ECMM Workshop 2010
Advances in the Understanding of the Pathogenesis of Opportunistic Fungal Diseases:

CANDIDOSIS

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Review article:

Candida, still number one – what do we know and where are we going from there?

Candida, immer noch Nummer Eins: Was wissen wir, und wie geht es weiter?

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Mycoses, 48, (Suppl. 1) 3–11, 2005

So, what is new since then?
This presentation will overview the state of art of the pathogenesis of candidosis, focusing on the present knowledge in the areas of:

1. **specific virulence factors** of the fungus, such as biofilm formation, tissue invasion, role of yeast-hyphal transition and site specific adaptation
2. **novel models for studying pathogenesis**: the Drosophila system - what can we learn from it
3. **epidemiologic, demographic changes in Candida species distribution**: Candida albicans vs non-Candida albicans species; are there explanations for the changes?
4. **intra-species genotypic** and possible correlations with the various clinical entities: are there differences in the virulence of strains and could it be possible to detect such differences and identify specific strains with defined virulence attributes?
5. **interaction between the fungus and the host**: events in the microbe and in the host following the interaction - transcriptional changes in the microbe and effects in the host, such as cytoskeletal rearrangements affecting cellular functions and host immune response
MAJOR CANDIDA VIRULENCE ATTRIBUTES

- Host recognition
- Adhesion to host tissue (Biofilm formation)
- Transition yeast → hyphae
- Tissue invasion: hydrolytic enzymes (e.g. SAPs, phospholipases)
- “Switching” (in *C. albicans*)
- Immunomodulation: overcomes host defense
WHAT IS A BIOFILM?

- A biofilm is an aggregate of **microorganisms** in which **cells** adhere to each other &/or to a surface. These cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).
- Biofilm EPS, is a **polymeric** conglomeration composed of extracellular **DNA**, **proteins** & **polysaccharides**.
- Biofilms may form on living or non-living surfaces and represent a prevalent mode of microbial life in natural, industrial and hospital settings.
- The microbial cells growing in a biofilm are **physiologically** distinct from **planktonic** cells.
- Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites, nutrients or exposure of planktonic cells to sub-inhibitory concentrations of antibiotics.

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CURRENT UNDERSTANDING OF FUNGAL BIOFILMS

Early 0–11 hr

Intermediate 12–30 hr

Maturation 31–72 hr

Adhesion

Filamentation (Als1, Als3, Ace2, Bcr1, Hwp1, Sun41, Eap1, Mnt1, Mnt2, Och1 and Pmr1)

Pathogenicity (Saps, PLBs)

Antifungal Resistance (Cdr1, Cdr2, Mdr1, MkC1, Erg11)

Persistors ?

Extracellular Matrix Production

Farnesol ← Quorum Sensing → Tyrosol

WHAT IS THE CLINICAL SIGNIFICANCE OF BIOFILMS?  
– BIOFILM FORMATION ON MEDICAL DEVICES

Candida has the capacity to adhere to inert surfaces of various polymeric materials of medical devices forming biofilms, e.g:  
* contact lenses  
* artificial dentures  
* urinary catheters  
* Intravenous catheters

<table>
<thead>
<tr>
<th>Device</th>
<th>Annual use in USA</th>
<th>Infection rate (%)</th>
<th>% Candida infection out of total (most common)</th>
<th>Mortality rate (%)</th>
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<tr>
<td>Vascular catheters</td>
<td>5x10⁶</td>
<td>3-8</td>
<td>10 <em>C. albicans</em>, <em>C. glabrata</em></td>
<td>26-38</td>
</tr>
<tr>
<td>Dialysis</td>
<td>2.4x10⁶</td>
<td>1-4</td>
<td>&lt;1 <em>C. albicans</em>, <em>C. parapsilosis</em></td>
<td>25-50</td>
</tr>
<tr>
<td>Cardiac Devices (valves)</td>
<td>8.5x10⁴</td>
<td>2.9</td>
<td>2-10 <em>C. albicans</em>, <em>C. glabrata</em>, <em>C. parapsilosis</em></td>
<td>33</td>
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<tr>
<td>Urinary catheters</td>
<td>3x10⁷</td>
<td>10-30</td>
<td>21 <em>C. albicans</em>, <em>C. glabrata</em></td>
<td>19.8-39</td>
</tr>
</tbody>
</table>

Attempts to intervene in biofilm formation on medical devices as tool for prevention of infection

**Principle:**

- **Inhibit adhesion:**
  - competitive binding to microbial adhesins
  - competitive binding to cellular receptors

- **Interfere in biofilm formation:**
  - antimicrobial agents
  - modification of surface properties of biomaterials
  - disinfectants or chemicals (silver coatings of catheters)
Inhibition of Candida adhesion on polymeric surfaces – Segal et. al

- A chitin soluble extract (CSE) was found to inhibit adhesion of Candida to various cells & tissues.
- In vivo CSE prevented development of Candida infection
- CSE inhibited adhesion to inert surfaces

Sionov et al., Mycoses, 2001
Rothman et al., Mycoses, 2003
Coating of polyurethane (polymer of IV catheters) with CSE

A schematic representation showing CSE chemical binding to polyurethane via spacer (WSC) molecule.

Polyurethane Coated With CSE To Prevent Candida Adherence

From:
Mean adherence of C. albicans to coated PU surfaces.

Examples of In Vivo Studies to prevent biofilm formation

- Demonstration of Antibiofilm and Antifungal Efficacy of Chitosan against Candidal Biofilms, Using an In Vivo Central Venous Catheter Model. Martinez, et al. 2010

A C. albicans CVC biofilm model was used for in vivo experiments. Chitosan-treated CVCs were generated by incubating polyethylene catheters in chitosan. Chitosan-treated catheters were inserted in Sprague-Dawley rats. Chitosan inhibits candidal biofilm formation in vivo.

- Treatment and prevention of Candida albicans biofilms with caspofungin in a novel central venous catheter murine model of candidiasis. Lazell, Lopez-Ribot; 2009

We developed murine model of CVC-associated candidiasis for in vivo biofilm formation. Following model development, caspofungin was instilled in the catheter to treat preformed biofilms. The results indicated that caspofungin treatment significantly reduced biofilm fungal load in the catheters and dissemination to kidneys compared with untreated controls. In a second set of experiments catheters were pre-treated by filling caspofungin before challenge with C. albicans via the CVC. Again, the results indicated a significant reduction in biofilm fungal load and dissemination to kidneys.
Examples of In Vitro Studies to prevent biofilm formation

• **Prevention of *Candida albicans* biofilm formation by covalently bound dimethylaminomethacrylate and polyethyleneimine. De Prijck et.al, 2010**
  Candida albicans biofilms are a major cause of *voice prosthesis* deterioration in laryngectomized patients. The aim of this study was to produce a surface capable of inhibiting *C. albicans* biofilm formation. Dimethylaminoethylmethacrylate (DMAEMA) and polyethyleneimine (PEI) moieties were covalently bound to the surface of polydimethylsiloxane (PDMS) or polymethylmethacrylate (PMMA) and subsequently quaternized. Physicochemical characterization of the grafted surfaces was carried out and their effect on *C. albicans* cell numbers was assessed. Covalently bound quaternized polyDMAEMA (polyDMAEMAq) and PEI (PEIq) inhibited biofilm growth, with reductions up to 92%.

• **Effect of tunicamycin on *Candida albicans* biofilm formation and maintenance. Pierce et al., 2009**
  The effect of tunicamycin, a nucleoside antibiotic that inhibits N-linked glycosylation affecting cell wall and secreted proteins, on *C. albicans* biofilm formation was studied. Tunicamycin displayed significant inhibitory effects on biofilm development.
The Lock Theory of Ghannoum

Rabbit Model of *Candida* Biofilm Infection: AAC 2004
Liposomal Amphotericin B Antifungal Lock Therapy

FIG. 1. Surgical placement of the intravenous catheter. (A to C) Catheter insertion into the external jugular vein; (D to F) attachment of the heparin lock device to skin; (G) postoperative venogram of catheter placement.
More Examples of Studies to prevent biofilm formation

• **Effective prevention of microbial biofilm formation on medical devices by low-energy surface acouistic waves.** Hazan et al., AAC, 2006
  Low-energy surface acoustic waves generated from electrically activated piezo elements are shown to effectively prevent microbial biofilm formation on indwelling medical devices. The development of biofilms by Candida species is prevented.

• **In vitro efficacies of caspofungin or micafungin catheter lock solutions on Candida albicans biofilm growth.** Cateau et al. J Ant Chem;2008
  The data suggest that caspofungin and micafungin could represent good candidates for the reduction or control of fungal biofilms associated with silicone medical devices, as part of an antifungal lock. They were able to induce a significant and persistent reduction in the yeast metabolic activity of intermediate and mature of intermediate and mature biofilms, 12 h and 5 days old, respectively, when used as catheter lock solutions.
Candida adhesion to host tissues

- Adhesion of a microbe to host cells is a major step in development of infection
- Observations indicate that adhesion is not a passive event: it causes changes in the cells & microbes

QUESTIONS TO BE ASKED:
- Events occurring in host cells post interaction with Candida?
- Events occurring in Candida post interaction with host cells?
Post Interaction Events in Candida Segal et al

- Interaction of *C. albicans* with HEp2 cells
  - DNA microarray technology (Georgetown University):
    - Genes from the ALS family up-regulated
  - Gene for manosyl transferase up-regulated
  - Down-regulated genes encoding actin proteins (ABP1, SAC6)
  - Down-regulated the gene for beta-glucosidase (SUN4)

From: Sandovsky-Losica H., Chauhan, N., Calderone, R. Segal, E. Gene transcription studies of Candida albicans following infection of HEp2 epithelial cells Med Mycol 2006
Post Interaction Events in Candida:
Segal et al

- Interaction of *C. albicans* with HEp2 cells

**RT-PCR STUDIES:**

- ALS genes up-regulated

Sandovsky-Losica *et al.*
Med Mycol 2006
Events in *C. albicans* during Infection or Post Interaction with Host (1)


> The exceptional adaptability of C. albicans is mediated by rapid alterations in gene expression in response to various environmental stimuli and this transcriptional flexibility can be monitored with tools such as microarrays.

> Using such technology it is possible to (1) capture a genome-wide portrait of the transcriptome that mirrors the environmental conditions, (2) identify known genes, signalling pathways and transcription factors involved in pathogenesis, (3) identify new patterns of gene expression and (4) identify previously uncharacterized genes that may be associated with infection.

> In this review, we describe the molecular dissection of three distinct stages of infections, covering both superficial and invasive disease, using in vitro, ex vivo and in vivo infection models and microarrays.
Events in *C. albicans* during Infection or Post Interaction with Host (2)


**Abstract:**

> In this study we examined the *C. albicans* SC5314 transcriptome from renal infections in the rabbit.
> Genes involved in adhesion, stress adaptation and the assimilation of alternative carbon sources were up-regulated in these cells compared with control cells grown in RPMI 1640, whereas genes involved in morphogenesis, fermentation and translation were down-regulated.
> When we compared the congenic virulent *C. albicans* strains NGY152 and SC5314, there was minimal overlap between their transcriptomes during kidney infections.
> This suggests that much of the gene regulation observed during infections is not essential for virulence. Indeed, we observed a poor correlation between the transcriptome and phenome for those genes that were regulated during kidney infection and that have been virulence tested.
Events in *C. albicans* during Infection or Post Interaction with Host (3)

- Adaptation of Candida albicans for growth in the mammalian intestinal tract, Rosenbach ….. Kumamoto; EUKARYOTIC CELL, 2010

Abstract:

> Most infections caused by *C. albicans* arise from organisms that were previously colonizing the host as commensals, and therefore successful establishment of colonization is a prerequisite for pathogenicity.

> transcription profile of *C. albicans* cells recovered from the intestinal tracts of mice was performed.

> Thus, gene expression both promoted the ability to grow rapidly and enhanced the ability to resist stresses

> Similarities in gene expression in commensal colonizing cells and cells invading host tissue during disease were found, showing that *C. albicans* cells adopt a particular cell surface when growing within a host in both situations.

> In addition, transcription factors Cph2p and Tec1p were shown to regulate *C. albicans* gene expression during intestinal colonization
Cellular actin is affected by *Candida albicans*

**Methodology:**
- Interaction of FITC labelled Candida with HEp2 cells
- Actin labelled with Rhodamine
- Confocal scanning laser microscopy (CLSM)

**Results:**
- Actin surrounds Candida at the site of contact with the HEp2 cells
- Actin is rearranged from the fibrillar form to an aggregate form at the site of contact between the fungus and host cell

- Actin rearrangement

Tsarfaty *et al.* FEMS Microbiol 2000
A C. albicans metabolite has a similar effect on HEp2 cells:

Characteristics of the metabolite

- Contains proteins (10%)
- Possesses proteolytic activity
- SDS gel chromatography: 9 bands – two (34 kDa & 45 kDa) showed effect on the cytoskeleton

- Proteomics of the two bands: presence of Sap, Als (Als 2, Als 4) & 1,3-beta-glucosidase
- Sap & Als known as molecules involved in recognition processes between fungus and host cells

Sandovsky-Losica H. at al. FEMS Microbiol 2002
**C. albicans** metabolite causes actin rearrangement in macrophages and affects phagocytosis

A. CLSM of macrophages exposed to **C. albicans** metabolite

B. CLSM – phagocytosis of **C. albicans** by macrophages in presence of **C. albicans** metabolite

From: Schindler B, Segal E. Med Mycol. 2008
Quantitative assessment of phagocytosis of Candida in presence of *C. albicans* metabolite

Candida metabolite reduces phagocytosis

**Conclusion:**

The data indicate that *C. albicans* secretes material that affects phagocytosis of murine macrophages by changes in the arrangement of actin

From: [Schindler B, Segal E. Med Mycol. 2008](#)
**GENERAL CONCLUSIONS**

- Interaction/Infection of Candida and non-phagocytic or phagocytic mammalian cells affects the cytoskeleton of the host cells involving reorganization of actin.
- Candida probably secretes biomolecules that induce a process involving the cellular signal transduction machinery culminating in actin reorganization.
- Concurrently transcriptional changes occur in Candida that may be related to the process in the host cell.

Thus, actin plays a role in Candida - host interaction and pathogenesis.
Events in the Host Post Interaction with *C. albicans*

- *Candida albicans* releases soluble factors that potentiate cytokine production by human cells through a protease-activated receptor 1- and 2-independent pathway. Cheng., Hube Netea; Infect Immun. 2010

**Abstract:**

> The innate immune system recognizes pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRR) and transduces downstream signaling to activate the host defense.

> Here we report that in addition to direct PAMP-PRR interactions, live *Candida albicans* cells can release soluble factors to actively potentiate interleukin-6 (IL-6) and IL-8 production induced in human mononuclear cells.
Events in the Host Post Interaction with \textit{C. albicans}


\textbf{Abstract:}

\begin{itemize}
  \item Candida albicans, which represents one of the most important humanpathogenic yeasts, is directly attacked by the host innate immune system upon infection.
  \item However this pathogen has developed multiple strategies to escape host immune defense.
  \item Here, we show that C. albicans secreted proteases interfere and inactivate host innate immune effector components, such as complement proteins.
  \item Secreted aspartic proteases (Saps) in the culture supernatant of C. albicans cells and also recombinant Sap1, Sap2 and Sap3 degrade host complement components C3b, C4b and C5 and also inhibit terminal complement complex (TCC) formation.
  \item The complement inhibitory role of Sap1, Sap2 and Sap3 was confirmed in hemolysis assays with rabbit erythrocytes and normal human plasma.
  \item Secretion of complement degrading proteases provides a highly efficient complement defense response of this human pathogenic yeast that acts after the immediate acquisition of host complement regulators to the cell surface.
\end{itemize}
PATHOGENESIS: CLASSICAL MODELS

In Vitro characteristic

- Biofilm - Producing strain
- Biofilm - Non-Producing mutant

14 days later

- Succumbed to infection
- Still alive & “well”

Conclusion: Biofilm formation is a virulence attribute
PATHOGENESIS: NOVEL MODELS
The *Drosophila melanogaster* model

- **What is it and why bother about it?**
  *Drosophila melanogaster* is a fruit fly, a little insect about 3mm long, of the kind that accumulates around spoiled fruit. It is also one of the most valuable of organisms in biological research, particularly in genetics and developmental biology. *Drosophila* has been used as a model organism for research for almost a century, and today, several thousand scientists are working on many different aspects of the fruit fly. Its importance for human health was recognised by the award of the [Nobel prize](https://www.nobelprize.org/prizes/) in medicine/physiology to Ed Lewis, Christiane Nusslein-Volhard and Eric Wieschaus in 1995.

- **Why work with *Drosophila***?
  Part of the reason people work on it is historical - so much is already known about it that it is easy to handle and well-understood - and part of it is practical: it's a small animal, with a short life cycle of just two weeks, and is cheap and easy to keep large numbers. Mutant flies, with defects in any of several thousand genes are available, and the entire genome has recently been sequenced.
Candida PATHOGENESIS: In the Drosophila melanogaster model

Recent Review: Kontoyiannis & Lewis, Microbe, July 2010: Studying Fungal Pathogenesis in Drosophila

- *Drosophila melanogaster* larvae are infected with *C. albicans* by injection into the fly abdomen
- Model serves for:
  > virulence attributes assessment
  > drug discovery assays
- **Advantages**: large screens can be conducted at low cost

**Examples of Studies in Drosophila**
1. Chamilos et al., 2006: A model for virulence mechanisms & antifungal drug efficacy
2. Chamilos et al., 2009: Cas 5, a regulator of cell wall integrity
3. Levitin et al., 2007: Drosophila Thor response to *Candida albicans* infection
4. Stroshein – Stevenson et al., 2009: Phagocytosis of *Candida albicans*....
The **Caenorhabditis elegans** model

- *Caenorhabditis elegans* is a free-living, transparent nematode (roundworm), about 1 mm in length which lives in temperate soil environments.
- Research into the molecular and developmental biology of *C. elegans* was begun in 1974 by Sydney Brenner.
- It has since been used extensively as a model organism.

**Laboratory uses**
- *C. elegans* is studied as a model organism for a variety of reasons.
- It is a multicellular eukaryotic organism that is simple enough to be studied in great detail.
- Strains are cheap to breed and can be frozen. When subsequently thawed they remain viable, allowing long-term storage.
- In addition, *C. elegans* is transparent, facilitating the study of cellular differentiation and other developmental processes in the intact organism.
Candida PATHOGENESIS:
In the Caenorhabditis elegans model

Model Used for:
- Virulence assessment
- Immune response assessment
- Antifungal drug research

Examples of Studies in C. elegans:
- Pukila –Vorley et al., Eukaryot, Cell: 2009: C. albicans hyphal formation and virulence assessed
- Tampakakis et al., Nat.Prot., 2008: A C. elegans whole animal in vivo screen for the identification of antifungal compounds
- Breger et al., PloS Pathog, 2007: Antifungal chemical compounds identified using a C.elegans pathogenicity assay
• ~200 species
• ~20 species pathogenic for man
• Major pathogen – *C. albicans*
• During the last decade a shift in the relative prevalence of species occurred: a decrease in prevalence of *C. albicans* as cause of infection and an increase in non-*albicans* species: e.g. *C. glabrata, C. krusei, C. parapsilosis*

<table>
<thead>
<tr>
<th>Species</th>
<th>1989</th>
<th>2001</th>
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<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>52</td>
<td>39</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>9</td>
<td>34</td>
</tr>
<tr>
<td><em>C. kefyr</em></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td></td>
<td>1</td>
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<tr>
<td><em>C. lusitaniae</em></td>
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</tr>
</tbody>
</table>

Adapted from: Vazquez 2004
EPIDEMIOLOGY:
THE GENUS CANDIDA – Candida species distribution in BSI
European data – up to 2005

Conclusions:
• *C. albicans* most frequent
• Non-CAS- geographic differences

From: Tortorano et al. 2006, Inter J AA
ECMM survey:
Species causing candidemia.
EPIDEMIOLOGY:
The GENUS CANDIDA – Candida species distribution in BSI
European data: 2005-2010

- FROM: Filioti J et al. Intensive Care Med, 2007: … Candida albicans continues to be the most prevalent isolate, However, an increasing role of non-C. albicans spp., particularly C. parapsilosis, C. tropicalis, account for almost half of pediatric IC cases.

- FROM Guery BP et al, Intensive Care Med, 2009: a review of the literature and a European expert panel discussion (adult ICU). Results: Candida albicans remains the most frequently isolated fungal species followed by C. glabrata.

- FROM Sabino, R. et al, Med. Mycol. 2010: Epidemiology of candidemia in oncology patients: a 6-year survey in a Portuguese hospital. … The most frequent species was C. albicans (48.7%), followed by C. parapsilosis (20.2%), C. tropicalis (8.4%), C. krusei (6.7%) and C. glabrata (5.0%)

CONCLUSIONS:
- Demographic differences
- Geographic differences
- No significant change: except of increase of C. parapsilosis
**EPIDEMIOLOGY:**

**THE GENUS CANDIDA – Candida species distribution in BSI**

The US data up to 2005

<table>
<thead>
<tr>
<th>Species</th>
<th>1997</th>
<th>2000</th>
<th>2003</th>
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<tr>
<td>C. albicans</td>
<td>73.3</td>
<td>68.1</td>
<td>62.3</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>11.0</td>
<td>9.5</td>
<td>12.0</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>4.6</td>
<td>7.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4.2</td>
<td>5.6</td>
<td>7.3</td>
</tr>
<tr>
<td>C. krusei</td>
<td>1.7</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>0.5</td>
<td>0.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Adapted from Pfaller & Diekema
Clin Microbiol Rev 2007
EPIDEMIOLOGY:

THE GENUS CANDIDA – Candida species distribution in BSI
USA data: 2005-2010

- From Horn et al. 2007

Data of 1710 patients with IFI studied 2006-2007

Candida spp. (%):
- C. albicans 47.9
- C. glabrata 25.7
- C. parapsilosis 14.8
- C. tropicalis 6.7
- C. krusei 2.6
- C. guilliermondii 0.4

Conclusion:
Marked increase in non-Candida albicans species

- Neofytos et al., Clin Inf Dis, 2009
- Lewis, Curr Med Res Opin, 2009

Similar conclusions
WHY IS THERE A CHANGE IN CANDIDA SPECIES PREVALENCE?

• **Antifungal susceptibility:**
  > Decreased susceptibility to azoles
  ➡️ increase in *C. glabrata* and *C. krusei*
  > Decreased susceptibility to echinocandins ➡️ increase in *C. parapsilosis*

• **Increased biofilm formation:**
  ➡️ increase in *C. parapsilosis*
Are all *C. albicans* strains the same?

Intra-species strain differentiation in Candida has been a persistent long-time goal – some examples:


  **Abstract:** An agar plate system for biotyping isolates of Candida albicans was evaluated in four laboratories for 18 coded yeast isolates, each tested in triplicate on duplicate series of agar plates. The results showed that the biotyping system gave excellent intralaboratory reproducibility. However, because the concordance of data among laboratories was poor, the method must be regarded as suitable only for research applications and not for routine use.

- **Merson-Davies LA, Odds FC:** A morphology index for characterization of cell shape in *Candida albicans*. J Gen Microbiol. 1989

- **Odds FC, Merson-Davies LA:** Colony variations in *Candida species*. Mycoses. 1989
C. albicans Intra-species strain differentiation
- Contemporary Methods (Genotyping):

- Multilocus sequence typing (MLST) (Most commonly used)
  > MLST based microarrays
  > Microsatellite typing
- DNA finger printing
- Electrophoretic Karyotyping (less common)
- Single nucleotide polymorphism (SNP) (less common)
- Flow Cytometry (less common)
GENOTYPING- Principles of Methods and Examples of Studies

  - Electrophoretic Karyotyping: to detect chromosomal rearrangements
  - Single Nucleotide Polymorphism (SNP): reveals allelic differences between strains
  - Flow Cytometry: a rapid and accurate method to analyze genome size changes (ploidy changes).

- Property differences among the four major *Candida albicans* strain clades. MacCallum, Gow, Odds; Eukaryotic Cell, 2009.


GENOTYPING - What do we learn from it: some examples

• Molecular phylogenetics and epidemiology of Candida albicans
  
  Odds; Future Microbiology, 2010
  
  - Molecular typing of C. albicans strains shows geographical evolutionary associations.
  
  - Individual patients usually carry a single C. albicans strain type, but this may undergo microvariation leading to detection of mixtures of closely related types.
  
  - There are also clade-related associations with lengths of tandem repeats in some cell-surface proteins, but not with virulence or type of infection.

• Progressive loss of echinocandin activity following prolonged use for treatment of Candida albicans oesophagitis
  
  Lavendiere……..Perlin ; J Antimicrob Chemother. 2006
  
  - Antifungal susceptibility profiles on isolates before and during therapy.
  
  - MLST on isolates before and during therapy.
  
  - Identical allelic homology for the isolates.
  
  - Lack of clinical response to micafungin was associated with increases in MICs to all three echinocandins in association with the acquisition of mutation in FKS1 gene.
Candidosis is an opportunistic fungal disease caused by the human commensal yeast, Candida. It is manifested in a diversity of clinical forms, ranging from muco-cutaneous infection to life threatening systemic disease, which affects primarily immunocompromised and debilitated patients.

This presentation attempted to overview the state of art of the pathogenesis of candidosis. It highlighted the current view and general understanding on how candidosis evolves, who is particularly at risk to develop the infection and what is the response to it.

Since it is believed that understanding the pathogenesis of an infection is a basis for a rational approach for diagnosis and therapy, reviewing the advances in the understanding of the pathogenesis of candidosis may contribute to the management of this complex clinical entity.
Thanks to all the students, post-docs & collaborators cited in this presentation and to the many, many authors of the studies overviewed today

AND

THANK YOU

for your attention