each month with the menses for six months or one 500-mg dose of clotrimazole intravaginally at the onset of symptoms. After six months, patients were switched (crossover) to the other regimen.

If a woman with an established diagnosis of recurrent vulvovaginal candidiasis does not respond to an imidazole, infection with a resistant non C. albicans species may be present. Terconazole vaginal cream (Terazol) is the agent of choice when infection with a species other than C. albicans is suspected. The potent interference of this agent with the cytochrome P450 isoenzymes makes C. tropicalis and C. glabrata more susceptible to treatment.

After the acute episode of recurrent vulvovaginal candidiasis has been treated, subsequent prophylaxis or maintenance therapy is essential. In one clinical trial, women with a history of recurrent vulvovaginal candidiasis were randomized to receive 400 mg of ketoconazole for 14 days or clotrimazole in the form of 100-mg vaginal suppositories for seven days (Sobel et al 1994). One week after treatment, the clinical cure rate was higher than 80 percent in both groups. Two months after treatment, in the absence of any maintenance therapy, 53 percent of women in the ketoconazole treatment group and 63 percent of those in the clotrimazole treatment group had recurrences.

Several maintenance regimens have been studied. In one clinical trial, 74 women with recurrent vulvovaginal candidiasis were treated for an acute episode with 400 mg of ketoconazole per day for 14 days (Sobel et al 1986). The women were then randomized to receive one of three treatments: placebo, 400 mg of ketoconazole administered orally for five days after the menses for six months, or 100 mg of ketoconazole administered orally each day for six months. The six-month
recurrence rates were 71 percent for the placebo group, 29 percent for the cyclic-regimen group and 5 percent for the daily-regimen group.

Maintenance therapy needs to be given frequently enough to prevent vaginal regrowth, but the optimal dosing interval is not clear. One study suggested that the weekly administration of 0.8 percent terconazole vaginal cream is nearly as effective as daily treatment with ketoconazole (Stein et al 1996). Similar efficacy has been noted for twice-weekly intravaginal treatment with 200 mg of clotrimazole (Fong 1992 b).

A monthly 150-mg dose of orally administered fluconazole has been shown to reduce the incidence of recurrences by 50 percent (Sobel 1992b). Itraconazole, in a dosage of 200 mg (Creatsas et al 1993) or 400 mg (Spinillo et al 1997b) administered orally once a month, also has been found to decrease the recurrence rate by approximately 50 percent. Boric acid, administered in a 600-mg vaginal suppository twice daily for two weeks and then daily during menstruation, has been effective in the treatment of women with resistant infection (Jovanovic et al 1991). However, the use of boric acid is limited by significant local irritation and the possibility of intoxication (Thai et al 1993).

Based on the findings of Erika (Erika 2000), ketoconazole (Nizoral) administered orally once a day, clotrimazole (Gyne-Lotrimin) administered intravaginally twice weekly, terconazole administered intravaginally once a week, and fluconazole (Diflucan) or itraconazole (Sporanox) administered orally once a month have been relatively effective in reducing the recurrence rate for vulvovaginal candidiasis.

Most studies recommend prophylaxis for six months. Then the woman is reevaluated. Many women have recurrences once prophylaxis is discontinued. Thus, they may need to stay on medication for a longer period.

A 1992 crossover study assessed the association between the daily ingestion of yogurt containing *Lactobacillus acidophilus* and the prevention of recurrent vulvovaginal candidiasis (Hilton et al 1992). Women were assigned to a yogurt-free diet or a yogurt-containing diet. Although only 13 of 21 women completed the protocol, the women who ingested yogurt had a threefold reduction in infection. The
authors of the study concluded that daily ingestion of 8 oz of yogurt containing *L. acidophilus* decreased the rate of candidal infection. A second study showed no difference in infection rates between women who ingested pasteurized yogurt and women who ingested yogurt that contained *L. acidophilus* (Shalev et al 1996). Additional evidence is needed before management recommendations can be made.

In addition to cost, other factors may determine the most appropriate regimen. Compliance rates are greater for medications that are taken orally rather than intravaginally. However, the potential for systemic toxicity and drug interactions is greater with orally administered medications.

Gastrointestinal side effects occur in 15 percent of patients treated with orally administered antifungal agents (Fong 1996). In addition, hepatic toxicity has been noted in one of 15,000 persons treated with orally administered ketoconazole (Titusville 1997). Although clotrimazole therapy may cause local discomfort, it is less frequently associated with systemic toxicity (headache occurs in 9 percent of recipients and abdominal pain is a Compared with ketoconazole, fluconazole is less likely to be toxic. Because fluconazole is administered orally, treatment compliance is better than with clotrimazole, which is administered intravaginally. Patients treated with fluconazole report headache (12 percent), abdominal pain (7 percent) and nausea (4 percent) (Sobel et al 1995).

Many antifungal agents have significant interactions with other medications. For example, interactions have been noted between fluconazole and warfarin (Coumadin), oral hypoglycemic agents, phenytoin (Dilantin), theophylline and rifampin (Rifadin) (Desai et al 1996). Other drugs reported to interact with fluconazole include cyclosporine (Sandimmune), zidovudine (Retrovir) and hydrochlorothiazide (Esidrix).

### 2. Neonatal Candidiasis

**Overview**

Invasive candidiasis in neonates is a serious and relatively common cause of late onset sepsis associated with a high mortality. A review of the first 26 cases reported in the English literature up to 1984 reported a mortality rate of 54%
Premature neonates and particularly low birth weight infants require invasive diagnostic and aggressive therapeutic interventions, many of which increase the risk factors for developing *Candida* infections. In addition the immaturity of the immune system especially among preterm neonates, which mainly involves T-cells and neutrophils, further predisposes this population to infections (Wilson 1986). Indeed, the anti-*Candida* activity of lung macrophages in neonates has been shown to be reduced (Bektas et al 1990; Frenkel et al 1987).

Neonatal candidiasis can be subdivided into two categories:

1. Catheter-related candidemia
2. Disseminated or invasive candidiasis

Catheter-related candidemia refers to infants with central vascular catheters in place and candidemia that resolves rapidly after catheter removal and initiation of therapy. Disseminated or invasive candidiasis refers to persistent candidemia if a catheter was in place and removed, and/or the isolation of *Candida* from other normally sterile body sites. However, as in adults, there are no clinical criteria or diagnostic tests that allow a differentiation of these two groups at presentation (Butler et al 1990). Up to 75% of cases of neonatal candidiasis present with infection of two or more organs. Unifocal osteomyelitis, meningitis, and renal candidiasis are the most common presentations, otherwise infection of any combination of blood, kidneys, meninges, heart, eye, (Johnson et al 1984).

A distinctive form of cutaneous candidiasis that may become invasive and that is known as Congenital Candidiasis is discussed separately below.

**Epidemiology**

The incidence of invasive candidiasis in neonatal care units ranges between 1.6 and 4.5% (Baley et al 1984b; Faix 1984; Johnson et al 1984). More recent data from the NICHD Neonatal Research Network centers on late-onset sepsis in very
low birth weight neonates, has reported that fungal pathogens explain 9% of all bloodstream infections (Stoll et al 1996). Neonates may acquire *Candida* by vertical or nosocomial transmission (Reef et al 1998; Waggoner-Fountain et al 1996). In vertical transmission, acquisition may occur either during gestation or at the time of delivery. In both cases an ascending route from the mother’s vagina is involved. Vaginal candidiasis occurs frequently among pregnant women, especially in the last trimester. Rates as high as 56% have been reported (Frerich et al 1977). *Candida* carriage rates among neonates vary between 30 to 60% (Pappu-Katikaneni et al 1990). Interestingly as gestational age falls, the rates of candidal colonization appear to rise (Sharp et al 1992). Acquiring *Candida* does not always translate into systemic infection, but previous colonization is a required step before the step of occurrence of invasive candidiasis.

Candidal nosocomial acquisition is being recognized as a very important form of transmission in the neonatal ICU (NICU) (Reef et al 1998). Indeed, important outbreaks of candidemia in NICU have been identified (Faix et al 1955; Finkelstein et al 1993; Reagan et al 1995). Such colonization is actually a general phenomenon: hand carriage rates among health workers in seven SICU across the USA have been found to be of around 30% (Rangel-Frausto et al 1999).

**Neonatal Candidiasis and Candida species**

Initial reports on neonatal candidiasis consistently found *Candida albicans* to be responsible for the majority of cases (Butler et al 1990; Faix et al 1989). However, similar to change of the epidemiologic patterns that has been observed in adults, a changing spectrum of species is being noted among neonates. This change is characterized by a progressive decrease in the rate of isolation of *Candida albicans* and an emergence of non-*albicans* species (Finkelstein et al 1993; Rangel-Frausto et al 1999).

Unlike the situation with adults however, it is *Candida parapsilosis* that is becoming the most prevalent species. In some centers it has replaced *C. albicans* as the most frequent species (Levy et al 1998).
Clinical Manifestations

The classic clinical picture of systemic candidiasis in neonates is indistinguishable from bacterial sepsis (Baley et al 1984b).

All of the following signs may be seen:

Temperature instability, hypotension, respiratory deterioration and apnea, abdominal distension, guaiac positive stools, carbohydrate intolerance.

Among these, respiratory dysfunction and apnea were the most common presenting signs in large series, being present in about 70% of cases (van den Anker et al 1994).

A significant proportion of neonates will present simultaneously with localized signs of candidal infection at one or more other sites:

1) Skin and mucous membranes

Mucocutaneous candidiasis in neonates may present with the classic thrush, diaper rash and/or any variety of this erythematous rash with papules and/or pustules affecting usually wet cutaneous surfaces (Faix et al 1989). Skin abscesses have also been described (Leibovitz et al 1992). However, the most common presentation is perineal candidiasis (Faix et al 1989).
A. Oral candidiasis

Oral candidiasis

Angular cheilitis

B. Candida skin and nail infections

Candidal nail infection

Superficial peeling from candida infection

Erosio interdigitalis blastomycetica

Intertrigo

Inflammatory candida causing pustules

Close-up of rash caused by candida

Fig. 1 Various Candida infections.
<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classic (also described in adults)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of multiple antibiotics</td>
<td>Length of antibiotic treatment is as important as the spectrum of the regimen used</td>
<td>(Weese-Mayer et al 1987)</td>
</tr>
<tr>
<td>Central venous catheters</td>
<td></td>
<td>(Weese-Mayer et al 1987)</td>
</tr>
<tr>
<td>Parenteral hyperalimentation and intravenous fat emulsion</td>
<td></td>
<td>(Weese-Mayer et al 1987)</td>
</tr>
<tr>
<td>Colonization with <em>Candida</em> and/or previous episode of mucocutaneous candidiasis</td>
<td></td>
<td>(Faix et al 1989; Pappu-Katikaneni et al 1990; Rowen et al 1994)</td>
</tr>
<tr>
<td><strong>Risk factors unique to this age group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight</td>
<td>~ 90% of affected neonates are very low birth weight (&lt;1500 gr)</td>
<td>(Faix et al 1989)</td>
</tr>
<tr>
<td>Endotracheal tubes and tracheostomies</td>
<td>Many neonates have some type of respiratory insufficiency</td>
<td>(Weese-Mayer et al 1987)</td>
</tr>
<tr>
<td>Peripheral venous catheters</td>
<td></td>
<td>(Leibovitz 1992)</td>
</tr>
<tr>
<td>Congenital malformations, GI malformations and congenital heart diseases are the most frequent</td>
<td>Most frequently seen in infants &gt; 2,500 gr at birth with prolonged NICU hospitalization</td>
<td>(Rabalais et al 1996)</td>
</tr>
<tr>
<td>Gastrointestinal tract diseases</td>
<td>Necrotizing enterocolitis and anatomical abnormalities requiring surgery</td>
<td>(Faix et al 1989)</td>
</tr>
</tbody>
</table>
It is important to emphasize that even when any of these forms of mucocutaneous candidiasis may precede the appearance of systemic disease; this is not a pathognomonic or required condition. Prospective evaluation of this association has found that 44% of neonates with invasive candidiasis never developed skin lesions (Faix et al 1989). However, the same analysis did demonstrate that neonates who developed mucocutaneous candidiasis had a higher risk of developing later deep organ candidal infection (Faix et al 1989).

Two unique varieties of neonatal candidal skin diseases are:

1. **Cutaneous Congenital Candidiasis.** This disease is discussed later.

2. **Invasive Fungal Dermatitis.** This recently described skin disorder (Rowen et al 1995) affects extremely low birth weight neonates. Characteristic ulcerative and erosive lesions with extensive crusting are seen. Even when other fungal pathogens have been implicated in this entity, *Candida* sp. is responsible for about 70% of cases (Rowen et al 1995). More than half of these *Candida*-related skin infections were associated with the occurrence of invasive candidiasis. Authors describing this picture and others have postulated that immature skin becomes in these cases the portal of entry for *Candida* (Rowen et al 1995; Melville et al 1996).

2) **Central nervous system**

Among neonates *Candida* meningitis is one of the most common manifestations of invasive candidiasis (Baley et al 1984b; Chesney et al 1978; Faix 1984). Up to 64 percent of neonates dying with invasive candidiasis have CNS involvement and more than 2/3 of these babies have positive CSF cultures at some point in their disease (Faix 1984). Neurologic clinical manifestations in this particular population are few and related to increased intracranial pressure (bulging fontanelle and splitting sutures). Instead, general signs of sepsis and progressive
clinical deterioration are commonly found (Faix 1984). In other words, *Candida* meningitis usually presents as part of the syndrome of invasive or disseminated candidiasis. Therefore, a physician dealing with sepsis in a high risk neonate, should suspect *Candida* meningitis if *Candida* sp. is recovered from the blood, urine or other site suggestive of heavy colonization (Faix 1984).

*Candida* meningitis carries a high rate mortality and for survivors a high incidence of severe sequelae (hydrocephalus, psychomotor retardation, and aqueductal stenosis) (Chesney et al 1978).

3) Eyes

The use of fundoscopic exam has been recommended as a tool for early diagnosis of invasive disease. The only prospective study evaluating neonates with either candidemia or CSF positive for *Candida* found an incidence of *Candida* endophthalmitis of 50% (Baley et al 1981).

4) Heart

Candidal endocarditis has been found to be the second most common form of endocarditis in this age group (Daher et al 1995; O'Callaghan et al 1988). Clinically, classic findings are expected, including cardiac murmurs, petechiae, skin abscesses, arthritis, hepatomegaly and splenomegaly (Daher et al 1995). Right-sided intracardiac fungal masses can manifest with heart failure or even with pulmonary fungal embolism (Johnson et al 1984; Gonzalez Dieguez et al 1992; Mayayo et al 1996).

5) Kidneys

Candidal UTI is the most frequent cause of urinary tract infection in the NICU (Phillips et al 1997). About half of these babies are found to have concomitant candidemia (Phillips et al 1997). In addition, this population is particularly predisposed to suffer renal candidiasis, which refers to renal fungus balls or renal fungal abscesses. Between 35 to 42% of neonates hospitalized at NICU with
candiduria will have renal candidiasis, and the large majority of these cases are indeed fungus balls (Phillips et al 1997; Viscoli et al 1991). Unilateral or bilateral renal obstruction may occur (Phillips et al 1997; Viscoli et al 1991). Renal insufficiency could be the first clinical manifestation of invasive candidiasis (Visser et al 1998).

6) Bones and Joints

_Candida_ sp. have been repeatedly listed among the three most common agents causing neonatal arthritis (Dan 1983; Omene et al 1984; Pittard et al 1976). Warmth and fusiform swelling of the lower extremities in combination with radiographic evidence of osteolysis and cortical bone erosion are the expected findings in cases of candidal osteomyelitis and/or arthritis in the neonate (Ward et al 1979; Weisse et al 1993).

Therapies

The ways in which neonates differ in terms of relevant strategies are increasingly appreciated. Important differences on the pharmacokinetics but particularly on the toxicity profile of available antifungal agents have been demonstrated. As we will discuss, the both classic but toxic antifungal agents, amphotericin B and 5-fluorocytosine, cause minimal side effects in neonates and the combination of these two agents for the treatment of neonatal candidiasis has been extensively used and advocated (Baley et al 1984b; Duffy et al 1983; Faix 1984; Johnson et al 1984; Leibovitz et al 1992; Weese-Mayer et al 1987).

1. Amphotericin B

Initial reports on the use of amphotericin B in neonates were somewhat alarming. In particular, the report by Baley implicated this agent in a high mortality rate in 10 infants with invasive candidiasis and caused skepticism among neonatologists (Baley et al 1984a; van den Anker et al 1995). However, confounding factors such as previous renal insufficiency and the simultaneous use of other nephrotoxic agents were not considered (van den Anker et al 1995).
Johnson (Johnson et al 1984) did not find a single case of significant renal toxicity with amphotericin B in a group of 21 infants (birth weight < 1,500 grams) with neonatal candidiasis treated with this agent. In addition, the classic infusion-related side effects, fever, chills, nausea and vomiting are especially seen in this population (Johnson et al 1984; Duffy et al 1983; Ward et al 1983) and even when amphotericin B is known to inhibit erythropoietin production, anemia has not been described as a significant finding among babies treated with this drug (Butler et al 1990; Lin et al 1990).

Indeed, the use of amphotericin B alone for the treatment of neonatal candidiasis has been advocated by some authors, in view of the lack of an intravenous formulation of 5-fluorocytosine and the immaturity of the GI tract in neonates (Butler et al 1990). A retrospective analysis of such approach revealed that transient azotemia, elevations in serum creatinine and hypoalkemia occurred in about half of cases, but all these complications were satisfactorily managed with short interruptions of therapy or adjustment in dosing intervals. In addition, a comparison of the mortality rate of these infants treated without 5-fluorocytosine with the one reported by authors using the classic combination revealed they were similar or even lower (Butler et al 1990).

2. **5-fluorocytosine**

Johnson (Johnson et al 1984) also emphasized the lack of cases of either bone marrow or liver toxicity when using 5-fluorocytosine for the treatment of neonatal candidiasis. They used doses of between 20 to 200 mg/kg/day. Many other reports reviewing this topic have favored the combined use of this agent with amphotericin B (Baley et al 1984; Johnson et al 1984; Leibovitz et al 1992; Weese-Mayer et al 1987).

3. **Fluconazole**

Limited data on the use of fluconazole in this population has been published. Most of the data are from anecdotal reports (Bilgen et al 1995; Huan et al 1998; Krzeska et al 1993; Merchant et al 1997; Narang et al 1996; Viscoli et al 1989;
1991; Wainer et al 1997). More recently a single randomized study compared fluconazole with amphotericin B for the treatment of 23 infants with neonatal candidiasis (Driessen et al 1996). However, a heterogeneous group of babies was included. Single stool culture positive for Candida was accounted as a criterion for invasive candidiasis in one case. Therefore, no major conclusions can be made from this study (Driessen et al 1996). There is no doubt that fluconazole deserves further evaluation.

In conclusion, amphotericin B alone or in combination with 5-fluorocytosine remains the standard of care for neonatal candidiasis. The optimal duration of therapy is unknown. However, it is recommended to complete a minimum of 10 to 15 mg/kg of amphotericin B in cases of uncomplicated catheter-related candidemia and between 25 to 30 mg/kg total for patients with invasive disease. When using 5-fluorocytosine, 100 mg/kg/day given in four equal doses is recommended (Butler et al 1990).

3. Chronic mucocutaneous candidiasis

Overview

Chronic mucocutaneous candidiasis (CMC) is the label given to a group of overlapping syndromes that have in common a clinical pattern of persistent, severe, and diffuse cutaneous candidal infections. These infections affect the skin, nails and mucous membranes. As suggested by Kirkpatrick's papers on this topic (Kirkpatrick et al 1971; 1976; 1979), the syndromes of CMC can be grouped as follows:

Classification of Chronic Mucocutaneous Candidiasis (CMC)

1. Chronic oral candidiasis
   o Related to denture stomatitis
   o HIV-associated candidiasis
   o Related to inhaled corticosteroid use
2. CMC and polyendocrinopathy
3. Localized CMC
4. Diffuse CMC
5. CMC with thymoma
6. CMC with interstitial keratitis
7. CMC associated with "KID" (keratitis, ichthyosis, deafness)
While the causative factors in the oral syndromes in group 1 are easily understood, patients affected with the other forms of CMC usually have one or more associated underlying disorders. Endocrine dysfunctions are the most common association, but almost any organ system can be involved. Of note, CMC almost always remains limited to the skin. The rare reported cases of invasive fungal infection in patients with CMC have been candidal and cryptococcal meningitis (Germain et al 1994; Kauffman et al 1981).

Immunopathogenesis

However, the true details of the defect(s) associated with the various CMC syndromes remain poorly understood. For example, cytokine production has only recently been assessed in patients with CMC. In the one study on this topic, patients with CMC showed reduced or absent IL-2 secretion and increased IL-6 secretion (Lilic et al 1996b). This pattern suggests that patients with CMC have a Th2-like response rather than a Th1-like response. As a Th1-like response is important to control at least some candidal infections (Cenci et al 1995; Roman et al 1991), demonstration of a Th2-like pattern in CMC is intriguing. In addition, some patients demonstrate abnormalities of antibody production and the susceptibility to viral and bacterial infections that is typical of antibody deficiency (Romani et al 1991). Hypogammaglobulinaemia associated with high levels of IgG and IgA antigen-specific anti-Candida antibody has also been described (Lilic et al 1996a).

Epidemiology

CMC is rare, but data giving a good estimate of its frequency are not available. Ahonen (Ahonen et al 1990) registered all patients diagnosed with CMC in Finland between 1910 and 1988. During this 78 year period, these authors found only 68 patients with CMC in combination with either hypoparathyroidism or hypoadrenalism. There is also an Iranian Jewish population with at least a moderately high frequency of these syndromes (Bjorses et al 1996). Sporadic cases have been noted in many other regions of the world (Bjorses et al 1996). As discussed below, a specific gene has been linked to the variety of CMC noted in the Finnish and Iranian Jewish populations.
Chronic mucocutaneous candidiasis is virtually always caused by *Candida albicans* (Kirkpatrick et al 1971).

**Clinical Manifestations**

Although CMC may become clinically apparent at any time in life, it typically presents before 3 years of age (Kirkpatrick 1989). Affected areas can be single or multiple and include the mouth, esophagus, nails, or skin (Kirkpatrick 1989).

Although the initial lesions are identical than the ones seen in the general population, over time patients with CMC characteristically develop unique disfiguring lesions (Kirkpatrick 1989).

**Chronic Oral Candidiasis**

This category is really quite distinct from all of the other forms of CMC. In the affected patients, persistence of well-known risk factors (either local or systemic) produces recurrent oropharyngeal candidiasis.

**CMC with Endocrinopathy**

This variety of CMC usually presents during infancy with persistent "diaper rash". Cutaneous candidiasis becomes more extensive later on, and onychomycosis also occurs. The endocrine dysfunctions can manifest any time during life, and usually follow a sequential pattern (Kirkpatrick 1993).

**Diagnosis**

From a diagnostic point of view, the evaluation of patients with suspected CMC is complex. Kirkpatrick has proposed the age-based approach to the patient (Kirkpatrick 1993).

**Therapy**

Current therapy for CMC principally revolves around prolonged use of antifungal agents. However, there have also been attempts to ameliorate the underlying immune defect of CMC.
1. **Antifungal Therapy**: The availability of effective oral agents, especially the azole antifungal agents, has dramatically changed the life of patients living with CMC (Kirkpatrick 1994). Ketoconazole was the first agent to be extensively used for CMC and proved to be very successful when used either continuously or intermittently (Fanconi et al 1982; Graybill et al 1980; Herrod 1990; Horsburgh et al 1983; Kirkpatrick et al 1980; Mobacken et al 1986; Montagnani et al 1986). However, liver toxicity soon was found to be a limitation (Tkach et al 1982). Itraconazole has also been used, although in fewer reports than for ketoconazole (Burke 1989; Tosti et al 1997). Fluconazole should also be effective, however there are only a few case reports describing its use (Hay et al 1988; Rybojad et al 1999; Shiraishi S et al 1994).

2. **Immunotherapy**: Given the underlying immunologic nature of CMC, immunologic treatments of various sorts have been attempted in selected patients:

   - Transfusion of a *Candida*-specific transfer factor has been reported to be very successful (remission for > 10 years) when combined with antifungal therapy (Ballow et al 1977; Kirkpatrick et al 1979).
   - Reports of successful treatment with bone marrow transplantation have also been published (Buckley et al 1968; Deeg et al 1986; Hoh et al 1996).
4. Oral candidiasis

Oral candidiasis is often known as thrush, because its white spots resemble the breast of the bird with the same name. Although candida is present in 50% of healthy mouths, it causes infection when increased numbers of yeast cells invade the mucosa (the name for the moist skin inside body openings).

Predisposing factors for infection include infancy or old age; serious underlying disease, such as cancer or infection with human immunodeficiency virus; dry mouth due to disease of the salivary glands or medications e.g. antihistamines, diuretics; dentures (especially if they are not regularly cleaned or fit badly); smoking; (Soysa et al 2005) injury to the mouth; nutritional deficiency e.g. iron &/or B-vitamin deficiency; inhaled corticosteroids used to treat asthma e.g. beclometasone, budesonide, fluticasone. (Ellepola et al 2000; Taillandier et al 2000; Slavinsky et al 2002)

5. Congenital Candidiasis

Congenital candidiasis is a rare clinical entity in which intrauterine Candida infection becomes manifest at birth. Congenital candidiasis is not related to vaginal delivery, premature rupture of membranes, prematurity, maternal age and duration of labor or parity (Dvorak et al 1966; Rudolph et al 1977). Intrauterine contraceptive devices have been frequently associated with this condition (Darmstadt et al 2000; Delprado et al 1982; Whyte et al 1982). Two forms of disease have been described (Pradeepkumar et al 1998):

1. Congenital cutaneous candidiasis

In this case an extensive skin rash becomes manifest within the first 12 hours of life (Rudolph et al 1977). A macular erythema that may evolve from a pustular, papular or vesicular phase finally results in extensive desquamation (Rudolph et al 1977; Glassman et al 1993). Paronychia and dystrophy of the nail plates have been also described Arbegast et al 1990; Raval et al 1995). The most commonly affected areas include the trunk, neck, face and extremities (Rudolph et al 1977). These
cutaneous lesions usually resolve spontaneously or after short courses of oral nystatin (Rudolph et al 1977).

2. Congenital systemic candidiasis

In certain cases, the picture may evolve to an invasive infection and death, particularly in very low birth weight infants (Johnson et al 1981). This form of the disease has a high mortality rate. Importantly, at least half of the cases do not develop the cutaneous phase previously described (Jin et al 1995). Pneumonia with respiratory distress is the most common presentation of systemic or invasive candidiasis (Cosgrove et al 1997; Glassman et al 1993; Jin et al 1995; Whyte et al 1982). Other presentations include candidal meningitis, candiduria and/or candidemia.

Diagnosis

Direct smear or culture of gastric aspirate, meconium, scrapings of skin vesicles or cord lesion can all be positive for Candida sp. Microscopic and even macroscopic examination of the placenta may disclose fungal Chorio-amnionitis (Whyte et al 1982). Elevated leukocyte count, with an increase in immature forms or persistent hyperglycemia are other frequently encountered findings (Darmstadt et al 2000; Whyte et al 1982).

Therapy

Systemic antifungal therapy with amphotericin B is warranted in infants with skin lesions suggestive of congenital cutaneous candidiasis and respiratory distress (Mamluk et al 1985).

6. Balanitis

Balanitis is an inflammation of the glans penis (head of the penis). Symptoms of balanitis include red swollen tender penis, blotchy rash may be present, occasionally penile discharge may be itchy and uncomfortable (Davis-Daneshfar et al 2000).
Causes of balanitis

Balanitis is not a sexually transmitted disease. It is usually caused by irritants and an overgrowth of organisms that are normally present on the skin of the glans (English et al. 1997). These include bacteria and Candida yeast. Certain circumstances may make a male more susceptible to getting balanitis, these include uncircumcised men, area under the foreskin is warm and moist, making it ideal conditions for the growth of organisms (usually Candida albicans) that cause balanitis; poor personal hygiene, diabetes, chemical irritants, e.g. soap, lubricating jelly; Obesity multiply and cause inflammation, particularly if the area under the foreskin is kept moist and uncleaned for a couple of days. (Sobel 1993).

7. Fungal nail infections

Fungal infection of the nails is known as Onychomycosis. It is increasingly common with increased age. It rarely affects children. Onychomycosis can be due to dermatophytes such as Trichophyton rubrum (T. rubrum), T. interdigitale. The infection is also known as tinea unguium (Williams 1993; Hay 2001). Candida infection of the nail plate generally results from paronychia and starts near the nail fold (the cuticle) (Williams 1993; Hay 2001). The nail fold is swollen and red, lifted off the nail plate. White, yellow, green or black marks appear on the nearby nail and spread. The nail may lift off its bed and is tender if you press on it.

Antifungal drugs

There are several classes of antifungal drugs (Dismukes 2000).

1. Polyene antifungals

A polyene is a circular molecule consisting of a hydrophobic and hydrophilic region. This makes polyene an amphoteric molecule. The polyene antifungals bind with sterols in the fungal cell membrane, principally ergosterol (Gallis et al. 1990).

Animal cells contain cholesterol instead of ergosterol and so they are much less susceptible. (Note: by reducing the hydrophobic chain on the polyene creates a more active sterol binding agent. Therefore, reducing the hydrophobic region too much results in binding to cholesterol, making it toxic to animals.) (Hiemenz et al 1996).
2. Imidazole and triazole antifungals

The imidazole and triazole antifungal drugs inhibit the enzyme Cytochrome P₄₅₀ 14 α-demethylase. This enzyme converts lanosterol to ergosterol, and is required in fungal cell wall synthesis. These drugs also block steroid synthesis in humans (Hoesley et al 1997).

Imidazoles:

- Miconazole, Ketoconazole, Clotrimazole - marketed as Lotrimin or Lotrimin AF, Econazole, Bifonazole, Butoconazole, Fenticonazole, Isoconazole, Oxiconazole, Sertaconazole, Sulconazole, Tioconazole.

The triazoles are newer, and are less toxic and more effective:

- Fluconazole, Ravuconazole, Itraconazole, Posaconazole, Voriconazole, Terconazole.

3. Allylamines

Allylamines inhibit the enzyme squalene epoxide, and other enzyme required for ergosterol synthesis.

**Details of some common antifungals used**

**Amphotericin B deoxycholate (AMB)**

**Trade & Generic Names & General Features**

Amphotericin B is a polyene antifungal agent, first isolated by Gold et al from *Streptomyces nodosus* in 1955. It is an amphoteric compound composed of a hydrophilic polyhydroxyl chain along one side and a lipophilic polyene hydrocarbon chain on the other. Amphotericin B is poorly soluble in water (Terrell et al 1992). Amphotericin B is now available in four formulations. The classic amphotericin B deoxycholate (Fungizone™) formulation has been available since 1960 and is a colloidal suspension of amphotericin B. A bile salt, deoxycholate, is used as the solubilizing agent.
This preparation has a number of toxicities that are partially ameliorated when a lipid carrier is used. Thus, three lipid preparations of amphotericin B have been licensed and are currently available (Hiemenz et al 1996). These are: Amphotericin B Colloidal Dispersion (ABCD; Amphocil™ or Amphotec™); Amphotericin B Lipid Complex (ABLC; Abelcet™); Liposomal Amphotericin B (L-AMB; Ambisone™).

Mechanism(s) of Action

Amphotericin B binds to sterols, preferentially to the primary fungal cell membrane sterol, ergosterol. This binding disrupts osmotic integrity of the fungal membrane, resulting in leakage of intracellular potassium, magnesium, sugars, and metabolites and then cellular death. The mechanism of action is the same for all the preparations and is due to the intrinsic antifungal activity of amphotericin B (Terrell et al 1992).

Susceptibility Patterns

A standard method of NCCLS is available for testing in vitro susceptibility to amphotericin B (National Committee for Clinical Laboratory Standards 1997; 1998). However, it lacks ability to readily discriminate the resistant isolates from the susceptible ones. While some investigators have reported the use of Antibiotic Medium 3 to be useful in this respect (Lozano-Chiu et al 1998; Rex et al 1995b), data obtained in other studies do not always support this theory (Nguyen et al 1998).
Using various modifications of the NCCLS methodology, moderately strong correlations between MIC and/or MLC (minimum lethal concentrations) and outcome have been reported (Nguyen et al 1998).

Generally speaking, amphotericin B has a very broad range of activity and is active against most pathogenic fungi. Notable exceptions include Trichosporon beigelii (Walsh et al 1990); Aspergillus terreus (Sutton et al 1999); Pseudallescheria boydii (Walsh et al 1992); Malassezia furfur (Francis et al 1992) and Fusarium sp. (Arikan et al 1999).

On occasion, however, isolate of any species may be found to be resistant. Among the Candida sp., isolates of C. albicans, C. guilliermondii, C. lipolytica, C. lusitaniae, C. norvegensis, C. tropicalis, C. glabrata, and C. krusei have been reported to be relatively resistant to amphotericin B (Karyotakis et al 1993; 1994; Meyer 1992; Terrell et al 1992). Reduced susceptibility has been observed specifically at fungicidal levels for C. parapsilosis (Seidenfeld et al 1983).

**Usual Doses**

Amphotericin B deoxycholate is administered by intravenous infusion at doses ranging from 0.3 to 1.5 mg/kg and over 1-4 h (Meyer 1992; Oldfield et al 1990).

**Side-Effects**

The most commonly observed infusion-related side effects of amphotericin B deoxycholate are fever, chills, and myalgia. These can be partially overcome by premedication with diphenhydramine and/or acetaminophen (Goodwin et al 1985). Nephrotoxicity is the major adverse effect limiting the use of amphotericin B. The manifestations of nephrotoxicity are azotemia, decreased glomerular filtration, loss of urinary concentrating ability, renal loss of sodium and potassium and renal tubular acidosis (Meyer 1992). The renal injury reduces erythropoietin production and leads to a normochromic, normocytic anemia (Lin et al 1990). Thrombophlebitis may occur at the site of infusion. Thrombocytopenia may rarely be observed (Chan et al 1982).
Routes

Amphotericin B and its lipid formulations are administered intravenously.

Current Status

Amphotericin B still remains as the mainstay of antifungal therapy. Its lipid formulations, on the other hand, are promising due to their ability to reduce the toxicity of amphotericin B. They are currently licensed for use when amphotericin B therapy fails or is unacceptably toxic. The use of lipid formulations in specific clinical settings is under continuing investigation (Wong-Beringer et al 1998).

Liposomal Nystatin

Trade & Generic Names & General Features

Liposomal nystatin is a lipid-based polyene antifungal agent. It is composed of nystatin incorporated into liposomes containing dimyristoyl phosphatidyl choline and di-myristoyl phosphatidyl glycerol.

Aiming to reduce the systemic toxicity of nystatin, liposomal nystatin was first developed in 1987 by Lopez-Berestein (Mehta et al 1987; Wallace et al 1997). It is being manufactured by Aronex Pharmaceuticals under the generic name liposomal nystatin and the trade name Nyotran™.
Mechanism(s) of Action

Similar to other polyene antifungal agents, nystatin binds to ergosterol in the fungal membrane. This binding disrupts osmotic integrity of the fungal membrane, resulting in leakage of intracellular potassium, magnesium, sugars, and metabolites and then cellular death. The lipid carrier of liposomal nystatin does not change this basic mode of action (Mehta et al 1987).

Susceptibility Patterns

When nystatin is incorporated into liposomes, its in vitro activity is maintained. Liposomal nystatin is active against a wide variety of yeasts and moulds, including Candida sp., Cryptococcus neoformans, and Aspergillus sp. (Jessup et al 1997; Mehta et al 1987). Comparison of nystatin and liposomal nystatin MICs produce variable results. Nystatin and liposomal nystatin MICs are either similar (Mehta et al 1987) or liposomal nystatin MICs are lower (Oakley et al 1999). The mechanism for generation of lower liposomal nystatin MICs is not known, and some authorities feel that testing should simply be done using the parent compound alone (Nystatin). As for Amphotericin B and its lipid formulations, the in vitro susceptibility testing method for liposomal nystatin needs to be improved. Liposomal formulation enhances the activity of the drug by enabling the entrapment of the drug in reticulo-endothelial system and delivery of it specifically to the site of infection. Thus, the ultimate enhanced activity of lipid formulation compared to the parent compound nystatin, is probably not an outcome of enhanced "in vitro"
activity but rather is that of improved efficacy "in vivo". Thus, the significance of susceptibility testing for liposomal nystatin is not known.

**Usual Doses**

In clinical trials done so far, liposomal nystatin has been intravenously administered at doses of 0.25 to 4 mg/kg (Williams 1999). Typical doses are not known yet.

**Side-Effects**

In contrast to nystatin, serious toxic reactions due to systemic administration of liposomal nystatin appear less likely. The most commonly encountered side effect is hypokalemia (Williams 1999). Nephrotoxicity also occurs, although details on the frequency of this side-effect are as yet unavailable (Williams 1999).

**Routes**

Liposomal nystatin is administered intravenously.

**Current Status**

Liposomal nystatin is in late Phase III clinical trials. Data obtained so far are promising for its future use in treatment of systemic fungal infections.

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**Fluconazole**

**Trade & Generic Names & General Features**

Fluconazole is a widely used bis-triazole antifungal agent. As with other triazoles, it has five-membered ring structures containing three nitrogen atoms. It is marketed as Diflucan® by Pfizer Pharmaceuticals (http://www.pfizer.com). Both oral and intravenous formulations of Fluconazole are available.
Mechanism(s) of Action

As with all azole antifungal agents, fluconazole works principally by inhibition of cytochrome P450 14 α-demethylase (P450 14 DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol (Lyman et al 1992 Marriott et al 1987; Odds et al 1986).

Fungal cell membrane synthesis is a multi-step process that involves the conversion of squalene to ergosterol. Fluconazole exerts its effect by selectively inhibiting the fungal cytochrome P450 14 α-demethylase. The result is a loss of normal fungal sterols and the accumulation of 14 alpha-methyl sterols within the fungus.

Usual Doses

For oropharyngeal candidiasis, and other forms of mucocutaneous candidiasis, fluconazole is typically dosed at 50-150 mg/day. For invasive candidiasis, the commonly applied dose is ~6 mg/kg/day, or 400 mg in the typical 70 kg adult. Doses of ~12 mg/kg are not FDA-approved, but are used with increasing frequency due to the desire to achieve higher blood levels and thus extend the range of use of the drug. Even higher doses (up to 30 mg/kg in one report) have been used safely (Berry et al 1992; Anaissie et al 1995; Haubrich et al 1994; Menichetti et al 1996; Voss et al 1999). Fluconazole is excreted by the kidneys, and the dose should be reduced in proportion to any reduction of kidney function (Debruyne et al 1993).
**Epidemiology and Risk Factors**

Fluconazole is generally quite well tolerated. In common with all azole antifungal agents, fluconazole may cause hepatotoxicity.

During the 1980s and 1990s, numerous reports emerged describing the development of fluconazole (Diflucan)-resistant oropharyngeal candidiasis in AIDS patients following prolonged exposure to fluconazole (Powderly et al 1999). During this time, the percentage of fluconazole-resistant *Candida* species ranged from approximately 5% to 33% (Powderly et al 1999; Barchiesi et al 2002; Law et al 1994). Investigators have identified low CD4 cell count, advanced immune suppression, greater number of fluconazole-treated episodes, and longer median duration of fluconazole therapy as the most important risk factors (Maenza et al 1996; 1997; White et al 1998). With the widespread use of fluconazole for oropharyngeal candidiasis in the late 1980s, the proportion of *C. albicans* isolates decreased and other *Candida* species, including *C. krusei*, *C. dubliniensis*, and *C. glabrata*, increased. Fluconazole-resistant oropharyngeal candidiasis has involved both *C. albicans* and the non-*albicans* species. In recent years, clinicians have observed a major decrease in the number of patients with fluconazole-resistant oropharyngeal candidiasis, predominantly as a result of the widespread use of HAART (Martins et al 1998).

**Routes**

Fluconazole has both oral and intravenous formulations.

**Current Status**

Fluconazole is a very widely used antifungal agent. It is one of the first-line drugs, particularly in treatment of infections due to *Candida* spp. other than *Candida krusei* and some *Candida glabrata* isolates. Fluconazole is commonly used also for prophylaxis in transplant patients (Patel 2000; Wolff et al 2000).
Itraconazole

Trade & Generic Names & General Features

Itraconazole (marketed as Sporanox® by Janssen Pharmaceutica) is a triazole antifungal agent that is prescribed to patients with fungal infections. The drug may be given orally or intravenously.

Mechanism of action

The mechanism of action of itraconazole is the same as the other azole antifungals: it inhibits the fungal cytochrome P	extsubscript{450} oxidase-mediated synthesis of ergosterol.

Indication

Itraconazole has a broader spectrum of activity than fluconazole (but not as broad as voriconazole or posaconazole). In particular, it is active against aspergillus, which fluconazole is not. It is also licenced for use in blastomycosis, histoplasmosis and onychomycosis. Itraconazole is over 99% protein bound and has virtually no penetration into cerebrospinal fluid. Therefore, it should never be used to treat meningitis or other central nervous system infections (Gilbert et al 2006). According to the Johns Hopkins Abx Guide, it has "negligible CSF penetration, however treatment has been successful for cryptococcal and coccidioidal meningitis" (The Safety Of Sporanox® Capsules And Lamisil® Tablets For The Treatment Of Onychomycosis 2001).

Dosing

Itraconazole is available as capsules or as an oral solution. The dose is 200 mg once a day, to 400 mg in severe infection. There is an intravenous preparation available in the US, but not in the UK. In the UK, if an intravenous preparation is required, then an alternative antifungal drug should be used.
The main problem with the use of itraconazole is its poor absorption, especially when given in capsule form. The oral solution is much better absorbed and should always be used in preference to the capsule. The cyclodextrin contained in the oral solution can cause an osmotic diarrhea, and if this is a problem, then half the dose can be given as oral solution and half as capsule in order to reduce the amount of cyclodextrin given. Itraconazole capsules should always be taken with food, as this improves absorption. Itraconazole oral solution should be taken an hour before food, or two hours after food (and likewise if a combination of capsules and oral solution are used). Itraconazole should be taken with orange juice or cola, as absorption is also improved by acid. Absorption of itraconazole is impaired when taken with an antacid, H₂-blocker or proton pump inhibitor.

In life-threatening situations, some doctors give an oral loading dose of 200 mg three times a day for three days, before dropping down to the usual dose. Because itraconazole absorption is unreliable, blood levels should be monitored at least once a week in those patients who are being treated for life-threatening (or potentially life-threatening) fungal infections.

In intravenous dosing, four doses of itraconazole 200 mg are given 12 hours apart, before changing the dose to once daily. There is no safety data for giving the intravenous preparation for more than 14 days continuously.

Adverse effects

Itraconazole is a relatively well-tolerated drug (although not as well tolerated as fluconazole or voriconazole) and the range of adverse effects it produces is similar to the other azole antifungals.

- Elevated alanine aminotransferase levels is found in 4% of people taking itraconazole.
- "Small but real risk of developing" Congestive Heart Failure (The Safety Of Sporanox® Capsules And Lamisil® Tablets For The Treatment Of Onychomycosis 2001).

The cyclodextrin that is used to make the syrup preparation can cause diarrhea. Side-effects that may indicate a greater problem include:
• nausea, vomiting, abdominal pain, fatigue loss of appetite, yellow skin (jaundice), yellow eyes, itching, dark urine, pale stool.

Production, marketing and Consumption

Itraconazole can be consumed orally in blue ¼ in (22 mm) capsules with tiny 1.5 mm blue pellets inside. Each tablet contains 100 mg and is usually taken multiple times a day such as every twelve hours. Sporanox, the brand name of Itraconazole, has been developed and marketed by Janssen Pharmaceutica Products, L.P. a subsidiary of Johnson and Johnson. Apparently, the three layer structure of these blue capsules are quite complex because Itraconazole is insoluble and is sensitive to pH. Parts of the processes of creating Sporinox were discovered by the Korean Patent Laid. The contents of the capsule, the tiny blue pellets, are manufactured in Beerse, Belgium (www.Fresh Patents.com. 2004; Janssen 2006).

Itraconazole is marketed as a treatment for at least three months and until test results find no fungal infections. It is also possible that Itraconazole treatment is prescribed indefinitely for some patients. Motivation to spend thousands of dollars for continued use is the risk and threat of the fungus regrowing in the body (Janssen 2006).

Clotrimazole

Clotrimazole is an antifungal medication commonly used in the treatment of fungal infections of both humans and animals such as vaginal yeast infections and ringworm. It also used to treat athlete's foot and jock itch.

General Information

Lotrisone lotion has been approved by the FDA for the treatment of symptomatic inflammatory tinea pedis, tinea cruris, and tinea corporis due to *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. Lotrisone contains two compounds - a synthetic corticosteroid (betamethasone dipropionate) and a synthetic antifungal agent (clotrimazole) - that target different aspects of fungal infection. Clotrimazole targets the cause of the
infection by inhibiting the growth of fungus on the skin, whereas betamethasone reduces fungal infection symptoms such as itching, swelling, and redness.

**Mechanism of Action**

Clotrimazole is a broad-spectrum, antifungal agent used for the treatment of superficial infections caused by species of pathogenic dermatophytes, yeasts, and *Malassezia furfur*. The mechanism of action involves inhibition of the synthesis of ergosterol, a major sterol in the fungal cell membrane. This leads to instability of the cell membrane and eventual death of the fungus.

Betamethasone dipropionate is a corticosteroid with anti-inflammatory, anti-pruritic, and vasoconstrictive properties. The exact mechanisms of action of corticosteroids in each disease are uncertain; however, betamethasone dipropionate has been shown to have dermatological and systemic pharmacologic and metabolic effects characteristic of this class of drugs. (from Mosby, Inc.)

**Side Effects**

Side effects of Lotrisone may include (but are not limited to) the following:-

- Blistering, Hives Infection, Irritated skin, Itching, Peeling, Skin eruptions and tingling sensation

Less common side effects may include the following:-

- Acne, burning, excessive hair growth and inflamed hair follicles
Ketoconazole

Trade & Generic Names & General Features

Ketoconazole is an imidazole antifungal agent. As with other imidazoles, it has five-membered ring structures containing two nitrogen atoms. It is marketed as Nizoral™ by Janssen Pharmaceutica (http://us.janssen.com/). Ketoconazole has oral tablet, cream and dandruff shampoo formulations. The oral formulation is available in USA since 1981. Ketoconazole is the only member of the imidazole class that is currently used for treatment of systemic infections.

Ketoconazole is a highly lipophilic compound. This property leads to high concentrations of ketoconazole in fatty tissues and purulent exudates. Expectedly, the distribution of ketoconazole into cerebrospinal fluid is poor even in the presence of inflammation. Its oral absorption and solubility is optimal at acidic gastric pH (Sheehan et al 1999; van der Merr 1980).

While ketoconazole was more widely used before the development of newer, less toxic, and more effective triazole compounds, fluconazole and itraconazole, its use has now been limited. It now appears as an alternative drug for specific indications.

Mechanism(s) of Action

As with all azole antifungal agents, ketoconazole works principally by inhibition of cytochrome P₄₅₀ 14 α-demethylase (P₄₅₀14 DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol (Lyman et al 1992). The affinity of ketoconazole for fungal cell membranes is less compared to that of fluconazole and itraconazole. Ketoconazole has thus more potential to effect mammalian cell membranes and induce toxicity (Como et al 1994).

Susceptibility Patterns

Ketoconazole is active against Candida sp., and Cryptococcus neoformans. However, its activity is limited compared to that of fluconazole and itraconazole (St-Germain et al 1995). Furthermore, due to its limited penetration to cerebrospinal fluid, it is clinically ineffective in meningeal cryptococcosis. Its activity against the
dimorphic moulds, *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis, Sporothrix schenckii, Paracoccidioides brasiliensis, and Penicillium marneffei* is favorable. However, fluconazole and itraconazole are at least as effective as ketoconazole against these fungi and are safer. Thus, ketoconazole remains as an alternative second-line drug for treatment of infections due to dimorphic fungi. Ketoconazole is not recommended for treatment of meningeal infections due to *Histoplasma capsulatum, Blastomyces dermatitidis,* and *Coccidioides immitis* due to its limited penetration to cerebrospinal fluid (Como et al 1994).

Ketoconazole is also active against *Pseudallescheria boydii* and is a good alternative for treatment of pseudallescheriasis (Sheehan et al 1999). It is also effective in pityriasis versicolor (Degreef et al 1994). Ketoconazole has practically no activity against *Aspergillus* sp., *Fusarium* sp., and zygomycetes order of fungi (Como et al 1994).

### Usual Doses

Oral ketoconazole is used at a dose of 200 to 400 mg/day in treatment of oral and chronic mucocutaneous candidiasis. The recommended dose is 400 mg/day in endemic mycoses due to dimorphic fungi in nonimmunosuppressed hosts (National Institute of Allergy and Infectious Diseases Mycosis Study Group 1985). The dose can be increased to 600-800 mg/day in patients not responding to regular doses. However, high doses carry the risk of high incidence of toxicity (Galgiani et al 1988).

Ketoconazole administered at a single dose of 400 mg/day is effective in treatment of pityriasis versicolor. The single dose therapy can be repeated once every month since recurrence is common with this infection (Degreef et al 1994).

### Side-Effects

The major drawbacks of ketoconazole therapy are from the occasionally seen adverse reactions. It may induce anorexia, nausea and vomiting (Como et al 1994; Dismukes et al 1983). Increase in transaminase levels and hepatotoxicity may occur (Lewis et al 1994; Walsh et al 1991). Ketoconazole may decrease testosterone and
cortisol levels, resulting in gynecomastia and oligospermia in men and women irregularities in women (O'Connor et al 2002; Thompson et al 1993).

The effects of ketoconazole on functions of the thyroid gland have also been investigated. There have been a number of reports suggesting that ketoconazole may have antithyroid function via impairment of thyroglobin iodination and iodothyrosine coupling. In vitro, ketoconazole has been found to form a complex with iodine and inhibit lactoperoxidase. In vivo experiments in rats have shown that the weight of thyroid gland increases in rats treated with ketoconazole (Comby et al 1994). In another study designed to investigate the influence of ketoconazole on the basal and TSH-stimulated iodide uptake in the rat thyroid cells, concentration-based variations were observed. Ketoconazole appeared to increase the basal iodide uptake slightly at lower concentrations, while it sharply decreased the uptake below the basal levels at higher concentrations. Under TSH stimulation, the inhibitory effects of the drug were observed even at low concentrations (Kohan et al 1992). In contrast to these animal data, studies carried on in a limited number of euthyroid, healthy individuals, hypothyroid patients, and patients with thyrotoxicosis failed to provide any data suggesting a potential inhibitory effect of ketoconazole on thyroid functions (De Pedrini et al 1988). These data suggest that possible antithyroid effect of ketoconazole demands further investigation and close follow-up.

Routes

Ketoconazole has oral tablet, cream and dandruff shampoo formulations.

Current Status

Although an effective compound, ketoconazole has become a second-line drug due to the ready availability of the somewhat safer agents, itraconazole and fluconazole.

Interestingly, administration of ketoconazole has successfully decreased the serum ionized calcium and 1,25-dihydroxyvitamin D levels in two patients with tuberculosis-associated hypercalcemia (Saggese et al 1993). Also, ketoconazole has provided biochemical and hormonal improvement in patients with paraneoplastic
Cushing's syndrome secondary to ectopic adrenocorticotropin (ACTH) production by malignant neoplasms (Winquist et al 1995).

**Definition of drug resistance**

Historically, clinical resistance has been defined as persistence or progression of an infection despite appropriate antimicrobial therapy. A successful clinical response to antimicrobial therapy typically not only depends on the susceptibility of the pathogenic organism but also relies heavily on the host immune system, drug penetration and distribution, patient compliance, and absence of a protected or persistent focus of infection (Galgiani et al 1987).

**Molecular mechanism of drug resistance**

Many different types of mechanisms are known to contribute to a drug-resistant phenotype in eukaryotic cells. The most frequent resistance mechanisms include reduction in the import of the drug into the cell; modification or degradation of the drug once it is inside the cell; changes in the interaction of the drug with the target enzyme (binding, activity); changes in other enzymes in the same enzymatic pathway; and an increased efflux of the drug from the cell.

**Drug Import**

Defects in drug import are a common mechanism of drug resistance. However, it is important to emphasize the distinction between the import of a drug into a cell and the gradual accumulation of the drug in the cell, which is the result of a balance between import into the cell and efflux of the drug from the cell. Analysis of drug import is difficult at best and requires that mechanisms of import be separated from efflux mechanisms. The standard method for studying drug import is to determine the amount of drug that associates with cells in extremely short periods (less than 10 s). This method has been used extensively in studying drug import in parasitic protozoans (Hedstrom et al 1999). The short incubation periods are achieved by mixing the cells and the drug above a layer of mineral oil. After the
short incubations, the cells are pelleted through the mineral oil and the amount of drug in the cell pellet is determined.

Studies of fungi have usually used labeled drug to monitor the amount of label that accumulates within the cell over several minutes. These accumulation studies have led to the identification of several fungal efflux mechanisms (see below). One study with *C. albicans* suggested that accumulation of [\(^3\)H] ketoconazole required glycolytically derived energy and was controlled by cell viability, environmental pH, and temperature (Boiron 1987). This study also showed that ketoconazole accumulation at low extracellular concentrations was saturable, implying a specific facilitator of import, while accumulation at high concentrations appeared to be by passive diffusion. Curiously, this study found no evidence for export, since the addition of unlabeled drug did not lower the concentration of labeled drug in the cells. Finally, data in this study suggested that other azole drugs and amphotericin B increased the accumulation of labeled ketoconazole in the cell (Boiron 1987). These results are not easily explained by the currently understood molecular mechanisms, which include an energy-dependent efflux pump for fluconazole. The energy requirement for ketoconazole accumulation described in this study is inconsistent with the energy-requiring efflux mechanisms: Elimination of energy levels would inhibit energy-dependent efflux pumps, resulting in increased drug accumulation, the opposite of what is seen with ketoconazole. These results suggest that active transport may be involved in ketoconazole import. It is possible that the ketoconazole and fluconazole are imported by two different mechanisms.

Drug import may also be affected by the sterol composition of the plasma membrane. Several studies have demonstrated that when the ergosterol component of the membrane is eliminated or reduced in favor of other sterol components such as 14α-methyl sterols, there are concomitant permeability changes in the plasma membrane and a lack of fluidity (Vanden Bossche et al 1987). These changes may lower the capacity of azole drugs to enter the cell.

Another common mechanism of drug resistance is modification of the target enzyme and/or other enzymes in the same biochemical pathway. For azole drugs,
that pathway is the ergosterol biosynthetic pathway. Analysis of the sterols of a cell can provide a wealth of information concerning the alterations that have occurred in a resistant strain. Modifications in the ergosterol pathway are likely to generate resistance not only to the drug to which the cells are exposed but also to related drugs.

**Molecular Alterations of the ERG11 Gene**

The predominant target enzyme of the azole drugs is lanosterol demethylase. The gene encoding this protein is currently designated *ERG11* in all fungal species, although it has previously been referred to as *ERG16* and *CYP51A1* in *C. albicans*. Several genetic alterations have been identified that are associated with the *ERG11* gene of *C. albicans*, including point mutations in the coding region, overexpression of the gene, gene amplification (which leads to overexpression), and gene conversion or mitotic recombination.

A point mutation results in the replacement of arginine with lysine at amino acid 467 of the *ERG11* gene (abbreviated R467K, where R = Arg and K = Lys). The mutation is positioned near the cysteine which coordinates the fifth position of the iron atom in the heme cofactor. The mutation is thought to cause structural or functional alterations associated with the heme (White 1997b).

Common mechanism of resistance in eukaryotic cells is gene amplification. The increase in the number of gene copies usually results in an increase in expression. Overexpression of the *ERG11* genes in *C. albicans* has not been associated with amplification of the *ERG11* gene (Sanglard et al 1995; White 1997a). However, in a clinical isolate of *C. glabrata*, increased levels of lanosterol demethylase were associated with a resistant phenotype (Vanden Bossche et al 1992). These increased levels of the protein were associated with *ERG11* gene amplification (Marichal et al 1997; Vanden Bossche et al 1994a) and correlated with an increase in *ERG11* mRNA levels (Vanden Bossche et al 1994b). In the same isolate, changes in drug efflux and in *ERG7* activity were also observed (Vanden Bossche et al 1994a). After 159 subcultures, the gene amplification, increased mRNA levels, enzyme levels, and drug efflux all reverted to normal. However, the
revertant retained partial resistance to fluconazole (Vanden Bossche et al 1994a). Recently, gene amplification of \textit{ERG11} in this isolate has been linked to a chromosome duplication, which results in overexpression of \textit{ERG11}, as well as altered expression of a variety of other proteins (Marichal et al 1997).

Two Major Types of Efflux Pumps

Eukaryotic cells contain two types of efflux pumps that are known to contribute to drug resistance: ATP binding cassette (ABC) transporters (ABCT) and major facilitators (MF) (Marger et al 1993; Michaelis et al 1995). Both types of pumps are known to cause drug resistance in other systems. The ABCT are frequently associated with the active efflux of molecules that are toxic to cells and are relatively hydrophobic or lipophilic, as is the case with most azole drugs. The MF has not been studied as extensively as the ABCT but is also associated with relatively hydrophobic molecules such as tetracycline (Marger et al 1993).

The ABCT are composed of four protein domains: two membrane-spanning domains (MSD), each consisting of six or seven transmembrane-spanning segments, and two nucleotide binding domains (NBD) (Michaelis et al 1995). The NBD of ABCT bind ATP through an ABC that consists of several conserved peptide motifs, including two Walker domains, a Signature domain and a Center domain. The ATP that is bound to the ABC is used as a source of energy for the ABCT, although the mechanism by which the ATP energy causes transport of the substrate molecule is unknown.

The MF does not contain NBD. They are composed primarily of 12 to 14 transmembrane segments (Marger et al 1993; Paulsen et al 1996). The MF uses the proton motive force of the membrane (gradient of \( \text{H}^+ \) across the membrane) as a source of energy. In general, the MF work by antiport; that is, protons are pumped into the cell and substrate molecules are pumped out.

Recently, the complete sequencing of the genome of \textit{S. cerevisiae} has allowed a determination of the total number of ABCT and MF in the genome of a eukaryotic cell. Thirty ABCT genes that contain an ABC have been identified
(Taglicht et al). Twenty-three of these genes appear to encode a transmembrane segment, which suggests that they could function as drug pumps and are closely related to known ABCT drug pumps. Twenty-eight MF genes were also identified in the *S. cerevisiae* genome by homology to the five known MF genes of fungi (Goff et al 1995). This suggests that *S. cerevisiae* contains at least 51 different genes encoding efflux pumps that could contribute to the drug resistance levels of the cell. There is good reason to believe that *Candida* and other fungi would have a similar number of efflux pumps in their genomes.
Chapter-2

Materials & Methods
Media and reagents used:

Steam sterilization of the prepared media, buffers and reagents was done. The following were the conditions:

**Pressure**: 15lb/sq inch

**Temperature**: 121°C

**Time**: 15 minutes

**Media:**

1. **Corn Meal Agar**:
   - Corn meal 50g/l
   - Infusion from agar 15g/l

2. **Saboraud Dextrose Agar**
   - Peptone special 10g/l
   - Dextrose 20g/l
   - Agar 17g/l

3. **HiChrome Candida Differential Agar**
   - Peptone special 15g/l
   - Yeast extract 4g/l
   - Dipotassium hydrogen phosphate 1g/l
   - Chromogenic mixture 7.22g/l
   - Chloramphenicol 0.5g/l
   - Agar 15g/l

4. **Yeast Peptone Dextrose**
   - Yeast extract powder 10g/l
   - Peptone 10g/l
   - D-glucose 20g/l
Reagents:

**Mac. Farland Reagent**

- \( \text{H}_2\text{SO}_4 \) 0.18M (99.5ml)
- \( \text{BaCl}_2 \) 0.048M (0.5ml)

**Methods:**

**Collection of samples:**

The present study was conducted for a period of 1 year 4 months from Nov. 2005 to March 2007 on samples isolated from Jawaharlal Nehru Medical College (JNMC) & Hospital, AMU and Feroz Specialist Hospital & Research Center (FSHRC), Aligarh.

A total of 1470 vaginal yeast cultures were collected from pregnant ladies in the labour room and from Gynaecology OPD of JNMC and FSHRC. Out of 1470 samples 600 samples were showing signs and symptoms of the disease. Of these 600 samples, only 175 samples were positive for *Candida*. From these 175 positive samples 200 Candida isolates were obtained.

A total of 1825 samples were collected from NICU, JNMC, AMU Aligarh. Out of 1825 samples 500 samples were showing signs and symptoms of the disease. Out of these 500 samples only 138 samples were positive for *Candida*. From these 138 positive samples 150 Candida isolates were obtained. These isolates were subjected to undergo morphological and biochemical identification and characterisation was further done. Clinical data such as age, sex, site of infection, predisposing factors, history of exposure to antifungals and clinical outcome of the patients are noted and shown in tables.
Morphological tests

1. Lactophenol Cotton Blue (LPCB)

Place a drop of lactophenol cotton blue (LPCB) on a clean glass slide with a dissecting needle, remove a small portion of the colony to be examined from agar surface and place it in the drop of LPCB. With two dissecting needles, gently tease apart the mycelial mass of the colony on the slide. Observe under microscope, first low power and then high power (Larone 1995).

Comparatively large and oval cells as compared to the small, round bacterial cells are distinguished. Pseudohyphae is also seen in case of yeast cell (San-Millan et al 1996).

2. KOH Preparation

Most organic substances that might be confused with fungi when seen through the microscope are converted to almost clear background in the presence of moderately strong alkali solutions. The fungi remain unaffected and are therefore easily demonstrated.

Some of the specimen is placed on a glass slide with 1-2 drops of 10% KOH. A coverslip is placed over the mixture and this slide is treated gently over a flame and then removed just before the mixture reaches the boiling point (Larone 1995).

3. Gram Staining

Fix the smear by passing it over a flame. Place crystal violet solution on the slide for 20 seconds. Wash gently in tap water. Decolorize quickly in solution of equal parts of acetone and 95% ethanol. Wash gently in tap water. Counterstain in Safranin for 10 second. Wash with tap water and air dry it. Observe the colour.

A purple color is a positive test for Candida (Larone 1995).
4. Corn Meal Agar

Cornmeal with Tween 80 is used in distinguishing the different species of candida. A quarter of the plate is used for each organism. A hard streak is made around the centre of the areas (slightly cutting the agar) and three or four soft streaks across the first one. Cover with a 22 X 22 coverslip and incubate at room temperature for three days. The plate without its lid is placed on the microscope. Different colonies show different morphology on CMA and they can be identified from the standard (San-Millan et al 1996).

5. Germ Tube Test

One of the most valuable and simplest tests for the rapid, presumptive identifications of Candida albicans. Using a capillary pipette, a portion of the yeast colony is emulsified in 0.5 ml of Fetal Bovine Serum. The preparation is incubated at 37 °C. A drop of serum is examined microscopically for the presence of germtubes (Larone 1995).

6. CHROM Candida Agar.

The media is dissolved in autoclaved distilled water. The conical flask with media in it is boiled on heater, cooled to 50 °C and poured in autoclaved petriplates. The specimen is then streaked on the solified plates, kept at 37 °C for 48 hours. Different species show colonies of different colour (Odds et al 1994).

Biochemical tests

1. Carbohydrate Fermentation Test

Fermentative yeasts recovered from clinical specimen produce CO₂ and alcohol. Production of gas and not a pH shift is indicative of fermentation. The test is done in tubes containing Durham tube inserts (small inverted tubes that trap CO₂). An aqueous suspension of yeast not exceeding a density of a Mac Farland standard is prepared and 0.1 ml is added to each of the fermentation broth tubes. The tubes are incubated at 30°C for 10-14 days, agitated daily. Positive fermentation is
evidenced by gas bubbles being trapped in durham tubes. Carbohydrates used are dextrose, maltose, sucrose, galactose, lactose and trehalose (Larone 1995).

2. Carbohydrate Assimilation Test

It measures the ability of a yeast to utilize a specific carbohydrate as the sole source of carbon in the presence of oxygen. Each tube is inoculated with a very dilute suspension of yeast cells with a very dilute suspension of yeast cells and incubated at room-temperature for 21 days. Assimilation is detected by the presence of growth (turbidity) in each tube. The major advantage is flexibility. Many more carbon sources in addition to the 12 standard can be tested (Salkin et al 1998).

**Antifungal susceptibility testing by Disc Diffusion method**

All the 350 positive Candida isolates were tested on Sabarauad Dextrose Agar for their sensitivity against six antifungals namely Amphotericin-B, Nystatin, clotrimazole, Itraconazole, Fluconazole and Ketoconazole.

5 ml of sterilized SDA broth were poured into autoclaved, plugged test tubes and each tube inoculated with positive samples of candida. The inoculated tubes are then kept in incubator at 37°C for 24 hours. Each inoculum is then spread over solidified SDA plates. Sterilized forceps were then used to apply antifungal discs and the sensitivity plates were incubated overnight at 37°C. The sensitivity was monitored by visualizing the zone of inhibition around the disc (Bauer et al 1996).
Table No. 4: List of Antifungals used

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Symbol</th>
<th>Concentration</th>
<th>Diameter of zone of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin-B</td>
<td>Ap</td>
<td>100 unit</td>
<td>10-18</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>Cc</td>
<td>10 mcg</td>
<td>12-18</td>
</tr>
<tr>
<td>Flucanazole</td>
<td>Fu</td>
<td>10 mcg</td>
<td>18-22</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>It</td>
<td>10 mcg</td>
<td>18-22</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Kt</td>
<td>10 mcg</td>
<td>18-22</td>
</tr>
<tr>
<td>Nystatin</td>
<td>Ns</td>
<td>100 mcg</td>
<td>15-23</td>
</tr>
</tbody>
</table>
Chapter-3
Isolation. Characterization &
Drug Resistance Pattern of
Candida Species Isolated from
Patients Attending
Gynecology OPD.
3.1 Introduction:

Limited data addresses the incidence of vulvovaginal Candidiasis. It suggests that approximately two-thirds of women experience at least one episode during their lifetime and nearly 50% of women have multiple episodes (Berg et al 1984; McCormack et al 1994). Although the majority of cases of vulvovaginal candidiasis are caused by C. albicans; however, episodes due to non-albicans species of Candida also appear to be increasing (Ozacan et al 2006, Kuriyama et al 2005; Sobel et al 1997; Cheng et al 2006). Most non-albicans Candida species have higher azole MICs, and infections caused by the non-albicans are often difficult to treat (Antunes et al 2004, Ostrosky et al 2003; Ribeiro et al 2000; Singh et al 2002; Sobel et al 1993).

A possible explanation for more frequent isolation of non-albicans species from vulvovaginitis patients may be the increased use of topical azole agents (Nyirjesy et al 1997; Marcia et al 2004). Patients who see a physician usually receive empirical therapy; vaginal cultures are not routinely obtained, and susceptibility testing is rarely performed.

Surveillance programs for candidemia have demonstrated that fluconazole resistance among C. albicans bloodstream isolates is rare (Pfaller et al 2002). It is analyzed that yeast isolates from vulvovaginitis patients have also shown the recovery of fluconazole-resistant C. albicans isolates to be an unusual event (Arzeni et al 1997; El-Din et al 2001; Antunes et al 2004, Ostrosky et al 2003; Ribeiro et al 2000; Sobel et al 2004), but they often have included a small number of isolates. The increased use of over-the-counter antifungals and prolonged therapy for recurrent candidiasis are risk factors for the emergence of azole resistance among C. albicans isolated from vulvovaginitis patients.

The purpose of this study was to determine the species distribution and prevalence of antifungal resistance among a large collection of Candida species isolates from patients with Vaginal Candidiasis.

There is mounting evidence suggesting that Candida bloodstream infections (BSI) have become a major problem in tertiary-care hospitals worldwide (Almirante et al 2005, Marchetti et al 2004, Pappas et al 2003, Tortorano et al 2004). Candidemia is generally difficult to diagnose and hard to treat, has a high attributable mortality rate, and is costly (Gudlaugsson et al 2003, Morgan et al 2005).
3.2 Material and methods:

The present study was conducted for a period of 1 year 4 months from Nov. 2005 to March 2007 on samples isolated from Jawaharlal Nehru Medical College (JNMC) & Hospital, AMU and Feroz Specialist Hospital & Research Center (FSHRC), Aligarh.

A total of 1470 vaginal yeast cultures were collected from pregnant ladies in the labour room and from Gynaecology OPD of JNMC and FSHRC. Out of 1470 samples 600 samples were showing signs and symptoms of the disease. Of these 600 samples, only 175 samples were positive for Candida. From these 175 positive samples 200 Candida isolates were obtained. Lactophenol Cotton Blue (LPCB), KOH & Gram staining slides were made of all the isolates to differentiate between yeast and bacteria. All the isolates having yeast like cells were plated on HiChrome Candida Differential Agar (Hi-Media chemicals, India), to ensure the detection of mix infections. Culture were incubated at 37 °C for 48 hrs. All the C.albicans show green colony on HiChrome Candida Differential Agar and they were further confirmed by the GTT(Germ Tube Test). GTT is positive for C.albicans only. For further morphological identification rest of the cultures were plated on Corn Meal Agar (Hi-Media chemicals, India). Further confirmation was done by sugar assimilation and sugar fermentation tests.

All the 200 positive Candida isolates were tested on Sabaraud Dextrose Agar for their sensitivity against six antifungals namely Amphotericin-B, Nystatin, Clotrimazole, Itraconazole, Fluconazole and Ketoconazole.

5 ml of sterilized SDA broth were poured into autoclaved, plugged test tubes and each tube inoculated with positive samples of candida. The inoculated tubes are then kept in incubator at 37°C for 24 hours. Each inoculum is then spread over solidified SDA plates. Sterilized forceps were then used to apply antifungal discs and the sensitivity plates were incubated overnight at 37°C. The sensitivity was monitored by visualizing the zone of inhibition around the disc (Bauer et al 1996).
3.3 Results:

This study was done on 200 isolates from 175 samples isolated from the 1490 patients attending gynecology OPD. Out of 200 isolates 129 (64.25 %) were C. albicans, 38 (19 %) were C. glabrata, 14 (7 %) C. parapsilosis, 10 (5 %) C. tropicalis, 6 (3 %) C. krusi, 3 (1.5 %) C. kyfer (Table 4, table 5.1, table 5.2). More comprehensive picture of % occurrence of each Candida species was shown in fig.3. Distribution of Candida species in different cultures with respect to age group of women is shown in table 6. Out of 175 women with positive Candida culture 20 (11.43 %) had the multiple positive cultures. From these 20 cultures, 45 Candida isolates were isolated. There are 155 cultures having single species 155 Candida species were isolated from these cultures. Out of 155 Candida isolates 113 (72.90 %) were C. albicans. The % of C. albicans in cultures having single species was higher than the % of C. albicans in total 200 (72.90 % vs 64.25 %). The age of the women at the 1st visit to gynecology OPD ranged from 15 years to 74 years. The maximum C. albicans infection was found in the women of age 15-54 years. The total patients in this age group were 163. Out of 163 patients C. albicans was isolated from 120 patients (73.61 %). 24 C. glabrata were isolated from 155 cultures. The % of C. glabrata in culture having single species was lower (15.48 %) than the % in total 200 Candida isolates (19 %). The maximum C. glabrata infection was found in women of age group 15-24 years. followed by 25-34 years. The total patients in this age group were 93. Of 93 patients, 26 patients were found to have C. glabrata (27.96 %). Total patients in age group of 35-44 years were 36. Out of 36 C. glabrata was isolated from 7 patients (19.44 %). 5.71 % infections of C. glabrata was found in the women of age group (45-54 years). 16.67 % of C. glabrata infection was found in older women (55-67 years). No C. glabrata infection was found in the women of age group, 65-74 years (table 6).

Out of 155 cultures having single species 9 strains of C. parapsilosis were isolated (5.88 %). Which is lower as compared to total 200 Candida isolates (7%). The maximum C. parapsilosis infection was found to be more prevalent in women of young age (15-24 years). The total patients in this age group were 56. Out of 56, C. parapsilosis was isolated from 7 patients (12.5 %) followed by 8.11 % among women of age group 25-34, 5.66 % in women of age group 35-44, 2.86 % in 45-54
age group women. No *C. parapsilosis* infection was found in the older women (55-74 years). 2 *C. tropicalis* were isolated from 155 cultures having single species (1.29%). It was lower than the % of *C. tropicalis* in total 200 cultures (1.29 % vs 5 %). Maximum (10.81 %), *C. tropicalis* infection was found in the women of age group 25-34 years. 8.93 % *C. tropicalis* infection was found in women of age group 15-24 years. 5.56 % *C. tropicalis* infection was found in women of age group 35-44 years. No *C. tropicalis* infection was found in the women of age group 45-74 years. % of *C. krusie* in cultures having single species is slightly higher (3.23%) than the % of *C. krusie* in total 200 isolates (3 %). Maximum (8.1 %), *C. krusie* infection was found in the women of age group 25-34 years. 3.57% *C. krusie* infection was found in women of age group 15-24 years. 2.78 % *C. krusie* infection was found in women of age group 25-34 years. No *C. krusie* infection was found in the women of age group 45-74 years. % of *C. kyfer* in cultures having single species is slightly higher than the % of *C. kyfer* in total 200 isolates (3.57 % vs 2.70 %). No *C. kyfer* infection was found in the women of age group 35-74 years. The % of non-albicans in total 200 isolates was slightly higher than the % of non-albicans in the cultures having single species (35.5 % vs 27.09 %). 15 out of 175 (8.57 %) patients were having infection with two species A total of 30 Candida isolates were yielded from these 15 cultures. Out of 30 isolates 11 isolates were *C. albicans* (36.67 %). The % of *C. albicans* in cultures having two species is lower (36.67%) than the % of *C. albicans* in cultures having single species (36.67 % vs 72.90 %), and % of *C. albicans* was quite less than the % of *C. albicans* in total 200 isolates (36.67 % vs 64.25 %). Out of 30 isolates total non-albicans were 19 (63.33 %). It was quite higher than the % of non-albicans in culture having single species (63.33 % vs 27.09 %) and the % non-albicans in total 200 isolates (63.33 % vs 35.5 %). Out of 15 cultures having two species 5 were having *C. albicans* and *C. glabrata*, 3 *C. albicans* and *C. tropicalis*, 2 *C. tropicalis* and *C. glabrata*, 2 *C. glabrata* and *C. parapsilosis*, 1 *C. albicans* and *C. kier*, and 1 *C. albicans* *C. krusie*. Out of 175 total cultures, there were 5 cultures having three species. Total isolates from these cultures were 15. Out of 15 isolates 5 were *C. albicans* (33.33 %). The % of *C. albicans* in total 200 isolates (33.33 %) was nearly half the % of *C. albicans* in culture having single species (64.25 %). The % of *C. albicans* in culture having three species(
33.33 %) was quite less than the % of *C. albicans* in culture having single species (72.90 %).% of *C. albicans* in culture having three species (33.33%) is slightly less than the % of *C. albicans* in culture having two species( 36.67%). % of non-albicans in cultures having three species was 66.67 %. It was quite higher than the % of non-albicans in total 200 isolates (35.5 %), it was higher than the % of non-albicans culture having single species (27.09 %) and it was slightly higher than the % of non-albicans in cultures having two species (63.33 %). More comprehensive picture of % occurrence of each species of Candida in different cultures is shown in (Table 7).

Majority of *C. albicans*, *C. glabrata*, *C. parapsilosis* were isolated from culture having single species as compared to culture mixture of two species and culture mixtutre of three species But in case of *C. tropicalis* majority were isolated from culture having two species(50 %) followed by culture having three species (30 %) and least from culture having single species (20 %). Majority of *C. krusie* and *C. kyfer* were isolated from culture having single species(83.33 %, 66.67 % respectively). No *C. krusie* and *C. kyfer* were isolated from culture having three species(table 8).

Every patients with multiple Candida isolates reported at least one of the symptoms like vulvovaginal itching, vulvovaginal pain, vulvovaginal discgarge. Every patients with vaginal candidiasis had at least one of the medical condition listed in (table 8), noted in their clinical profile on her visit to gynecology OPD. Out of 175 majority (57 %) of the patients had vulvar contact dermatitis, urinary tract infections (30 %), diabetes (25 %), Vulvar Intraepithelial Neoplasia (VIN) (24 %) vulvar vestibulitis (20 %), hormon replacement therapy (10 %), chronic sinussitis (4 % ), acne (3 %) , CIN (2.5 %) and urinary incontinence (1.5 %). Most of UTI infections were found in women of age group (45-54 years) followed by 25-34 years (12 out of 60), chronic sinussitis was found commonly in women of age group 15-24 years (5 out of 8), no chronic sinussitis was found in women of age group 55-74 years. Majority of acne was found in women of age group 15-24 years (5 out of 6), no acne was found in women of age group 35-74 years. Majority of contact dermatitis was found in women of age group 45-54 years (5 out of 6), followed by 35-44 years and least was found in 55-74 years. It was found that vulvar vestibulitis was found in women of age group 15-24 years (20 out of 40 years), and no results
were found in women, of age group 55-74 years. VIN was most common in women of age group 35-44 years (15 out of 48) followed by 45-54 years (12 out of 48). Least was found in age group 65-74 years. Lichen sclerosis was most common in women of age group 15-24 years (8 out of 20). No case was found in women of age group 55-74 years. Diabetes was most common in women of age group 45-54 years (11 out of 25) and least in women of age group 15-24 years (1 out of 25). CIN was also most common in women of age group 45-54 years (3 out of 5). No case was found in women of age group, 15-34 and 65-74 years. Urinary incontinence was found equally in all women of age group 35-64 years and no case have been detected among the women of age group 15-34 and 65-74 years. Hormon replacement therapy was most common in women of age group 45-54 years (10 out of 20), followed by 55-64 years (8 out of 20). No case was found in women of age group 15-44 years (table 8).

Most of strains were isolated from vagina as compared to cervical (70 %) including C. krusie (5 out of 6; 83.33 %), followed by C. glabrata (73.68 %). Other species were also predominantly isolated from vagina, compared to cervical. (table 10) Candida infection was found more prevalent among pregnant women as compared to non-pregnant women (55 %). % occurrence of C. albicans, C.glabrata, C. parapsilosis during pregnancy was higher than the % occurrence in non-pregnant women (55.81 % vs 44.74 %, 55.20 % vs 44.74 %, 64.29 % vs 35.71 % respectively). C. krusie was found to be present equaly among pregnant and non-pregnant women. Where as C. tropicalis and C. kier were isolated maximaly among non-pregnant women (60 % vs 40 %).

Susceptibility test for each species are shown in (table 11). Susceptibility testing was performed using disc diffusion method. Our results revealed that 63 % isolates were resistant against Itraconazole, followed by Ketoconazole (46 %), Fluconazole (20 %), Nystatin (17 %), Clotrimazole (16.5 %) and Amphotericin-B (7.5 %). More comprehensive picture is shown in (fig. 17). Itraconazole resistance was observed maximum in C. glabrata (78.95 %), followed by C. albicans (62.01 %), C. parapsilosis (50%), C. tropicalis (50 %), C. krusie (50 %) and least in C. kyfer (33.33 %). Ketoconazole resistance was observed maximum in C. glabrata (52.63 %), followed by C. albicans (46.51 %), C. parapsilosis (42.86 %), C. krusie (33.33 %), C. kyfer (33.33 %) and least C. tropicalis (30 %). Nystatin resistance was
observed maximum in *C. albicans* (23.26 %) followed by *C. tropicalis* (10 %) and least in *C. glabrata* (2.33 %), no resistance was observed in *C. parapsilosis*, *C. krusie*, *C. kyfer*. Maximum Clotrimazole resistance was observed maximum in *C. albicans* (23.26 %) followed by *C. glabrata* (2.23 %).

Maximum Fluconazole resistance was observed in *C. krusei* (66.67 %) followed by *C. tropicalis* (21.43 %), *C. krusie* (20 %), *C. albicans* (19.38 %). No resistance against Fluconazole was observed in *C. krusie*, and *C. glabrata*. Maximum Amphotericin-B resistant was observed in *C. parapsilosis* (21.43 %), followed by *C. glabrata* (15.79 %), *C. tropicalis* (10 %), and *C. albicans* (3.88 %). *C. krusie* (1 %). No resistance against Amphotericin-B was observed in *C. kyfer*. 
Table 4: Etiology and characterization of Candida species based on the morphology.

<table>
<thead>
<tr>
<th>Total no. Candida species isolated</th>
<th>% of total isolates</th>
<th>Morphology on KOH slide</th>
<th>Morphology on Hi-chromo Candida agar</th>
<th>Microscopic morphology on CMA at 37°C</th>
<th>Growth on 37°C on SDA</th>
<th>GTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>64.5% (129)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Green</td>
<td>Pseudohyphae with terminal chlamydospores; clusters of blastospores at septa</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>19% (38)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>White, Pink, Purple</td>
<td>No pseudohyphae, cells small, terminal budding</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>7% (14)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>White, Pale, Pink</td>
<td>Blastospores along curved pseudohyphae; giant mycelial cells</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>5% (10)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Black blue to blue gray with dark halo in centre</td>
<td>Blastospores anywhere along pseudohyphae</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>3% (6)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Pale, Pink, Purple</td>
<td>Pseudohyphae with “cross-sticks” blastospores</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. kyfer</td>
<td>1.5% (3)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Large, rough, pink to levenendor, often with darkened centers</td>
<td>Elongated blastospores resembling “logs in a stream along pseudohyphae”</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

No values are given in parenthesis
Total no. of patients = 175
Total no. of Candida isolates are 200
+ = positive
- = negative
GTT = Germ Tube Test
Fig. 2 Morphology of various Candida species on chrome Candida agar and Corn meal agar. (left chromeagar, right corn meal agar at 100X, at 25°C and 72 hrs. incubation
Fig. 2.2 Morphology of various Candida species on GTT slide and KOH slides at 100 X.
Table 5: Biochemical characterization of isolated specimen

5.1 Sugar Fermentation

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<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>64.5% (29)</td>
<td>AG</td>
<td>AG</td>
<td>A</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>19% (38)</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>7% (14)</td>
<td>AG</td>
<td>-</td>
<td>-</td>
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<td>AG</td>
</tr>
<tr>
<td><em>C. tropicalis.</em></td>
<td>5% (10)</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>3% (6)</td>
<td>AG</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td><em>C. kyfer</em></td>
<td>1.5% (3)</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>-</td>
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</tr>
</tbody>
</table>

No. are given in parenthesis
Total no. of patients = 175
Total no. of Candida isolates are 200

AG = Acid + Gas (+ve)
A = Acid formation (+ve)
- = No Acid No Gas (-ve)
Dex = Dextrose
Malt = Maltose
Lact = Lactose
Suc = Sucrose
Galact = Galactose
5.2 Sugar Assimilation

<table>
<thead>
<tr>
<th>Candida species isolated</th>
<th>% of total isolates</th>
<th>Dextrose</th>
<th>Maltose</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Galactose</th>
<th>Melibiose</th>
<th>Cellobiose</th>
<th>Inositol</th>
<th>Xylose</th>
<th>Raffinose</th>
<th>Trehalose</th>
<th>Dulcitol</th>
<th>KNO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>64.5% (129)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>C. glabrata</td>
<td>19% (38)</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>7% (14)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>-</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>5% (10)</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C. krusei</td>
<td>3% (6)</td>
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</tr>
<tr>
<td>C. kyfer</td>
<td>1.5% (3)</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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</table>

No. are given in parenthesis
Total no. of patients = 175
Total no. of Candida isolates are 200
+ = Positive
- = Negative
Fig. 3: Distribution pattern of different Candida species isolated from gynecology OPD
Table 6: Distribution of Candida species in different cultures with respect to age group of patients attending gynecology OPD

<table>
<thead>
<tr>
<th>Culture having single species</th>
<th>Total No. of Candida isolates</th>
<th>Total cultures</th>
<th>15-24 yrs</th>
<th>25-34 yrs</th>
<th>35-44 yrs</th>
<th>45-54 yrs</th>
<th>55-64 yrs</th>
<th>65-74 yrs</th>
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<tbody>
<tr>
<td>C. albicans (129)</td>
<td>113</td>
<td>28</td>
<td>17</td>
<td>25</td>
<td>32</td>
<td>4</td>
<td>6</td>
<td></td>
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<tr>
<td>C. glabrata (38)</td>
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<td>10</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>1</td>
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<tr>
<td>C. parapsilosis (14)</td>
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<td>4</td>
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<td>2</td>
<td>1</td>
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<td>C. tropicalis (10)</td>
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<td>C. krusei (6)</td>
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<td>3</td>
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<td>C. kyfer (3)</td>
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<td>Culture having two species</td>
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</tr>
</tbody>
</table>

a = No. of each species is given in parenthesis
Total no. of patients is 175
Total no. of Candida isolates 200
* = Age at first visit to gynecology OPD
Table 7: Distribution of Candida species in single culture and mix cultures

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Culture with Single species</th>
<th>Culture with Mixtures of Two species</th>
<th>Culture with Mixtures of three species</th>
<th>Total no. of each Candida species from all cultures</th>
<th>% of total isolates from all cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of Candida species isolated</td>
<td>% of total no. of each Candida species isolated</td>
<td>No of Candida species isolated</td>
<td>% of total no. of each Candida species isolated</td>
<td>No of Candida species isolated</td>
</tr>
<tr>
<td>C. albicans</td>
<td>113</td>
<td>87.60</td>
<td>11</td>
<td>8.5</td>
<td>5</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>24</td>
<td>63.16</td>
<td>9</td>
<td>23.68</td>
<td>5</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>9</td>
<td>64.29</td>
<td>3</td>
<td>21.43</td>
<td>2</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>2</td>
<td>20</td>
<td>5</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>C. krusei</td>
<td>5</td>
<td>83.33</td>
<td>1</td>
<td>16.67</td>
<td>0</td>
</tr>
<tr>
<td>C. kyfer</td>
<td>2</td>
<td>66.67</td>
<td>1</td>
<td>33.33</td>
<td>0</td>
</tr>
</tbody>
</table>

Total no. of patients is 175
Total Candida isolates from all cultures are 200
Table 8: Clinical profile and age distribution of 175 patients attending gynecology OPD

<table>
<thead>
<tr>
<th>Clinical states of patients</th>
<th>% of total 175</th>
<th>15-24 yrs</th>
<th>25-34 yrs</th>
<th>35-44 yrs</th>
<th>45-54 yrs</th>
<th>55-64 yrs</th>
<th>65-74 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract infection</td>
<td>30% (60)</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>25</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chronic sinusitis</td>
<td>4% (8)</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acne</td>
<td>3% (6)</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vulvar contact dermatitis</td>
<td>57% (114)</td>
<td>24</td>
<td>20</td>
<td>26</td>
<td>29</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Vulvar vestibulitis</td>
<td>20% (40)</td>
<td>20</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VIN</td>
<td>24% (48)</td>
<td>8</td>
<td>5</td>
<td>15</td>
<td>12</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Lichen sclerosis</td>
<td>10% (20)</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12.5% (25)</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CIN 1</td>
<td>2.5% (5)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td>1.5% (3)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td>10% (20)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

* = Age at first visit to gynecology OPD
Total no. of patients = 175
a = Total no. of Candida isolates 200
No. are given in parenthesis
CIN 1 = Cervical Intraepithelial Neoplasia 1
VIN = Vulvar Intraepithelial Neoplasia
Table 9: Occurrence of Candida species in vagina and cervical

<table>
<thead>
<tr>
<th>Candida species isolated</th>
<th>Candida isolates(^a)</th>
<th>No. of Candida isolates from cervical</th>
<th>No. of Candida isolates from vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>129 (64.5%)</td>
<td>40 (31%)</td>
<td>89 (68.99%)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>38 (19%)</td>
<td>10 (26.32%)</td>
<td>28 (73.68%)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>14 (7%)</td>
<td>5 (35.71%)</td>
<td>9 (64.29%)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>10 (5%)</td>
<td>3 (31%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>6 (3%)</td>
<td>1 (16.67%)</td>
<td>5 (83.33%)</td>
</tr>
<tr>
<td>C. kyfer</td>
<td>3 (1.5%)</td>
<td>1 (33.33%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>Total isolated species of Candida</td>
<td>200</td>
<td>60 (30%)</td>
<td>140 (70%)</td>
</tr>
</tbody>
</table>

Total no. of patients = 175
\(^a\) = Total no. of Candida isolates 200
% are given in parenthesis
Table 10: Distribution of Candida species in pregnant / non-pregnant women

<table>
<thead>
<tr>
<th>Candida species isolated</th>
<th>No. of Candida isolates(^a)</th>
<th>No. of Candida species isolated from pregnant women</th>
<th>No. of Candida species isolated from non-pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>129 (64.5%)</td>
<td>72 (55.81%)</td>
<td>57 (44.19%)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>38 (19%)</td>
<td>21 (55.26%)</td>
<td>17 (44.74%)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>14 (7%)</td>
<td>9 (64.29%)</td>
<td>5 (35.71%)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>10 (5%)</td>
<td>4 (40%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>6 (3%)</td>
<td>3 (50%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>C. kyfer</td>
<td>3 (1.5%)</td>
<td>1 (33.33%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>Total isolated species of Candida</td>
<td>200</td>
<td>110 (55%)</td>
<td>90 (45%)</td>
</tr>
</tbody>
</table>

Total no. of patients = 175

\(^a\) = Total no. of Candida isolates 200

% are given in parenthesis
Table 11: % Resistance of Candida species against different antifungals

<table>
<thead>
<tr>
<th>Candida species isolated</th>
<th>% occurrence of each Candida speciesa</th>
<th>% Resistance of each species against It</th>
<th>% Resistance of each species against Kt</th>
<th>% Resistance of each species against Ns</th>
<th>% Resistance of each species against Cc</th>
<th>% Resistance of each species against Fu</th>
<th>% Resistance of each species against Ap</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>64.25% (129)</td>
<td>62.01% (80)</td>
<td>46.51% (60)</td>
<td>23.26% (30)</td>
<td>23.26% (30)</td>
<td>4.56% (6)</td>
<td>3.88% (5)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>19% (38)</td>
<td>78.95% (30)</td>
<td>52.63% (20)</td>
<td>2.33% (3)</td>
<td>2.33% (3)</td>
<td>7.89% (3)</td>
<td>15.79% (6)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>7% (14)</td>
<td>50% (7)</td>
<td>42.86% (6)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>21.43% (3)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>5% (10)</td>
<td>50% (5)</td>
<td>30% (3)</td>
<td>10% (1)</td>
<td>0% (0)</td>
<td>20% (2)</td>
<td>10% (1)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>3% (6)</td>
<td>50% (3)</td>
<td>33.33% (2)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>66.67% (4)</td>
<td>66.67% (1)</td>
</tr>
<tr>
<td>C. kyfer</td>
<td>1.5% (3)</td>
<td>33.33% (1)</td>
<td>33.33% (1)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Total resistance of each antifungal drug</td>
<td>63% (126/200)</td>
<td>46% (92/200)</td>
<td>17% (34/200)</td>
<td>16.5% (33/200)</td>
<td>7.5% (15/200)</td>
<td>8% (16/200)</td>
<td></td>
</tr>
</tbody>
</table>

a = Total no. of Candida isolates 200
Total no. of patients is 175
No. are given in parenthesis
It = Itraconazole (10 mcg)
Kt = Ketoconazole (10 mcg)
Ns = Nystatin (100 mcg) (10 mcg)
Cc = Clotrimazole (10 mcg)
Fu = Fluconazole (10 mcg)
Ap = Amphotericin-B (100 mcg)
Fig. 4: Comparison of total resistant of different antifungals against different Candida species isolated from gynecology OPD.
3.4 Discussion:

In this study, *C. albicans* was the most prevalent species associated with vaginal candidiasis (64.25 %). Which is similar to results in other European (54-66 %) and Americans (45-55 %) countries (Pfaller et al 2001, Matteo et al 2006, Luzzati et al 2000). Candida *glabrata* was the second most frequently isolated yeast (25.7%), consistent with the results of other data (Linhares et al 2001, Cernicka et al 2006). This species is represented in 16.3% and 38.1% of the cases in the studies of Bauters et al. (Bauters et al 2002) and Verghese et al. (Verghese et al 2001), respectively.

The overall percentage of non-albicans vaginitis (35.5 %) is higher than in two previously reported data (Holland et al 2003, Cheng et al 2006). Spinillo et al. reported 17% of 209 isolates from symptomatic patients referred to an Italian vulvovaginitis clinic were non-albicans species in 1995 (Cheng et al 2006). An Australian study found vulvovaginal yeast carriage among 21% of 5, 802 women, receiving a pelvic exam in a primary care setting; non-albicans species were isolated in only 11% of the positive cultures (Holland et al 2003). This difference likely reflects a bias towards more complicated patients in the current study who are symptomatic and had prior episodes before being referred to a gynecology OPD. Non- albicans species are reported as lower rate in previous study (Ozacan et al 2006, Kuriyama et al 2005) copared to the current study.

The subset of isolates from patients with multiple positive cultures during the study period included a higher percentage of non-albicans species (64.44 %) than the isolates from patients with only one positive culture (27.09 % non-albicans). The marked difference in species distribution between these two groups may be the result of a higher level of antifungal exposure among patients with multiple positive cultures providing selective pressure for non-albicans species that are more likely to be azole resistant. An alternative explanation is that patients who develop vulvovaginitis from a non-albicans species are more likely to fail therapy and have multiple positive cultures.
The prevalence of *C. albicans* noted among the subset of patients with multiple positive cultures in the current study (38.55%) was similar to the prevalence of *C. albicans* reported by Candida bloodstream infection surveillance studies (52 to 56%) (Pfalle et al 1999). The prevalence of *C. glabrata* (17.78%) among patients with multiple positive vaginal cultures was similar to that reported among bloodstream isolates (8 to 18%) (Pfalle et al 2000).

The distribution of candida species in patients of different age group is similar to those of previous studies based on similar groups of host and body sites (Inping et al 2004; Bedini et al 2006). The percentage occurrence of different medical conditions among the patients is within the range of previous studies (Sandra et al 2005).

In the present study, statistically highly significant difference in incidence of vaginal candidiasis was observed between pregnant and non-pregnant women (Table 2). Vaginal candidiasis is more common and difficult to eradicate during pregnancy (Marcia et al 2004, Chander 2002). This is probably due to high level of reproductive hormones during pregnancy, which provides an excellent carbon source for growth of Candida. High incidence of vaginal candidiasis observed in patients using oral contraceptives, is similar to the earlier coworkers (Marcia et al 2004, Chander 2002). Significant influence increasing use of antibiotics in vaginal candidiasis was observed in the present study. Antibiotics are known to destroy the normal protective vaginal flora and help in colonization with Candida. *C. tropicalis* and *C. kyfer* are more frequently isolated from non-pregnant women. According to in vitro antifungal susceptibility testing, 8% and 7.5% of isolates are considered resistant to amphotericin B and fluconazole, respectively. The difference in rate of resistance between fluconazole and amphotericin B is due to different mechanisms of antifungal activity (Cernicka et al 2006, Pfaller et al 2002), frequency of use of different molecular mechanisms of drug resistance (Morgan, 2005, Cernicka et al 2006).

Fungal infections caused by non-albicans Candida species have been increasing dramatically (Bedini et al 2006, Slavin et al 1995, Kuriyama et al 2005).
C. krusei and C. glabrata have been considered intrinsically more resistant to fluconazole (Bedini et al 2006, Tortorano et al 2004). In this study, no C. krusei isolate was susceptible to fluconazole, but C. glabrata was less resistant to fluconazole than in other studies (10% vs 13% or 45.4%) (St. Germain et al 2001, Safdar et al 2001). Overall, non-albicans Candida species have higher rates of resistance to fluconazole than did C. albicans.

The increasing rate of fluconazole resistance in C. tropicalis (19%) is important because C. tropicalis is one of the most commonly found non-albicans Candida species (Ozcan et al 2006, Holland et al 2003). C. tropicalis develops drug resistance in the presence of fluconazole much more rapidly than does C. albicans (Barchiesi et al 2000, Antunes et al 2004). These findings may explain why C. tropicalis (19%) had a higher rate of resistance to fluconazole than C. albicans (5%) and C. glabrata (10%).


C. albicans exhibiting high-level resistance to fluconazole may express several mechanisms of resistance (Perea et al 2001, Morgan, 2005). Those isolates of C. albicans with a phenotype of high-level resistance to both fluconazole and itraconazole may overexpress both MDR and CDR efflux pumps with or without ERG16 mutations or overexpression, whereas strains with high-level resistance to fluconazole and susceptibility to itraconazole may over express the MDR pump but not the CDR pump (Perea et al 2001, Morgan, 2005).

Resistance of different Candida species to other antifungals (Ketoconazole, Nystatin, Clotrimazole, Amphotericin-B) are within the range of previous studies (Sandra et al 2005). Thus our study and previous studies (Sandra et al 2005, Antunes et al 2004) suggests Fluconazole and Amphotericin B as the most effective antifungal drugs in patients attending gynecology OPD.
Chapter-4
Isolation. Characterization &
Drug Resistance Pattern of Candida Species Isolated from NICU.
4.1 Introduction:

*Candida* species is important nosocomial pathogens in the newborn population, particularly among the preterm. Colonization of the neonatal skin and gastrointestinal tract is the first step in the pathogenesis of invasive Candidiasis (Pittet at al 1994) *C. albicans* is the most commonly isolated species in colonized or infected infants. However, in the past decade infection and colonization with other species of *Candida* has risen dramatically and high rates of yeast carriage in neonates, especially preterm has been reported by many workers (Sharp at al 1992; Singh at al 1999; Baley at al 1986) This has been attributed to the advancement in technology, life support systems, relative immunodeficiencies in the preterm, high prevalence of hand carriage of *Candida* in health care workers,(Phelps at al 1986; Burnie at al 1985) ability of *Candida* to survive on environmental surfaces (Pfaller 1995) and colonization of maternal vagina (Seeling 1996) Colonization of the infant occurs early in life and this is affected by a variety of common practices in the neonatal intensive care unit (NICU). Presently, there is a paucity of information on fungal colonization in preterm infants. Keeping the above facts in view this study had been undertaken in preterm admitted in NICU of a rural tertiary care hospital to find out the rate, risk factors and source of colonization by various species of *Candida*. Colonization with Candida species is an important risk factor for systemic infection in very low birth weight (VLBW; <1500 g) and extremely low birth weight (ELBW, <1000 g) infants. ELBW infants are at a higher risk than VLBW infants for fungal sepsis and its associated mortality, but few studies have examined fungal colonization exclusively in ELBW infants (Kaufman et al 2006). Invasive Candia infections have become the third most common cause of late-onset infection among very low birth weight infants in most neonatal intensive care units. Significant risk factors include birth weight less than 1000 g, exposure to more than two antibiotics, third generation cephalosporin exposure, parenteral nutrition including lipid emulsion, central venous catheter, and abdominal surgery. The majority of neonatal Candida infections are caused by *C. albicans* and *C. parapsilosis*, although other nonalbicans species are being reported more frequently (Chapman 2007).
4.2 Material and methods:

The present study was conducted for a period of 1 year 4 months from Nov. 2005 to March 2007 on samples isolated from Jawaharlal Nehru Medical College (JNMC) & Hospital.

Lactophenol Cotton Blue (LPCB), KOH & Gram staining slides were made of all the isolates to differentiate between yeast and bacteria. All the isolates having yeast-like cells were plated on HiChrome Candida Differential Agar (Hi-Media chemicals, India), to ensure the detection of mixed infections. Culture were incubated at 37°C for 48 hrs. All the *C. albicans* show green colony on HiChrome Candida Differential Agar and they were further confirmed by the GTT (Germ Tube Test). GTT is positive for *C. albicans* only. For further morphological identification rest of the cultures were plated on Corn Meal Agar (Hi-Media chemicals, India). Further confirmation was done by sugar assimilation and sugar fermentation tests.

All the 150 positive Candida isolates were tested on Sabouraud Dextrose Agar for their sensitivity against six antifungals namely Amphotericin-B, Nystatin, Clotrimazole, Itraconazole, Fluconazole and Ketoconazole.

5 ml of sterilized SDA broth were poured into autoclaved, plugged test tubes and each tube inoculated with positive samples of candida. The inoculated tubes are then kept in incubator at 37°C for 24 hours. Each inoculum is then spread over solidified SDA plates. Sterilized forceps were then used to apply antifungal discs and the sensitivity plates were incubated overnight at 37°C. The sensitivity was monitored by visualizing the zone of inhibition around the disc (Bauer et al 1996).
4.3 Results:

A total of 1825 samples were collected from NICU, JNMC, AMU Aligarh. Out of 1825 samples 500 samples were showing signs and symptoms of the disease. Out of these 500 samples only 138 samples were positive for *Candida*. From these 138 positive samples 150 Candida isolates were obtained at, AMU and Feroz Specialist Hospital & Research Center (FSHRC), Aligarh. Out of 150 isolates 79 (52.67 %) were *C. albicans*, *C. tropicalis* 27 (18 %), *C. parapsilosis* 6 (12.67 %), *C. glabrata* were 19 (10 %), *C. krusie* 10 (5 %), *C. kyer* 3 (1.5 %) (Table 12, 13, 14). More comprehensive picture of % occurrence of each Candida species is shown in fig.3.

Out of total 150 isolates, 55 (36.67 %) were from blood, 28 % from urine, 12 % from eye lid, 12.67 % from body surface, 6.67 % from umbilical tip and 4 % from CSF (Cerebrospinal fluid). Predominantly isolated species from blood was *C. albicans* (41.81 %) followed by *C. tropicalis* (27.27 %). Predominantly isolated species from urine was *C. albicans* (50 %) followed by *C. glabrata* (23.80 %). Predominantly isolated species from eye lid was *C. albicans* (55.56 %) followed by *C. krusie* (16.67 %). Predominantly isolated species from body surface *C. albicans* (63.16 %) followed by *C. tropicalis* (15.79 %). Predominantly isolated species from umbilical tip was *C. albicans* (70 %) followed by *C. tropicalis* (20 %). *C. albicans* was the only species isolated from CSF (Cerebrospinal fluid) (Table 15).

Maximum *C. albicans* infection was found in CSF followed by umbilical tip. Maximum *C. tropicalis* infection was found in blood (22.27 %) followed by umbilical tip. (20 %). Maximum *C. glabrata* infection was found in urine (23.8 %) followed by blood (12.72 %). Maximum *C. krusie* and *C. parapsilosis* infection was found in urine (23.8 %) followed by blood (10.9 %). Maximum *C. gluilliermondi* infection was found in body surface (Table 15).

Non-albicans species (58.18 %) was dominant over *C. albicans* (41.8 %) in blood isolates. Non-albicans species and *C. albicans* species are equally isolated from urine cultures. *C. albicans* species is dominated over the non-albicans species in eye lid, body surface, umbilical tip, and CSF cultures. (Table 15).
Total 150 Candida isolates were isolated from 138 neonates. Out of 150 Candida isolates, 90 (60%) were isolated from male neonates and 60 (40%) were isolated from female neonates. In male and female neonates C. albicans was predominantly isolated followed by C. tropicalis (Table 16).

The age of neonates at the time of sample collection vary from 5 days to 7 days. The frequency of C. albicans was observed in neonates of age 5 days followed by 6 days and 7 days (70%, 53.33%, and 45.45% respectively). Maximum Candida infections were observed in neonates of age of 7 days followed by 6 days and 5 days (73.33%, 20% and 6.67% respectively). The % of infection due to non-albicans species increases as the age of neonates increases (table 16).

The weight of the neonates at the time of sample collection ranges from 400 gm to 4000gm. Maximum (73.33%) candida infection was observed in neonates of weight group 400 gm-1000gm and least was observed in 3401-4000gm (2%). C. albicans was predominantly isolated from neonates of weight group 400-1000gm (50.90%) and least in 3401-4000gm (33.33%). Prevalence of non-albicans species increases as weight increases. The prevalence of Candida infection was higher (96.67%) in neonates having vaginal birth as compared to non-vaginal birth.

The gestational age varies from 24 weeks to 46 weeks. The neonates of gestational age 24 weeks to 35 weeks were preterm, 36-41 weeks term and 42-46 weeks post term. The prevalence of Candida infection was maximum in preterm of gestational age 24-29 weeks (53.33%) followed by 30-35 weeks (26.67%) and least Candida infection was observed in term neonates and postterm neonates (10% each). Frequency of C. albicans was maximum in 24-29 weeks gestational age neonates (56.25%) followed by 30-35 (26.67%) and least in 30-46 weeks gestational age. % of non-albicans increases as the gestational age increases (table 16).

Out of 138 neonates, 128 (92.75%) neonates were having infection of single species of Candida, 8 (5.78%) have infection of two species and 2 (1.45%) have infection of three species (table 17). Total 128 Candida isolates were isolated from culture having single species Out of 128 Candida isolates, 73 (57.03%) were C. albicans. The % of non-albicans in cultures having single species was 42.97%. Out of 8 patients having infection of two species 2 were having infection of C. albicans and C. glabrata, 2 C. albicans and C. parapsilosis, 2 C. tropicalis and C.
parapsilosis, 1 C. tropicalis and C. albicans and 1 C. tropicalis and C. glabrata. The % of C. albicans in cultures having two species was 31.25 %; it is quite lower than the % (57.03 %) of C. albicans in cultures having single species. The % of non-albicans in cultures having two species was 68.75 %; it is quite higher than the % (42.79 %) of non-albicans species in cultures having single species. Out of 2 patients having infection of three species, 1 was having infection of C. albicans and C. glabrata and C. tropicalis and 1 was having C. kruisie and C. parapsilosis and C. tropicalis. Total 6 Candida isolates were isolated from these two patients having infection of three species. Out of 6 Candida isolates, 1 (16.67 %) were C. albicans. The % of C. albicans in culture having three species was nearly half the of % C. albicans in culture having two species (31.25 %) and the % of C. albicans in culture having three species was nearly one third of the % C. albicans in culture having two species (57.03 %). The % of non-albicans in culture having three species was (83.33 %). It was higher than the % of non-albicans in cultures having two species (68.75 %) and the % of non-albicans in cultures having single species (42.97 %) (Table 17).

Susceptibility test for each species are shown in (table 18). Susceptibility testing was performed using disc diffusion method. Our results revealed that 52.67 % isolates were resistant against Itraconazole, followed by Ketoconazole (34 %), Fluconazole (18.67 %), Nystatin (13.33 %), Clotrimazole (10.67 %) and Amphotericin-B (5.70 %). More comprehensive picture is shown in (fig. 19). Itraconazole resistance was observed maximum in C. tropicalis (66.67 %), followed by C. kruisie (53.33 %), C. glabrata (52.63 %), C. albicans (50.63 %), C. parapsilosis (33.33 %) and least in C. gluilliermondi (25 %). Ketoconazole resistance was observed maximum in C. albicans (44.30 %), followed by C. parapsilosis (33.33 %), C glabrata (26.31 %), C. kruisie (26.67 %), C. gluilliermondi (25 %) and least C. tropicalis (14.81 %). Nystatin resistance was observed maximum in C. albicans (20.25 %) followed by C. parapsilosis (16.67 %) and no resistance was observed in C. gluilliermondi. Maximum Clotrimazole resistance was observed maximum in C. albicans (18.99 %) followed by C. tropicalis (3.70 %). No resistance was found in C. glabrata, C. kruisie, C. parapsilosis and C. gluilliermondi. (Table 18)
Maximum Fluconazole resistance was observed in C. *krusei* (44.67 %) followed by C. *albicans* (17.72 %), C. *glabrata* (15.78 %), C. *tropicalis* (14.81 %). No resistance against Fluconazole was observed in, C. *parapsilosis* and C. *gluilliermondi*. Maximum Amphotericin-B resistant was observed in C. *krusie* (16.67 %), followed by C. *tropicalis* (15.79 %), C. *albicans* (1.27 %), and no resistance against Amphotericin-B was observed in C. *glabrata*, C. *parapsilosis* and C. *gluilliermondi*. (Table 18).

Out of 150 Candida isolates, 40 Candida isolates were from neonates which were studied for their mode of transmission from mother's vaginal mucosa to her neonates (table 19). Out of 40 samples, 20 were from blood, 13 from urine and 7 from skin of neonates. The samples were also taken from their corresponding mother's vaginal mucosa. Of 40 neonates, 35 had vaginal delivery and remaining were through non-vaginal delivery. It was found that among 40 neonates; 9 (22.5 %) neonates showing to have same species as isolated from mother's vaginal mucosa. Among these species having concordance, only 7 were found to have same sensitivity pattern (77.78 %). The over all concordance was 7/40 (17.5 %). No concordance was found in neonates having non-vaginal delivery. Maximum concordance of Candida species was found in urine cultures (38.46 %) followed by skin cultures (28.57 %) and blood (20 %). Maximum susceptibility concordance was observed in urine cultures (23.67 %) followed by blood (75 %) and skin (14.29 %) (Table 19).
Table 12: Characterization of Candida species based on the morphology.

<table>
<thead>
<tr>
<th>Candida species isolated</th>
<th>% of total isolates</th>
<th>Morphology on KOH slide</th>
<th>Morphology on Hi-chromo candida agar</th>
<th>Microscopic morphology on CMA at 37°C</th>
<th>Growth on 37°C on SDA</th>
<th>GTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>52.67 (79)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Green</td>
<td>Pseudohyphae with terminal chlamydomspores; clusters of blastospores at septa</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>18 (27)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Black blue to blue gray with dark halo in centre</td>
<td>Blastospores anywhere along pseudohyphae</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>12.67 (6)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>White, Pale, Pink</td>
<td>Blastospores along curved pseudohyphae; giant mycelial cells</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>10 (19)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>White, Pink, Purple</td>
<td>No pseudohyphae, cells small, terminal budding</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>4 (15)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Pale, Pink, Purple</td>
<td>Pseudohyphae with “cross-sticks” blastospores</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. guillermondii</td>
<td>2.67 (4)</td>
<td>Round, oval, big, yeast like cells, budding cells</td>
<td>Large, rough, pink to leevendor, often with darkened centers</td>
<td>Fairly short, fine pseudohyphae, clusters of blastospores at septa</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

No. are given in parenthesis
Total no. of Candida isolates are 150
+ = positive
- = negative
GTT = Germ Tube Test
CMA = Corn Meal Agar
Table 13: Biochemical characterization of isolated specimen

Sugar Fermentation

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>52.67 (79)</td>
<td>AG</td>
<td>AG</td>
<td>A</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>18 (27)</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>12.67 (6)</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>10 (19)</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4 (15)</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td>C. guilliermondi</td>
<td>2.67 (4)</td>
<td>AG</td>
<td>-</td>
<td>AG</td>
<td>-</td>
<td>AG</td>
</tr>
</tbody>
</table>

No. are given in parenthesis
Total no. of patients is 138
Total no. of Candida isolates are 150
AG = Acid + Gas (+ive)
A = Acid formation (+ive)
- = No Acid No Gas (-ive)
Dex = Dextrose
Malt = Maltose
Lact = Lactose
Suc = Sucrose
Galact = Galactose
Table 14: Sugar Assimilation

<table>
<thead>
<tr>
<th>Candida species isolated</th>
<th>% of total isolates</th>
<th>Dextrose</th>
<th>Maltose</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Galactose</th>
<th>Melibiose</th>
<th>Cellobiose</th>
<th>Inositol</th>
<th>Xylose</th>
<th>Raffinose</th>
<th>Trehalose</th>
<th>Dulcitol</th>
<th>KNO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>52.67 (79)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>18 (27)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>12.67 (6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>10 (19)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4 (15)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. guillhermondi</td>
<td>2.67 (4)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

No. are given in parenthesis
Total no. of patients is 138
Total no. of Candida isolates are 150
+ = Positive
- = Negative
Fig. 5: Distribution pattern of different candida species isolated from NICU.
Table 15: Distribution of candida species at different sites

<table>
<thead>
<tr>
<th>Site of isolation of organism</th>
<th>Total positive isolate</th>
<th>C. albicans</th>
<th>C. tropicalis</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. parapsilosis</th>
<th>C. guilliermondii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>55</td>
<td>23</td>
<td>15</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Urine</td>
<td>42</td>
<td>21</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Eye lid</td>
<td>18</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Body surface</td>
<td>19</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Umbilical tip</td>
<td>10</td>
<td>7</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CSF*</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total no. of Candida isolates</td>
<td>150</td>
<td>79</td>
<td>27</td>
<td>19</td>
<td>15</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

% are given in parentheses
Total no. of Candida isolates are 150
CSF = *Cerebrospinal fluid
Table 16: Clinical profile of Candida species Among the neonates with respect to their sex, age, weight, gestational age & nature of birth

<table>
<thead>
<tr>
<th>Medical history</th>
<th>Total no. 150</th>
<th>C. albicans</th>
<th>C. tropicalis</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. parapsilosis</th>
<th>C. guilliermondii</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>90</td>
<td>45</td>
<td>15</td>
<td>13</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>34</td>
<td>12</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Age</strong> (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>16</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>110</td>
<td>50</td>
<td>20</td>
<td>18</td>
<td>14</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Weight</strong> (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400-1000</td>
<td>110</td>
<td>56</td>
<td>18</td>
<td>15</td>
<td>13</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1001-1600</td>
<td>20</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1601-2200</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2201-2800</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2801-3400</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3401-4000</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Vaginal Birth</strong></td>
<td>145</td>
<td>72</td>
<td>26</td>
<td>18</td>
<td>15</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><strong>Non-vaginal Birth</strong></td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Gestational age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-29 Preterm</td>
<td>80</td>
<td>45</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>30-35 Preterm</td>
<td>40</td>
<td>18</td>
<td>5</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>36-41 term</td>
<td>15</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>42-46 post term</td>
<td>15</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total no. of isolates of each species</strong></td>
<td>79 (52.67%)</td>
<td>27 (18.0%)</td>
<td>19 (12.67%)</td>
<td>15 (10.0%)</td>
<td>6 (4.0%)</td>
<td>4 (2.67%)</td>
<td></td>
</tr>
</tbody>
</table>

Total no. of patients is 138
Total no. of Candida isolates are 150
* = age at the time of sample collection
** = Weight at the time of sample collection
Table 17: Candida species distribution in different cultures

<table>
<thead>
<tr>
<th>Distribution of Candida species</th>
<th>No. ( %) of total culture</th>
<th>Total Candida isolates from culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture having single species</td>
<td>(128) 92.75</td>
<td>128</td>
</tr>
<tr>
<td>C. albicans</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C. gluilliermondi</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Culture having two species</td>
<td>(8) 5.78</td>
<td>16</td>
</tr>
<tr>
<td>C. albicans, C. glabrata</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C. albicans, C. parapsilosis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis, C. parapsilosis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C. albicans, C. tropicalis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C. glabrata, C. tropicalis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Culture having three species</td>
<td>(2) 1.45</td>
<td>6</td>
</tr>
<tr>
<td>C. albicans, C. glabrata &amp; C. tropicalis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis, C. parapsilosis &amp; C. krusei</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

No. are given in parenthesis
Total no. of patients is 138
Total no. of Candida isolates are 150
<table>
<thead>
<tr>
<th>Total no. of Candida species</th>
<th>% occurrence of each species</th>
<th>% Resistance of each species against It</th>
<th>% Resistance of each species against Kt</th>
<th>% Resistance of each species against Ns</th>
<th>% Resistance of each species against Fu</th>
<th>% Resistance of each species against Ap</th>
<th>% Resistance of each species against Ce</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>52.67 (79)</td>
<td>50.63 (40)</td>
<td>44.30 (35)</td>
<td>20.25 (16)</td>
<td>17.72 (14)</td>
<td>1.27 (1)</td>
<td>18.99 (15)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>18.0 (27)</td>
<td>66.67 (18)</td>
<td>14.81 (4)</td>
<td>3.70 (1)</td>
<td>14.81 (4)</td>
<td>3.70 (1)</td>
<td>3.70 (1)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>12.67 (19)</td>
<td>52.63 (10)</td>
<td>26.31 (5)</td>
<td>5.26 (1)</td>
<td>15.78 (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>10 (15)</td>
<td>53.33 (8)</td>
<td>26.67 (4)</td>
<td>6.67 (1)</td>
<td>46.67 (7)</td>
<td>16.67 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4 (6)</td>
<td>33.33 (2)</td>
<td>33.33 (2)</td>
<td>16.67 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. gluilliermondi</td>
<td>2.67 (4)</td>
<td>25.0 (1)</td>
<td>25.0 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total % resistant of each antifungal drug</td>
<td>150 (79/150)</td>
<td>52.67% (51/150)</td>
<td>34.0% (20/150)</td>
<td>13.33% (28/150)</td>
<td>18.67% (3/150)</td>
<td>5.70% (16/150)</td>
<td></td>
</tr>
</tbody>
</table>

Total no. of patients is 138  
a = Total no. of Candida isolates 150  
No. are given in parenthesis  
It = Itraconazole (10 mcg)  
Kt = Ketoconazole (10 mcg)  
Ns = Nystatin (100 mcg) (10 mcg)  
Cc = Clotrimazole (10 mcg)  
Fu = Fluconazole (10 mcg)  
Ap = Amphotericin-B (100 mcg)
Fig. 6: Comparison of total resistant of different antifungal against different Candida species isolated from NICU
Table 19: Transmission of Candida species from mother to neonates

<table>
<thead>
<tr>
<th>Neonates</th>
<th>Site of Specimen</th>
<th>Nature of delivery</th>
<th>Species from neonates</th>
<th>Concordant species from mother’s vagina</th>
<th>Sensitivity against antifungals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood</td>
<td>Vaginal</td>
<td>C. albicans</td>
<td>C. albicans</td>
<td>same</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>Non-Vaginal</td>
<td>C. glabrata</td>
<td>Different species</td>
<td>different</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. tropicalis</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. glabrata</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. glabrata</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. glabrata</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. glabrata</td>
<td>C. glabrata</td>
<td>same</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. tropicalis</td>
<td>C. tropicalis</td>
<td>different</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>Non-Vaginal</td>
<td>C. kruse</td>
<td>Different species</td>
<td>&quot;</td>
</tr>
<tr>
<td>11</td>
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<td>Vaginal</td>
<td>C. albicans</td>
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<td>&quot;</td>
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<tr>
<td>12</td>
<td>&quot;</td>
<td>&quot;</td>
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</tr>
<tr>
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<td>&quot;</td>
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<td>C. albicans</td>
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<td>&quot;</td>
</tr>
<tr>
<td>14</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. parapsilosis</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>15</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>16</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. tropicalis</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>17</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. tropicalis</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>18</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. parapsilosis</td>
<td>C. parapsilosis</td>
<td>same</td>
</tr>
<tr>
<td>19</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>Different species</td>
<td>different</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>21</td>
<td>Urine</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>22</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. tropicalis</td>
<td>C. tropicalis</td>
<td>same</td>
</tr>
<tr>
<td>23</td>
<td>&quot;</td>
<td>Non-Vaginal</td>
<td>C. albicans</td>
<td>Different species</td>
<td>different</td>
</tr>
<tr>
<td>24</td>
<td>&quot;</td>
<td>Vaginal</td>
<td>C. glabrata</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>25</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>26</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>C. albicans</td>
<td>different</td>
</tr>
<tr>
<td>27</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. parapsilosis</td>
<td>Different species</td>
<td>&quot;</td>
</tr>
<tr>
<td>28</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. tropicalis</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>29</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>30</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>C. albicans</td>
<td>same</td>
</tr>
<tr>
<td>31</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. gluilliermondi</td>
<td>Different species</td>
<td>different</td>
</tr>
<tr>
<td>32</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. glabrata</td>
<td>C. glabrata</td>
<td>same</td>
</tr>
<tr>
<td>33</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>Different species</td>
<td>different</td>
</tr>
<tr>
<td>34</td>
<td>Skin</td>
<td>&quot;</td>
<td>C. kruse</td>
<td>Different species</td>
<td>different</td>
</tr>
<tr>
<td>35</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>C. albicans</td>
<td>same</td>
</tr>
<tr>
<td>36</td>
<td>&quot;</td>
<td>Non Vaginal</td>
<td>C. tropicalis</td>
<td>C. albicans</td>
<td>different</td>
</tr>
<tr>
<td>37</td>
<td>&quot;</td>
<td>Vaginal</td>
<td>C. parapsilosis</td>
<td>C. glabrata</td>
<td>&quot;</td>
</tr>
<tr>
<td>38</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. glabrata</td>
<td>C. parapsilosis</td>
<td>&quot;</td>
</tr>
<tr>
<td>39</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>C. kruse</td>
<td>&quot;</td>
</tr>
<tr>
<td>40</td>
<td>&quot;</td>
<td>&quot;</td>
<td>C. albicans</td>
<td>C. tropicalis</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

a = Total antifungals are six
b = All the six antifungals have the same sensitivity
4.4 Discussion:

Earlier studies found *C. albicans* as the most common isolate from neonates followed by *C. tropicalis* and *C. parapsilosis* (Fridkin et al 2006). In our study, *C. albicans* was found to be the most common isolate like others reports (Narain 2003, Mendiratta et al 2006, Sharp et al 1992, Kaufman et al 2006). *C. albicans* was 52.67% it is slightly lower the previous reports (Mendiratta et al 2006, Narain 2003). Prevalence of other Candida species is in agreement of previous reports (Chen et al 2005, Colombo et al 2006), (table 12, 13, 14). More comprehensive picture is shown in fig. 18.

In our study *C. albicans* is the most common species isolated from all sites of isolation followed by *C. tropicalis* except urine and eye lid here *C. glabrata* and *C. krusie* are the 2nd most common species respectively, *C. tropicalis* 3rd most common species and *C. gluilliermondi* was the rarest species from all sites of isolation .Our findings are supported by the other co-workers (Malini et al 2005. Colombo et al 2006, Nucci et al 2007, Chen et al 2005).

Our findings of common infections of *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. gluilliermondi*, and *C. krusie* on different sites are quite similar to previous reports (Malini et al 2005, Clerihew et al 2006). In our study non-albicans species are predominantly isolated from blood cultures while albicans species are dominated in other cultures. Our findings are supported by previous reports (Malini et al 2005, Remya et al 2004). This may lead to resistance or a shift towards intrinsically resistant non- albicans species (table 15).

In our study male neonates are at high risk of Candida infections (60 %) as compared to female neonates (40 %). Our findings are supported by other workers (Pasqualotto et al 2005, Malini et al 2005, Richard et al 2007), (table 16). The frequency of *C. albicans* decreases as the age of neonates increases while infection by non-albicans increases as the age of neonate increases. Our findings are supported by the findings of other co-workers (Almirante et al 2007, Clerihew et al 2006, Schelonka et al 2003, Johnsson et al 2004). This may be because *C. albicans* are more susceptible to antifungals while non-albicans are less susceptible to
antifungals. As the age of neonates increases, the frequency of antifungal also increases, so the frequency of C. albicans decreases and non-albicans increases.

As the weight of neonates increases the rate of fungal infection decreases. Our findings are in agreement of with earlier published reports (Austin et al 2004, McGuire et al 2003, Kaufman et al 2006, Clerihew et al 2007). The frequency of C. albicans infection was higher in VLBW (very low birth weight) infants as compared to high birth weight infants and the frequency of non-albicans infection was lower in VLBW infants and higher in high birth weight infants. Our findings are in agreement with the findings of other co-workers (Nucci et al 2007, Colombo et al 2006). This could be related to the use of antifungals prophylaxis. C. albicans are becoming more susceptible to antifungals as compared to non-albicans species.

The prevalence of Candida infection is higher in vaginally birth neonates as compared to non-vaginally birth neonates. Our findings are supported by the other workers (Caramalac et al 2007). The neonates having vaginal birth have higher frequency of Candida infections because they acquire some Candida infections from mother’s vaginal mucosa and some infections from NICU.

The prevalence of Candida infection s increases as the gestational age decreases. The frequency of C. albicans infections decreases as the gestational age increases. The frequency of non-albicans infections increases as the gestational age increases. Our findings are in agreement with the previously published reports (Johnsson et al 2004, Clerihew et al 2004). This may because preterm neonates have low immunity (table 16).

In our study C. albicans were predominantly isolated from cultures having single species as compared to non-albicans species. While non-albicans species were predominantly isolated as compared to albicans from cultures having two species and three species. Our findings are in agreement with the reports of other co-workers (Mohan et al 2007, Clerihew et al 2007). In our study it shown that the frequency of C. albicans decreases with increase of age of neonates. The infection of more than one species also increases as the age increases. Here the % of C. albicans decreases.
in mix infections. Our study are supported by the findings of other co-workers (Qi QG et al 2005, Nucci et al 2007).

According to in vitro antifungal susceptibility testing, 5.70 % and 18.67 % of isolates are considered resistant to amphotericin B and fluconazole, respectively. The difference in rate of resistance between fluconazole and amphotericin B is due to different mechanisms of antifungal activity (Cernicka et al 2006, Pfaller et al 2002), frequency of use of different molecular mechanisms of drug resistance (Morgan, 2005, Cernicka et al 2006) (table 18).

Fungal infections caused by non-albicans Candida species have been increasing dramatically (Bedini et al 2006, Colombo et al 2006, Kuriyama et al 2005). C. krusei and C. glabrata have been considered intrinsically more resistant to fluconazole (Bedini et al 2006, Tortorano et al 2004). In this study, no C. krusei isolate was susceptible to fluconazole, but C. glabrata was less resistant to fluconazole than in other studies (15.78 % or 45.4%) (St. Germain et al 2001, Safdar et al 2001). Overall, non-albicans Candida species have higher rates of resistance to fluconazole than did C. albicans.

The increasing rate of fluconazole resistance in C. tropicalis (14.81 %) is important because C. tropicalis is one of the most commonly found non-albicans Candida species (Nucci et al 2006, Kaufman et al 2006). C. tropicalis develops drug resistance in the presence of fluconazole much more rapidly than does C. albicans (Barchiesi et al 2000, Antunes et al 2004). The use of azole antifungals, particularly fluconazole, may also lead to a pathogen shift with increased incidence of the inherently resistant Candida species such as C. glabrata and C. krusei (Antunes et al 2004, Ostrosky et al 2003). The widespread use of azoles has led to an increase in the prevalence of fluconazole-resistance among non-albicans species, especially C. glabrata (Sobel 1998, Vazquez et al 1999, Nguyen et al 1996, Cross et al 2000, Alexander et al 2005, Morgan, 2005). The use of fluconazole did not modify the relationship between colonization and the subsequent development of invasive fungal infection. Prophylactic fluconazole reduces the incidence of colonization and invasive candida infection in neonates weighing less than 1500 g at birth.
C. albicans exhibiting high-level resistance to fluconazole may express several mechanisms of resistance (Perea et al 2001, Morgan, 2005). Those isolates of C. albicans with a phenotype of high-level resistance to both fluconazole and itraconazole may over express both MDR and CDR efflux pumps with or without ERG16 mutations or over expression, whereas strains with high-level resistance to fluconazole and susceptibility to itraconazole may over express the MDR pump but not the CDR pump (Perea et al 2001, Morgan, 2005).

Resistance of different Candida species to other antifungals (Ketoconazole, Nystatin, Clotrimazole, Amphotericin-B) are within the range of previous studies (Almirante et al 2007). Thus our study and previous studies (Almirante et al 2007, Antunes et al 2004) suggests Clotrimazole and Amphotericin B as the most effective antifungal drugs in neonates in NICU.

For vaginal route group, the rate of mother-neonates concordance at the level of species was 22.5 % and no concordance was found in cesarean birth neonates. Concordance of neonates at species level is slightly lower (22.5 %) than the concordance (23.5 %) reported by previously published reports (Caramalac et al 2007, Fridkin et al 2006). The over 11 concordance (17.5 %) in our study is higher than the concordance (6 %) in previous reports (Caramalac et al 2007, Mendiratta et al 2006).

Our study indicates that transmission of Candida from mother to neonates is not the major cause of Candida infection in neonates but non-perinatal nosocomial transmission of Candida species is the predominant mode of acquisition by neonates in NICU, at AMU. Mother may be colonized with multiple strains of Candida simultaneously, colonizing Candida strains can cause invasive disease in neonates; and molecular biology based techniques are necessary to determine epidemiologic relatedness of maternal and infant Candida isolates and to facilitate mode of transmission.
General Discussion
In NICU and Gynecology OPD C. albicans was the most common species. In case of women C. glabrata was the 2nd most species (Clerihew et al 2007) but in case of NICU C. tropicalis was the 2nd most common species (Colombo et al 2006). In case of neonates infections due to non-albicans species like C. tropicalis, C. parapsilosis are increasing as the age of neonates increases (Nucci et al 2006). In women the maximum C. albicans frequency is observed in school age women (15-21 years) followed by 22-35 years (Mohan et al 2007). Non-albicans infections are common in women above age of 35 to 70 years. As the age become above 45 years the frequency of Candida infection decreases. In case of neonates the some of Candida infection are transmitted from mother’s vaginal mucosa and some are acquired from NICU. As age increases the infections due to multiple Candida species increases (Clerihew et al 2004).

Antifungal pharmacology in neonates compared with adults. Pharmacokinetic data suggest dosing differences in children versus adult patients with some antifungals, but not all agents have been fully evaluated. The available pharmacokinetic data on the Amphotericin-B deoxycholate formulation in neonates exhibit considerable variability; nevertheless, the dosage regimen suggested in the neonatal population is similar to that used in adults. More pharmacokinetic information is available on the liposomal and lipid complex preparations of amphotericin B and fluconazole, and it supports their use in neonates; however, the optimal dosage and duration of therapy is difficult to establish. All amphotericin-B formulations, frequently used in combination with flucytosine, are useful for treating disseminated fungal infections and Candida meningitis in neonates. Fluconazole, with potent in vitro activity against Cryptococcus neoformans and almost all Candida species, has been used in neonates with invasive candidiasis at dosages of 6 mg/kg/day, and for antifungal prophylaxis in high-risk neonates. There are limited data on itraconazole, voriconazole, and posaconazole use in neonates. Caspofungin, which is active against Candida species and Aspergillus species, requires higher doses in children relative to adults, and dosing is best accomplished based on body surface area (Almirante et al 2007).
Compared to adults, pediatrics received more frequently broad-spectrum antibiotics, vasopressors, blood transfusions, arterial catheter, chest tube, cardiothoracic surgery, mechanical ventilation, and parenteral nutrition. Candidaemia caused by Candida parapsilosis was more common in pediatrics, as was the isolation of Candida species from catheters (Pasqualotto et al 2005).
Conclusion
The aim of this study was isolation, characterization and drug resistance pattern of Candida species isolated from NICU and patients attending gynecology unit. In patients attending gynecology OPD it is observed that the *C. albicans* is the most common pathogenic fungi followed by *C. glabrata*. The college age women are at higher risk of *C. albicans* infection while the women of child bearing age are at higher risk of non-albicans infections. Over all menopause women are at higher risk of Candida infections as compared to post menopause women. The frequency of non-albicans mix infections increases as the age of menopause women increases. *C. albicans* are predominantly isolated from cultures of single species while the non-albicans are predominantly isolated from mixed cultures. Hormone replacement therapy, use of antibiotics and contraceptive are major risk factors for Candida infections in women. Candida species are predominantly isolated from vagina as compared to Cervical because vagina has warm and most moist environment which is favorable to Candida growth and colonization. Most of the Candida species are predominantly isolated from pregnant woman as compared to non-pregnant woman *C. tropicalis* and *C. krusie* are predominantly isolated from non-pregnant woman.

Amphotericin-B and Fluconazole are the most susceptible antifungal drugs in case of women. In case of neonates also *C. albicans* are the most common fungi. In non-albicans *C. tropicalis* is the 2nd most common species. Blood is the most common site of infection. The lowest frequency of *C. albicans* infection is observed in blood as compared to other sites of infection. Male neonates are at higher risk of Candida infection as compared to female neonates. The frequency of mixed infections due to non-albicans species increases as the age of neonates increases. VLBWC (very low birth weight) neonates or SGA (small for gestational age) are higher risk of Candida as compared to LGAC (large for gestational age) neonates. Preterms are having more Candida infection as compared to terms neonates due to their low immunity and often are having problems in gaining weight.

Amphotericin-B and Clotrimazole are the most successful antifungal drugs in case of neonates. Our study indicates that transmission of Candida from mother to neonates is not the major cause of Candida infection in neonates but non-perinatal nosocomial transmission of Candida species is the predominant mode of acquisition
by neonates in NICU, at AMU. Mother may be colonized with multiple strains of Candida. Simultaneously, colonizing Candida strains can cause invasive disease in neonates. Molecular biology based techniques are necessary to determine epidemiologic relatedness of maternal and infant Candida isolates and to facilitate mode of transmission.


Immunologic and hematologic reconstitution after allogeneic bone marrow transplantation. Transplantation. 41:583-6.


108. Dismukes WE. 2000. Clinical Infectious Diseases Introduction to Antifungal Drugs From the Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham School of Medicine. 30:653-657.


179. Infection in preterm infants. Cochrane Database Syst Rev; 1


