Oral *Candida* biofilm model and *Candida* – Staph interactions

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Background – *C. albicans*

- *Candida albicans* is a polymorphic fungus
  - Yeast
  - Pseudohyphae
  - Hyphae (pH, temperature, serum, N)

Background – S. aureus

- **Staphylococcus aureus**
  - G+ coccus with low G+C content
  - non-spore forming, non-motile
  - facultative anaerobe
  - normal resident of nares (30-70%)
  - Re-emerging human pathogen
    - Rapid development of antimicrobial resistance
    - increased use of indwelling medical devices.
  - Capable of forming biofilms
  - Causes a variety of diseases
    - Skin and soft tissue infections
    - Keratitis
    - Endocarditis
    - Toxic Shock Syndrome
    - Food poisoning
    - Indwelling medical device infections
    - Bacteremia

*Staphylococcus aureus*
Candida albicans and Staphylococcus aureus

- Both capable of forming biofilms and currently rated among the top 3 bloodstream pathogens (CDC).

- Implications for interaction:
  - Co-isolated from the oral, vaginal, gastrointestinal mucosa.
  - Blood cultures from candidemic patients are associated (11%) with S. aureus co-infection. (Klotz, et. al. 2007)
  - Commonly associated in otitis externa, VAP, diabetic foot wound infections, keratitis, and on catheter lines and denture surfaces.

Polymicrobial biofilm on catheter disc material.
C. albicans enhances S. aureus pathogenicity

Carlson, et al.

Infect Immun. 38(3): 921-4

Infect Immun. 50(3): 655–659
Patient records from 2003-2006 were screened for various criteria:

- HIV+ (AIDS - CD4+ T cell count < 200)
- Documented *S. aureus* bacteremia
  - 85% of these traced to i.v. needle use, CVC lines
  - 15% of unknown etiologies
    - 25% had current candidiasis
    - 50% had previous documented cases of candidiasis
Co-localization in the oral cavity


In vivo dual-species infection model

Amp.

Prednisolone

CD-1

5x10^6 CFU

C. albicans 529L

S. aureus

C. albicans + S. aureus
In vitro interaction of *C. albicans* and *S. aureus* in biofilms

Images of biofilm probed with (A) Tamra-labeled Universal Yeast probe and FITC-labeled *S. aureus* probe and (B) Tamra-labeled *S. aureus* probe and FITC-labeled *C. albicans* probe.
Physical interaction of *C. albicans* and *S. aureus* in biofilms

Differential *S. aureus* binding to *C. albicans* biofilm

![Graph showing differential binding of S. aureus to C. albicans biofilm](image)

Bacterial adherence to *C. albicans* hyphae

- Percent of cells attached to *Candida albicans* hyphae:
  - S. aureus: 57.4%
  - S. pyogenes: 24.6%
  - S. epidermidis: 24.7%
  - P. aeruginosa: 15.1%
  - B. subtilis: 2.4%
  - E. coli (DH5-α): 5.6%

*Avg of 10 fields per slide, 100X (#attached cells / #total cells)*
**in vitro co-infection**

- HaCaT human keratinized epithelial cell line
- Infect with $5 \times 10^6$ CFU *C. albicans*/*S. aureus*

**Imaging:**

- **SA** (20x): *S. aureus* (green)
- **CA + SA** (20x): *C. albicans* (red) and *S. aureus* (green)
- **CA + SA** (100x): Magnified view of *C. albicans* (red) and *S. aureus* (green) interaction
Dual-species *in vitro* infection using HaCaT keratinized epithelium
Adherence ELISA assay

*C. albicans* strain tested
Atomic force microscopy (AFM)

Bastiann Krom, et al., UMC Groningen, Netherlands.
Atomic force microscopy

Bastiann Krom, et al., UMC Groningen, Netherlands.
**ex vivo model of co-infection**

1. CD-1
   - Tongue
     - S. aureus
     - C. albicans S. aureus
   - 1h, wash, RPMI, 12h, wash
   - One half homogenize
     - Culture on selective media
   - One half fix
     - Embed in paraffin, section, stain

2. CD-1
Conclusions

- **Over half** of all *S. aureus* bacteremias have no known portal of entry (del Rio, *et al.* 2007)

- *C. albicans* is a risk factor for *S. aureus* bacteremia but not *vice versa* (Klotz, *et al.* 2007)