Candida Biofilms

Perspectives from a Clinician

CHEN, PAO-YU, MD
Attending physician
Department of Traumatology
National Taiwan University Hospital
Aug. 28th , 2016

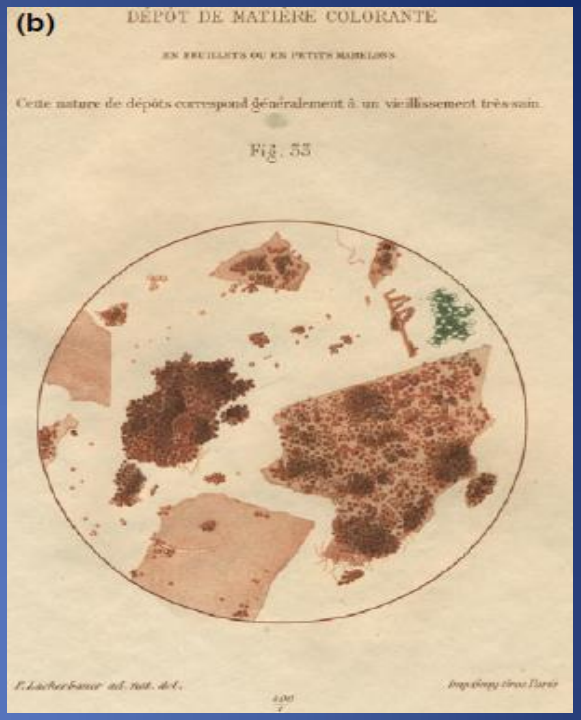
BIOFILM VS. BIOFILM INFECTIONS

Bio-film

- Referring to bacterial adhesion, aggregation and multiplication on surfaces, was used in marine microbiology to distinguish adhering (sessile) bacteria from free swimming (planktonic) bacteria as early as 1933

Høiby N *Pathog Dis* 2014

Luis Pasteur
(1822-1895)

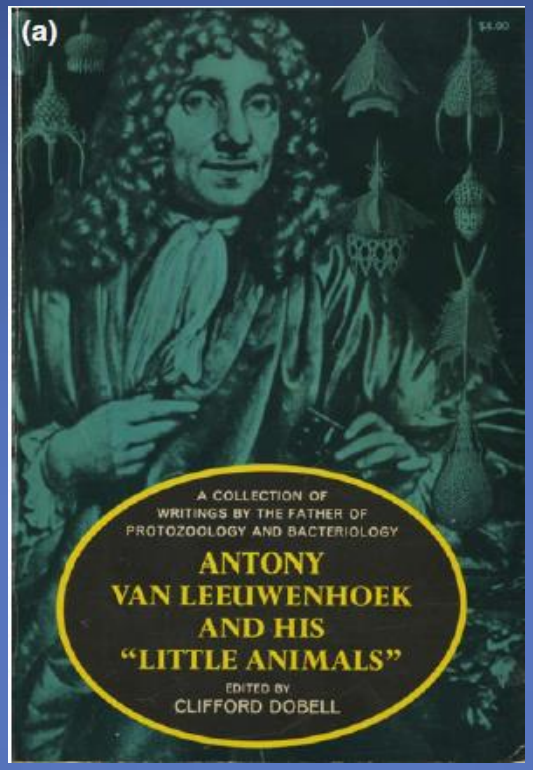


An aggregate of microbial cells adherent to a living or nonliving surface, embedded within a matrix of extracellular polymeric substances of microbial origin.

Høiby N *Pathog Dis* 2014

Flemming HC et al. *Nat Rev Microbiol* 2010

Antony van Leeuwenhoek
(1632-1723)

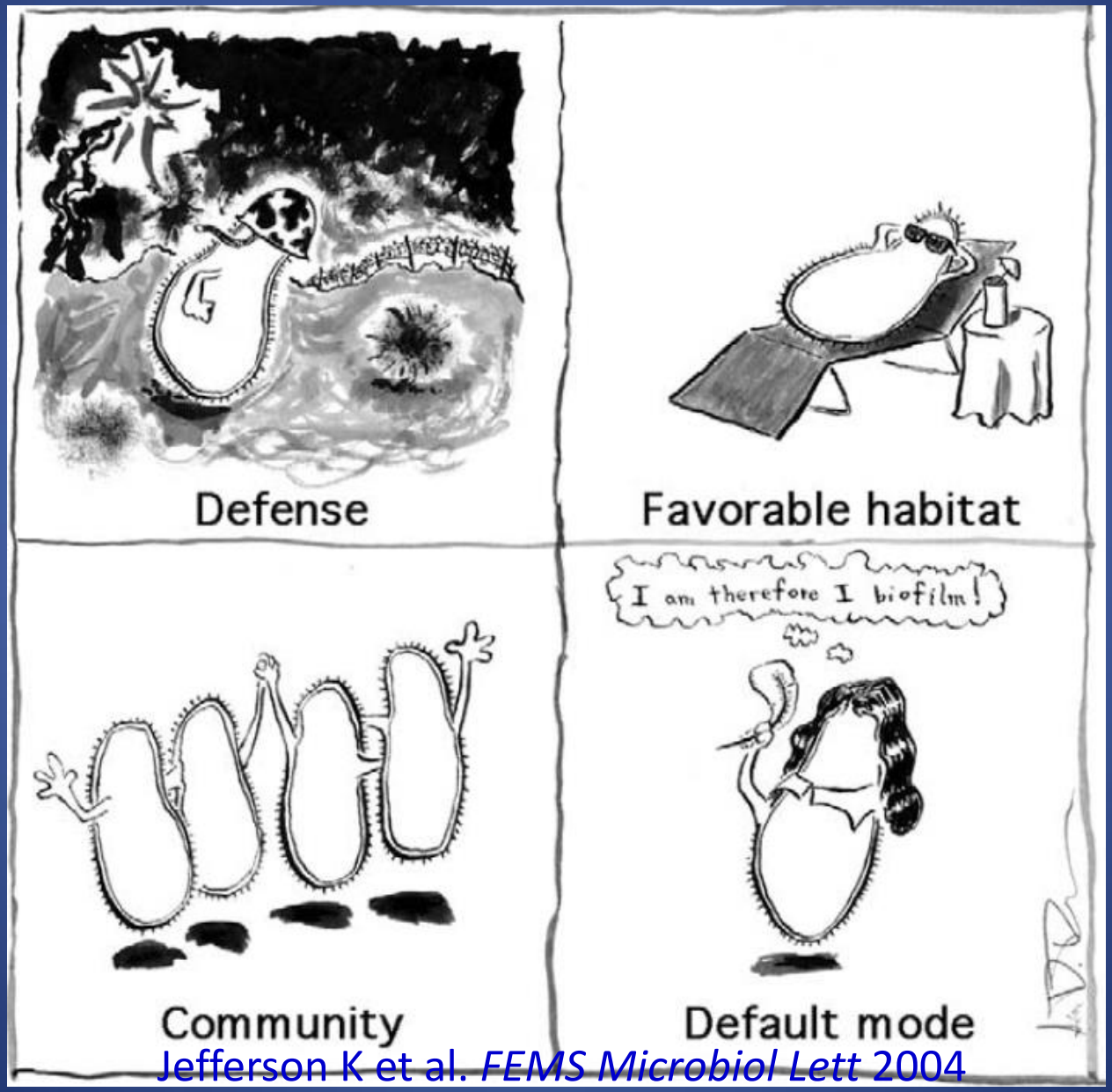


Aggregated, microbial cells surrounded by a polymeric self-produced matrix, which may contain host components.

Høiby N *Pathog Dis* 2014

Hall-Stoodley L et al. *FEMS Immul Med Micriobiol* 2012

Driving Forces for Biofilm Formation



Jefferson K et al. *FEMS Microbiol Lett* 2004

Biofilm in Medicine

- 1970-1972, cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection.

  Høiby N et al. *Acta Pathol Mcirobiol Scand B* 1973

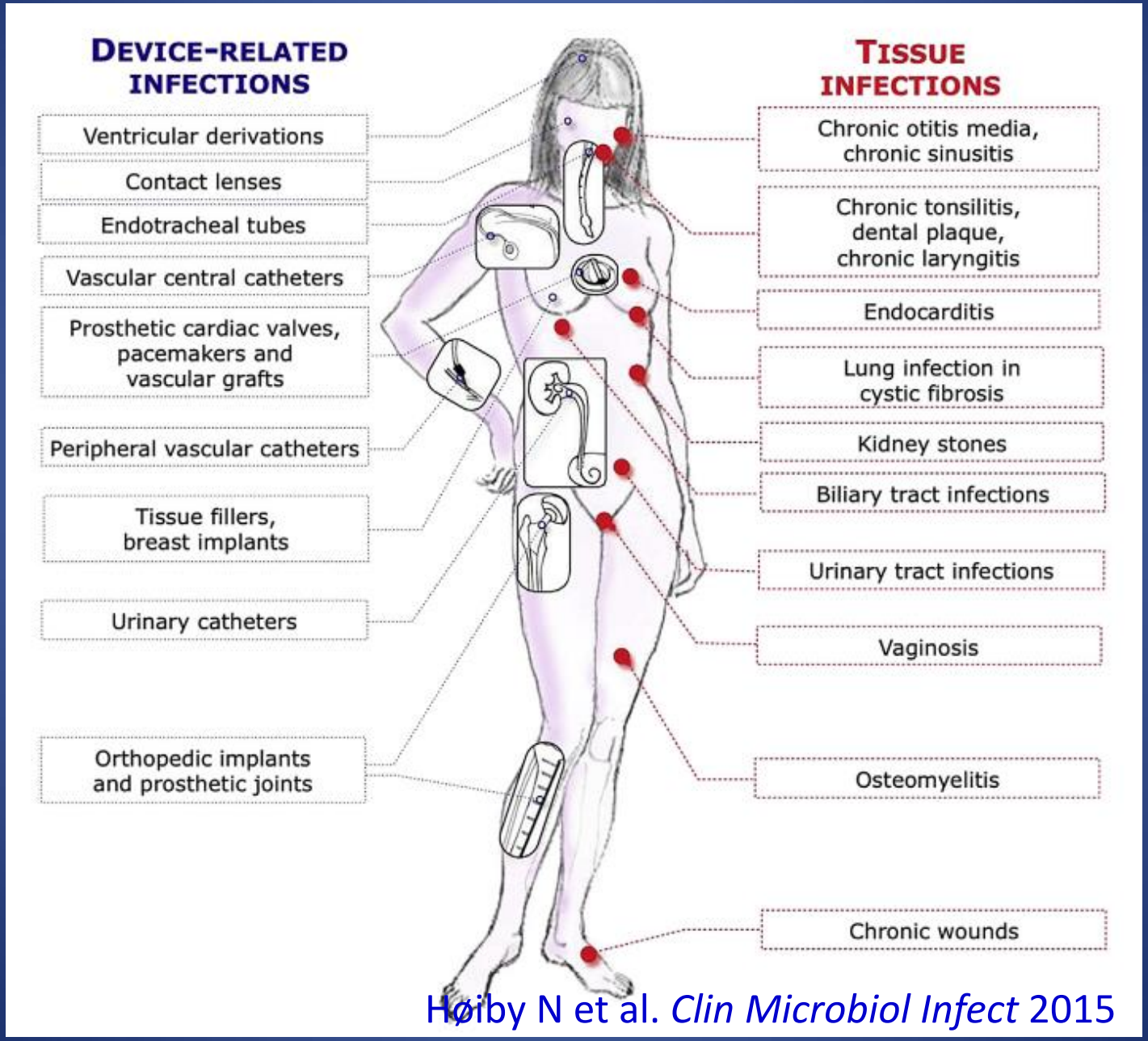
  Høiby N *Acta Pathol Mcirobiol Scand B* 1974

- 1978-1980, *Candida*-induced denture stomatitis

  Budtz-Jörgensen *J Am Dent Assoc* 1978

  Theilade J et al. *J Biol Buccale* 1980

Biofilm Associated Infections



Høiby N et al. *Clin Microbiol Infect* 2015

Candida infections of Medical Devices

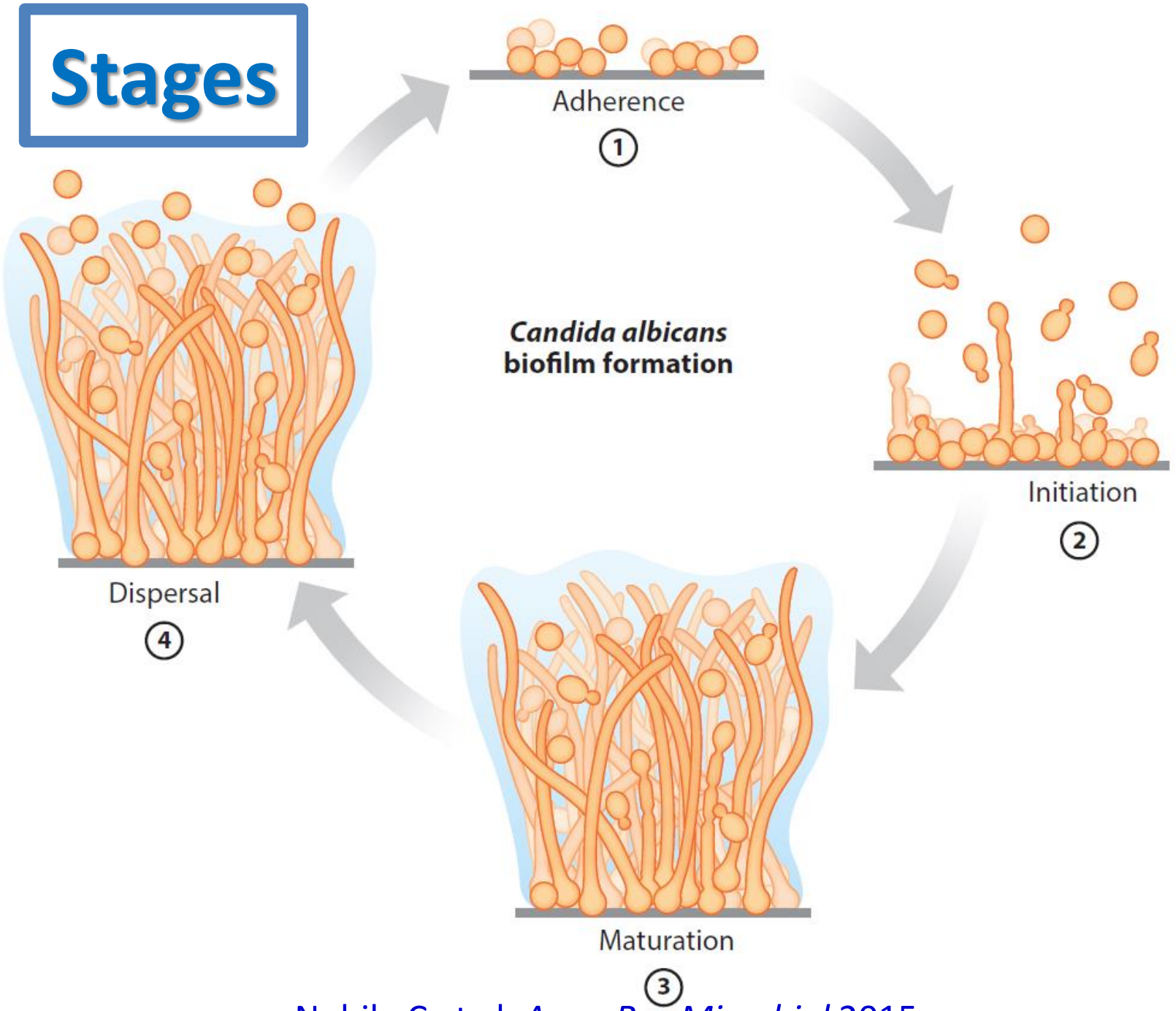
Device	Infection rate (%)	Proportion of *Candia* spp. Infection (%)	Risk factor
Vascular catheters	3-8	10	Similar with risk factors of candidemia
Prosthetic valves	2.9	2-10	Prior bacterial IE; Prolonged antibiotics use; IV cath; IVDU
Pacemakers	0.5-7	4.5	Nil.
VP shunt	6-15	1	Prior or concurrent meningitis; Broad spectrum antibacterials; Bowel perforation; abdomen surgery
PD catheters	23	2.4-7	Recent bacterial peritonitis; Prior antibacterials; SLE
Joint prostheses	1-3	<1	Nil.

* Removal needed to achieve cure for all medical devices

Cauda R et al. *Drug*, 2009

BASICS OF CANDIDA BIOFILM

Stages



Nobile C et al. *Annu Rev Microbiol* 2015

How to Quantify Biofilms?

Crystal violet stain

- Staining the metabolically active and inactive cells in mature biofilms
- **Biomass** production

XTT reduction assay

- XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2*H*-tetrazolium-5-carboxanilide]
- Yellow salt that is reduced by dehydrogenases of **metabolically active** cells to a colored formazan product

Measured colorimetrically with a microtiter plate reader

Taff HT, et al. *Med Mycol* 2012

Factors affecting *Candida* Biofilm *in vitro*

1. Fluid flow shear
2. Substrate
3. Nutrients
4. *Candida* species and strains
5. Microbial cohabitants

Chandra J et al. *Microbiol Spectr* 2015

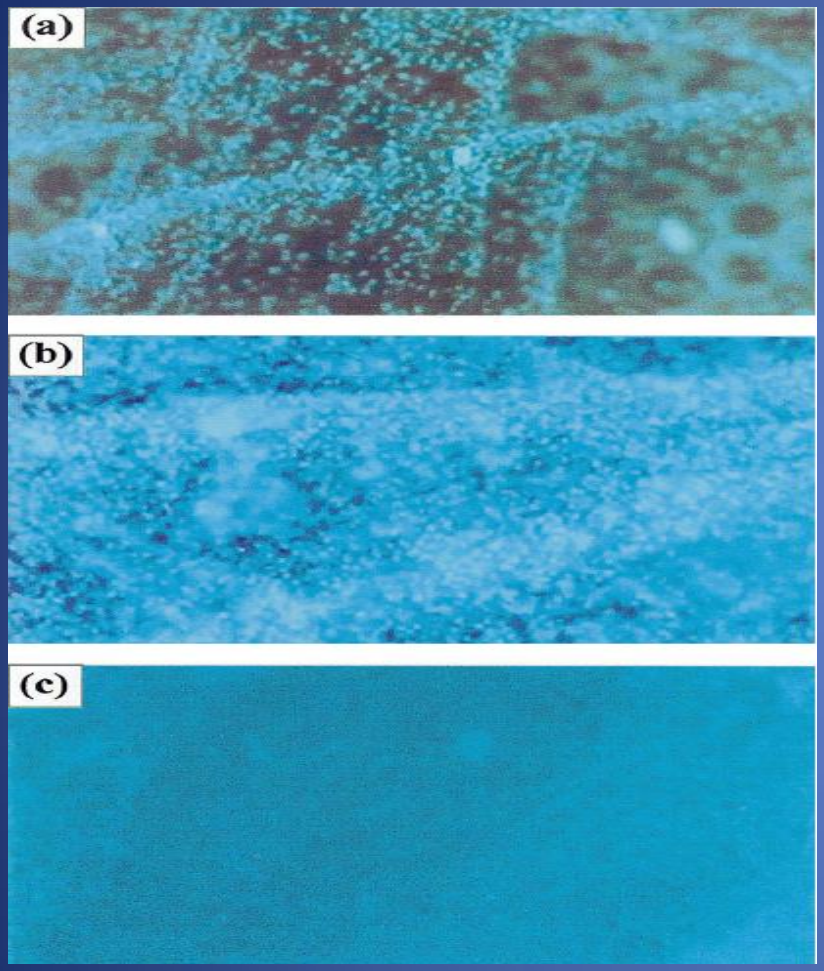
Comparisons of Visualization Methods

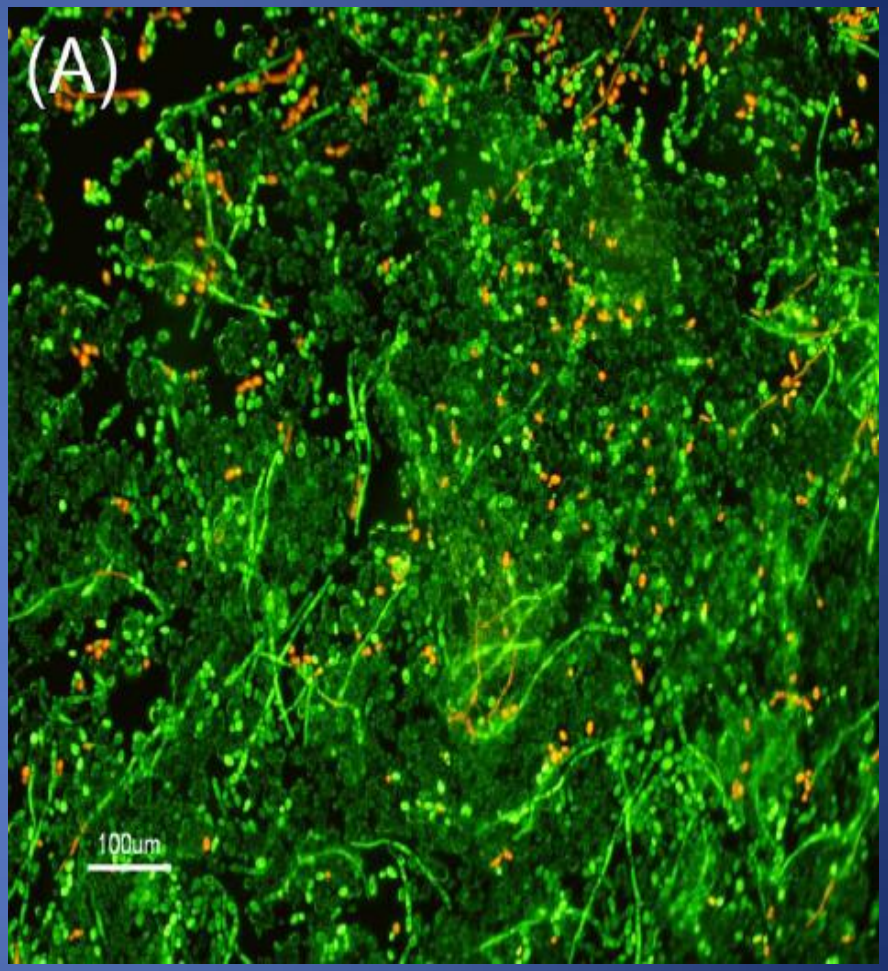
Biofilm Morphology & Architecture

	Fluorescence microscopy	Scanning electron microscopy (SEM)	Confocal scanning laser microscopy (CSLM)
Advantages	Quick method as a screening tool	Surface topography of biofilms at very high magnification	three-dimensional reconstruction of undisturbed biofilm
Disadvantages	Low magnification	Dehydration artifacts in the biofilm matrix	

Mukherjee P et al. *Med Mycol* 2005

Fluorescence microscopy





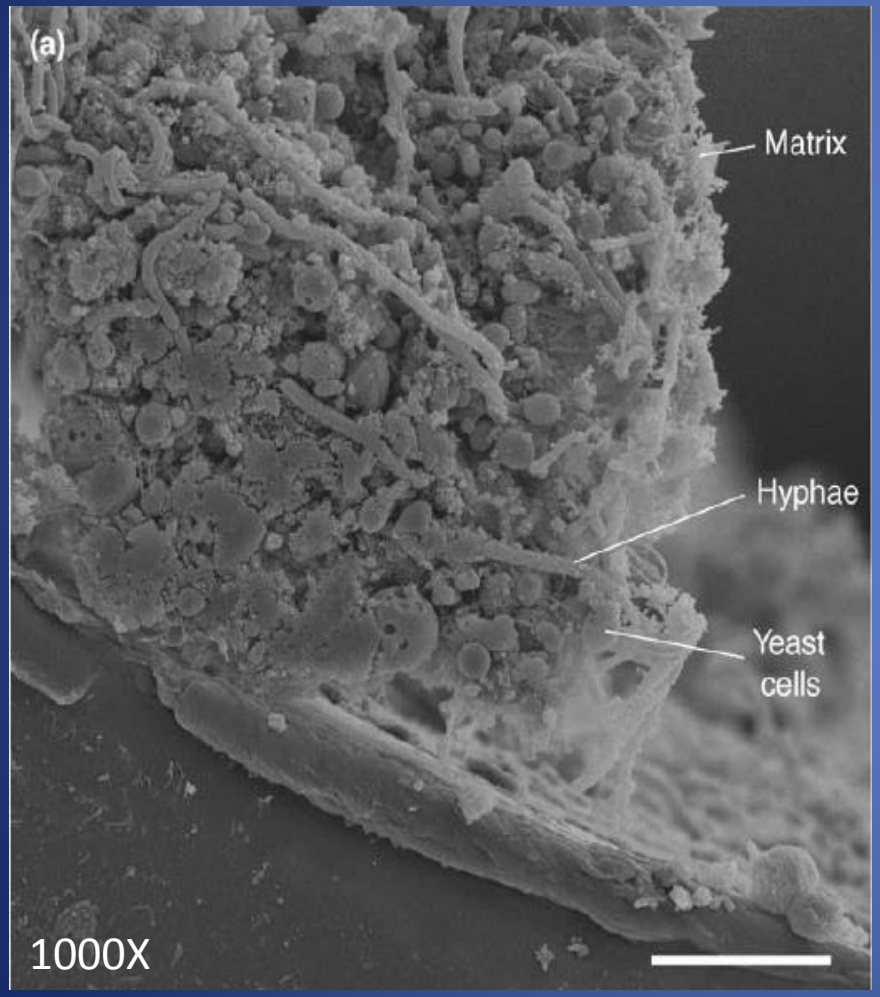
Calcofluor-White

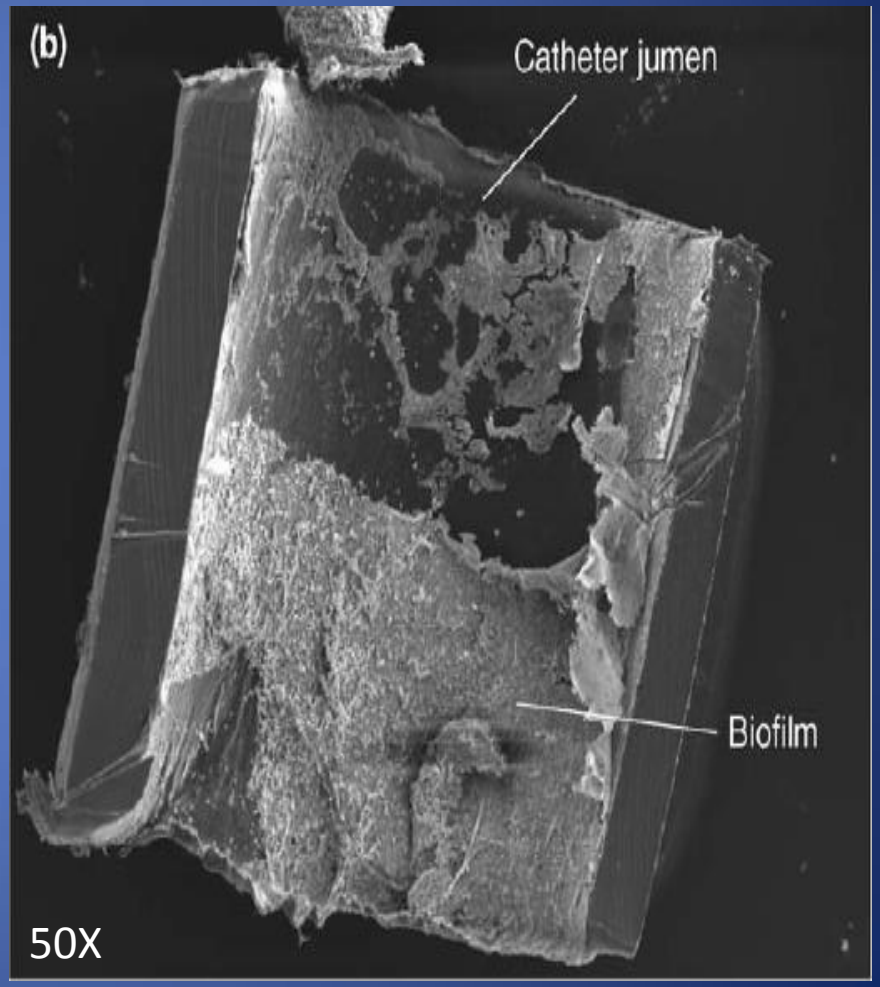
Chandra J et al. *J Bacteriol* 2001

SYTO 9 dye/ Propidium iodide

Tøndervik A et al. *PLoS One* 2014

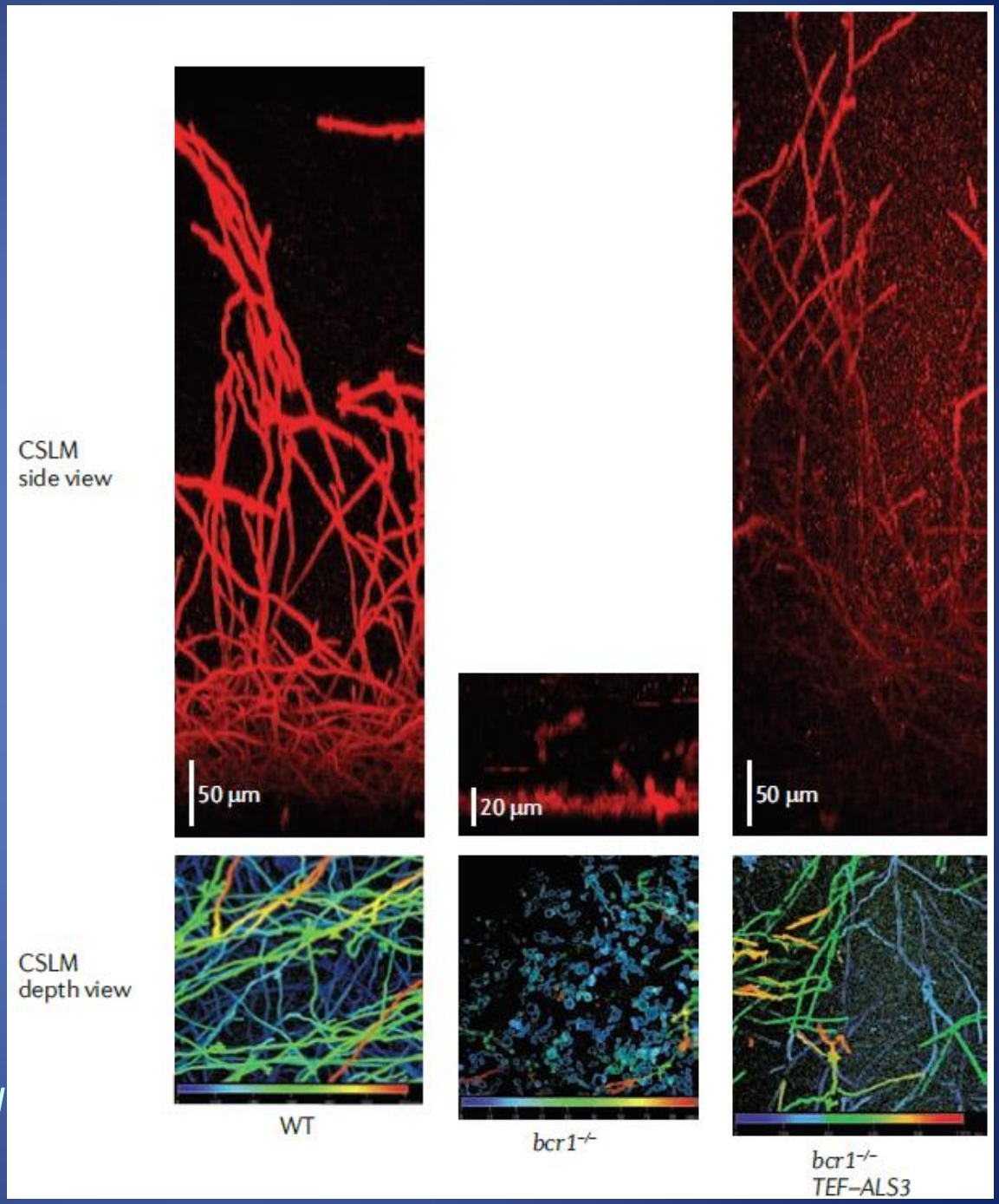
Scanning Electron Microscopy





Nett J et al. *Curr Opin Microbiol* 2006

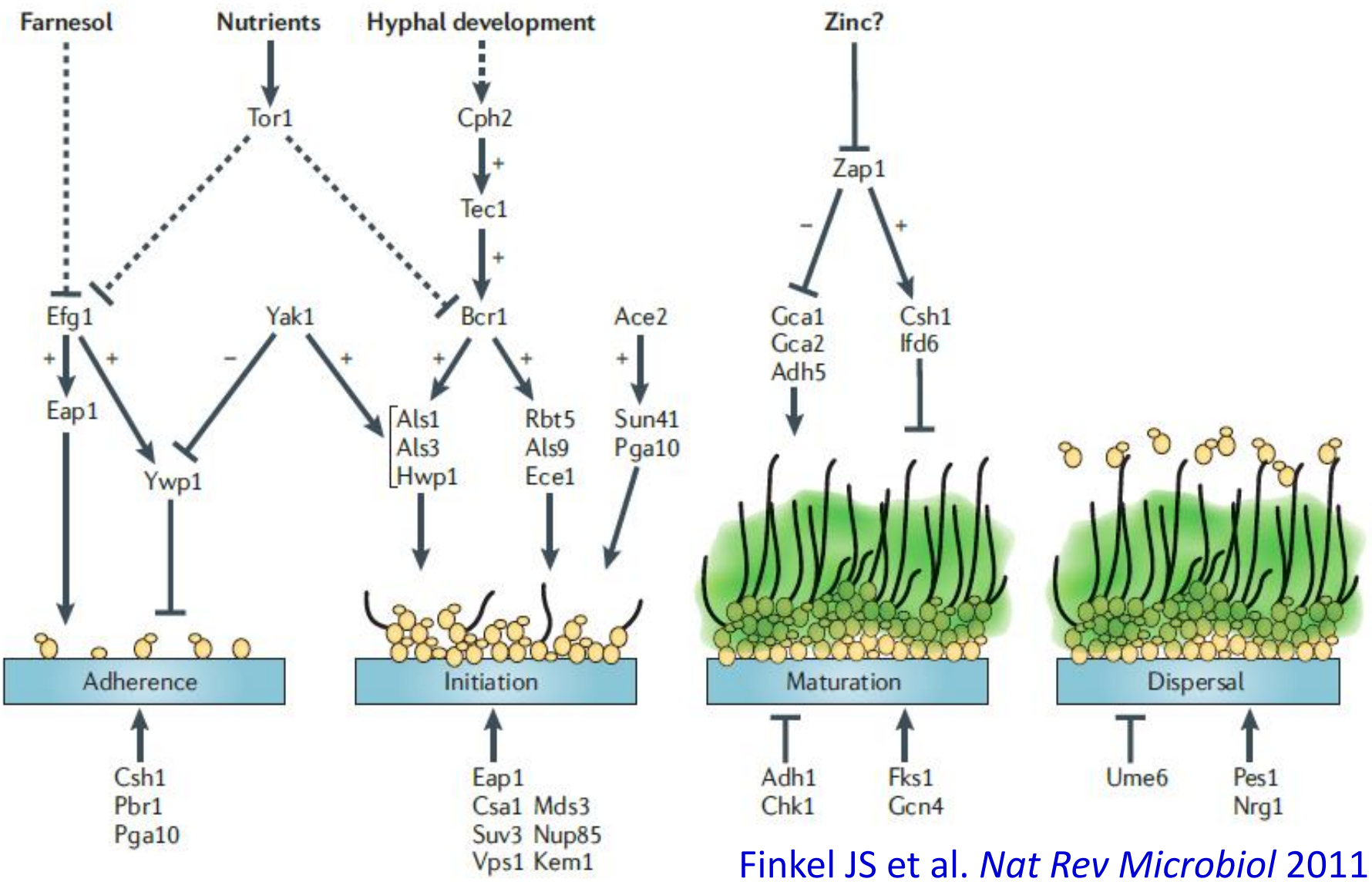
Confocal Scanning Laser Microscopy



Concanavalin A-Alexa Fluor

Finkel J et al. *Nat Rev Microbiol* 2011

Proteins that Function in Biofilm Development



Finkel JS et al. *Nat Rev Microbiol* 2011

Selected Genes in Biofilm Development

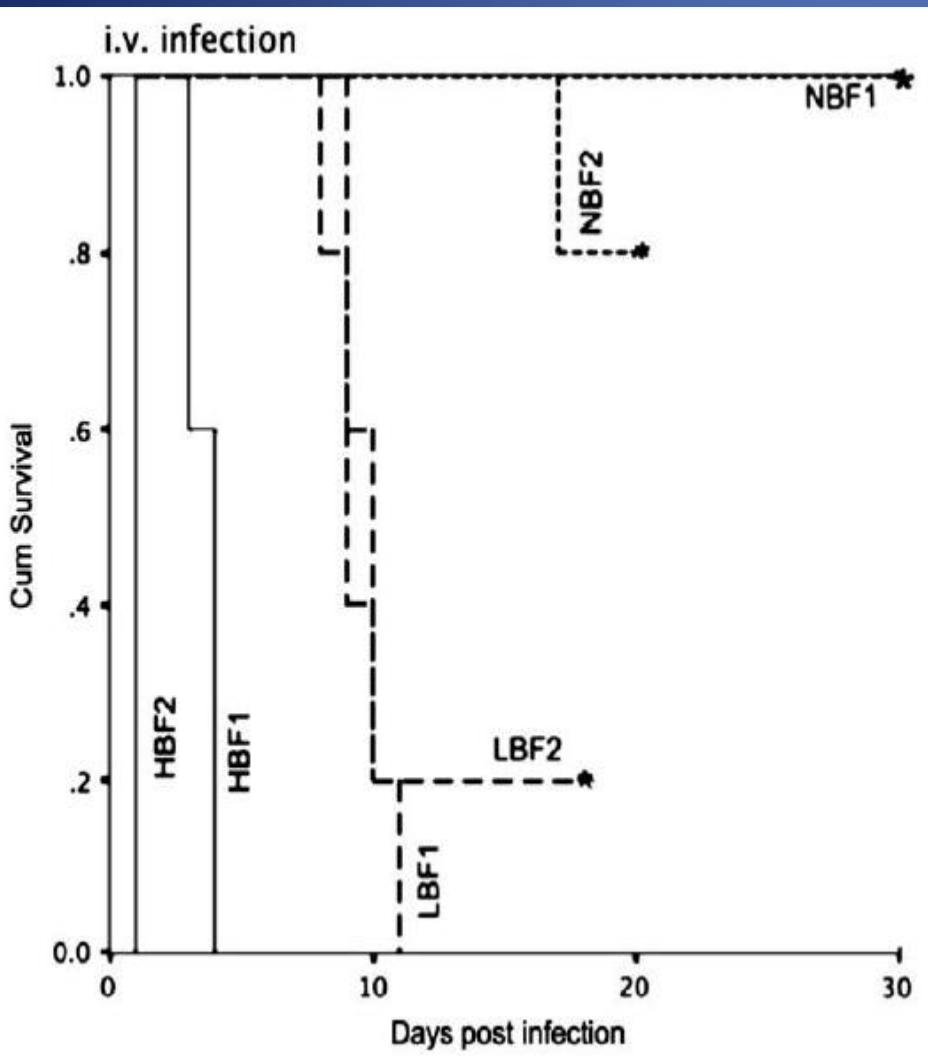
Molecular function of gene products*	Role of gene product	Genes
Transcription factors	Positive	*ACE2*‡, *BCR1*, *CPH1*, *CZF1*‡, *EFG1*‡, *FLO8*‡, *GCN4*, *TEC1*‡, *UME6*‡ and *NRG1*‡
	Negative	*ZAP1*
Cell wall-related proteins	Positive	*ALS1*, *ALS2*‡, *ALS3*, *ALS4*, *ALS5*, *ALS7*, *ALS9*, *CSA1*, *EAP1*, *FKS1*, *HWP1*, *HWP2*, *OCH1*, *PGA1*, *PGA10*‡, *PMT1*‡, *PMT2*‡, *PMT4*, *PMT6*, *RBT1*, *RBT5* and *SUN41*‡
	Negative	*YWP1*
Alcohol dehydrogenases	Positive	*ADH5*
	Negative	*ADH1*, *CSH1* and *IFD6*
Protein kinases	Positive	*CBK1*‡, *GIN4*‡, *IRE1*‡, *MKC1* and *YAK1*‡
	Negative	*CHK1* and *TOR1*,
Drug efflux pumps	Positive	*CDR1*, *CDR2* and *MDR1*
Glucoamylases	Positive	*GCA1* and *GCA2*
Other functions§	Positive	*CAT2*, *ECE1*, *KEM1*‡, *MDS3*‡, *NDH51*, *NUP85*‡, *PBR1*, *PES1*, *PDX1*, *RIX7*, *SUV3*‡, *VAM3*‡ and *VPS1*‡

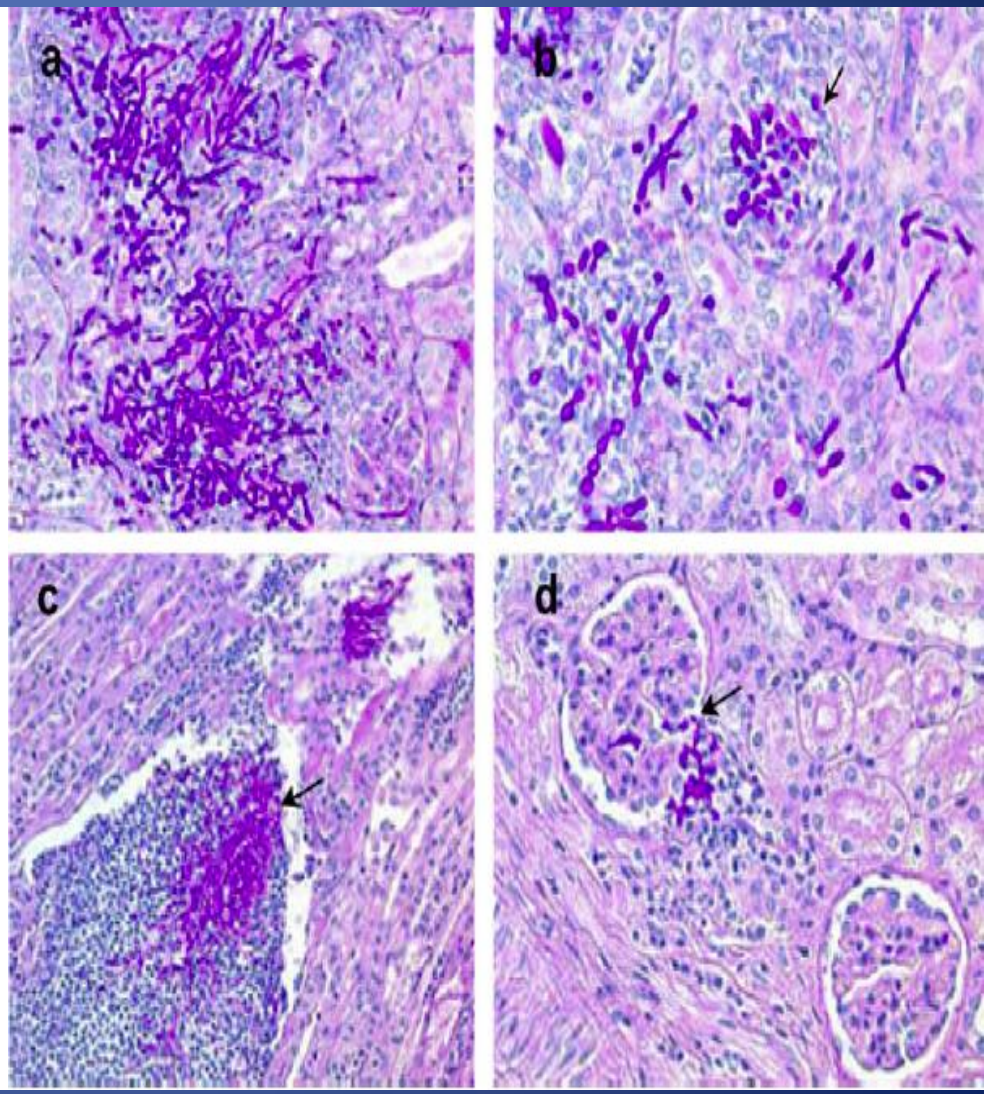
‡ A regulator of filamentation

Finkel JS et al. *Nat Rev Microbiol* 2011

BIOFILM PATHOGENESIS IN *CANDIDA* INFECTION

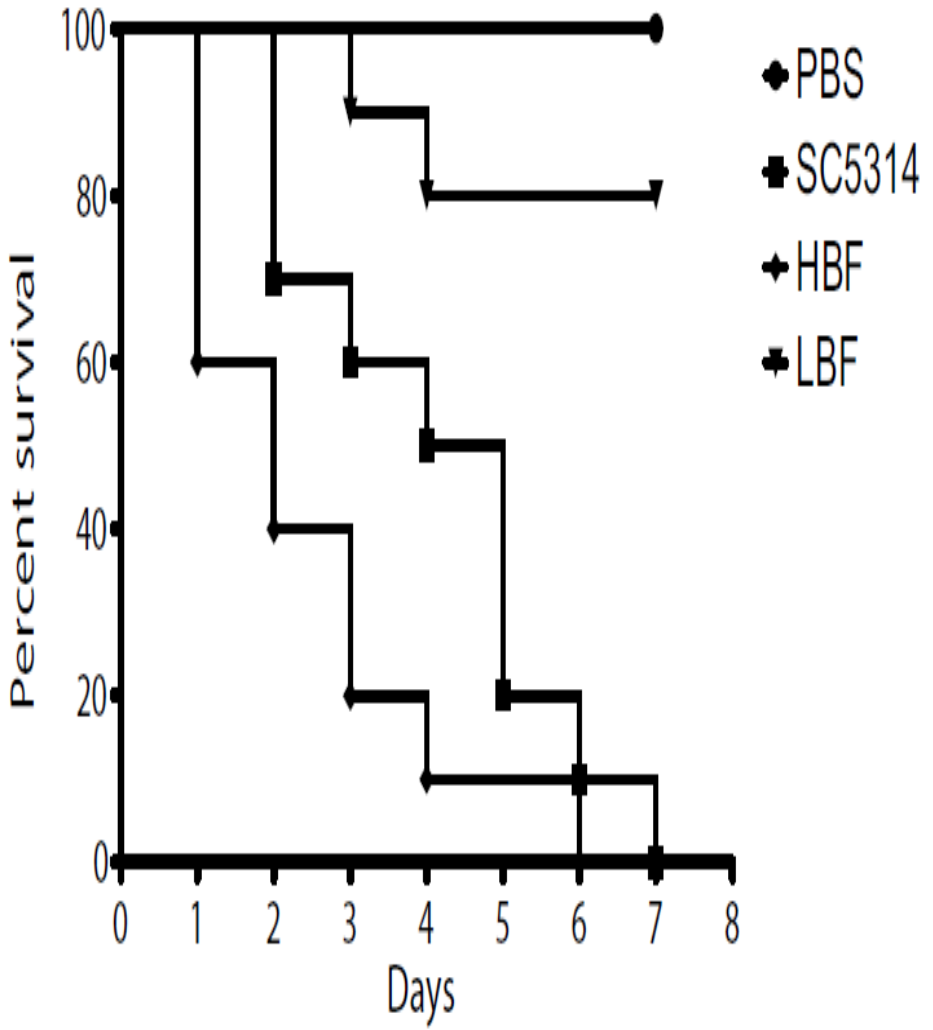
Virulence in Intravenous Murine Infection Model

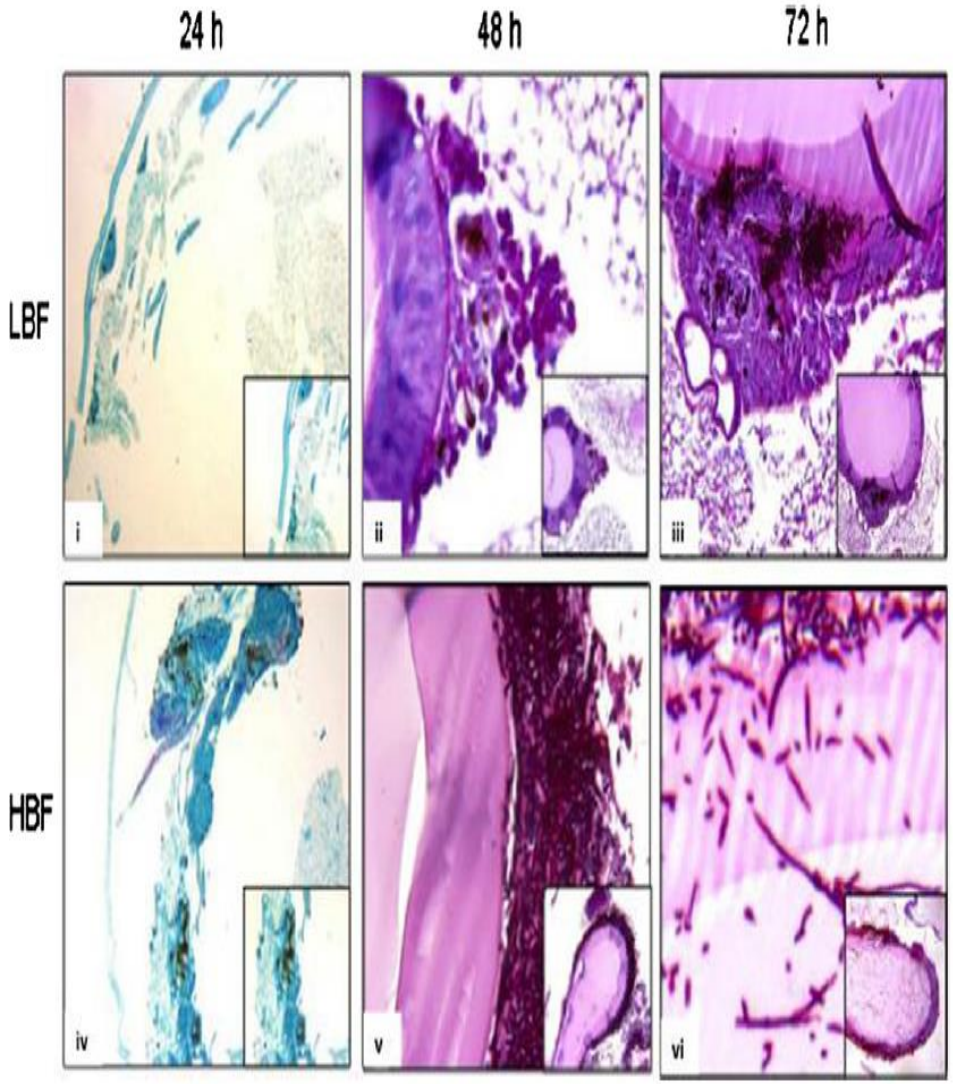




Hasan F et al. *Microbe Infect* 2009

Virulence in *Galleria mellonella* Infection Model





Sherry L et al. *BMC Microbiol* 2014

Planktonic vs. Sessile, Azole

Species	No. of isolates tested	Type of MIC[a]	No. of isolates for which indicated MIC (μg/ml) was:											
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>1,024
C. albicans	12	MIC for planktonic cells			3	4	1	3			1				
		MIC_{50} for sessile cells									1	1	2	1	7
		MIC_{80} for sessile cells													12
C. parapsilosis	12	MIC for planktonic cells				1	4	4	3						
		MIC_{50} for sessile cells											2		10
		MIC_{80} for sessile cells													12
C. tropicalis	10	MIC for planktonic cells			1	6	1	2							
		MIC_{50} for sessile cells									2				8
		MIC_{80} for sessile cells													10
C. glabrata	9	MIC for planktonic cells								1	2	5	1		
		MIC_{50} for sessile cells									1				8
		MIC_{80} for sessile cells													9

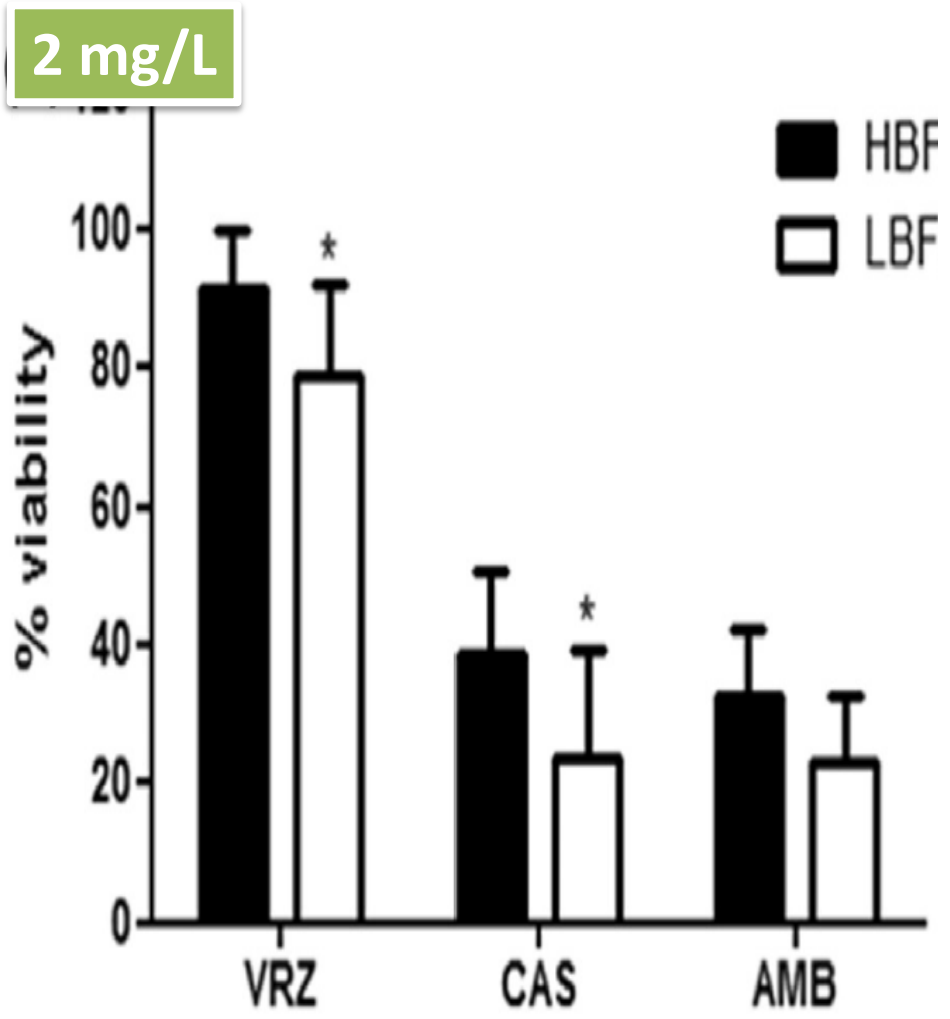
Choi HW et al. *Antimicrob Agent Chemother* 2007

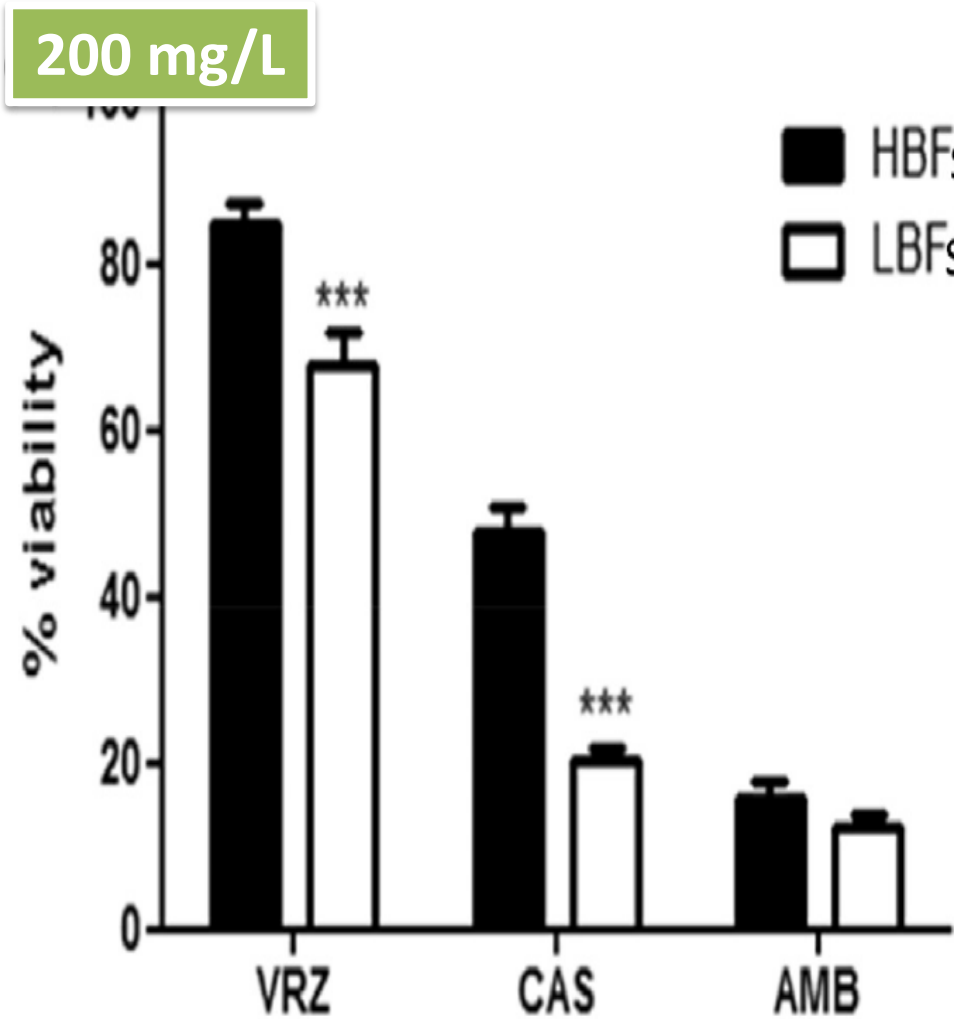
Planktonic vs. Sessile, Candin

Drug	Species	No. of isolates tested	Type of MIC[a]	No. of isolates for which indicated MIC (μg/ml) was:										
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
Caspofungin	*C. albicans*	12	MIC for planktonic cells	6	4	2								
			MIC_{50} for sessile cells		1	1	1	9						
			MIC_{80} for sessile cells					9	3					
	C. parapsilosis	12	MIC for planktonic cells				6	5	1					
			MIC_{50} for sessile cells						1	2	2	2		5
			MIC_{80} for sessile cells											12
	C. tropicalis	10	MIC for planktonic cells	4	5	1								
			MIC_{50} for sessile cells						6	2				2
			MIC_{80} for sessile cells											10
	C. glabrata	9	MIC for planktonic cells	2	5	2								
			MIC_{50} for sessile cells			1	1	6	1					
			MIC_{80} for sessile cells					3	6					

Choi HW et al. *Antimicrob Agent Chemother* 2007

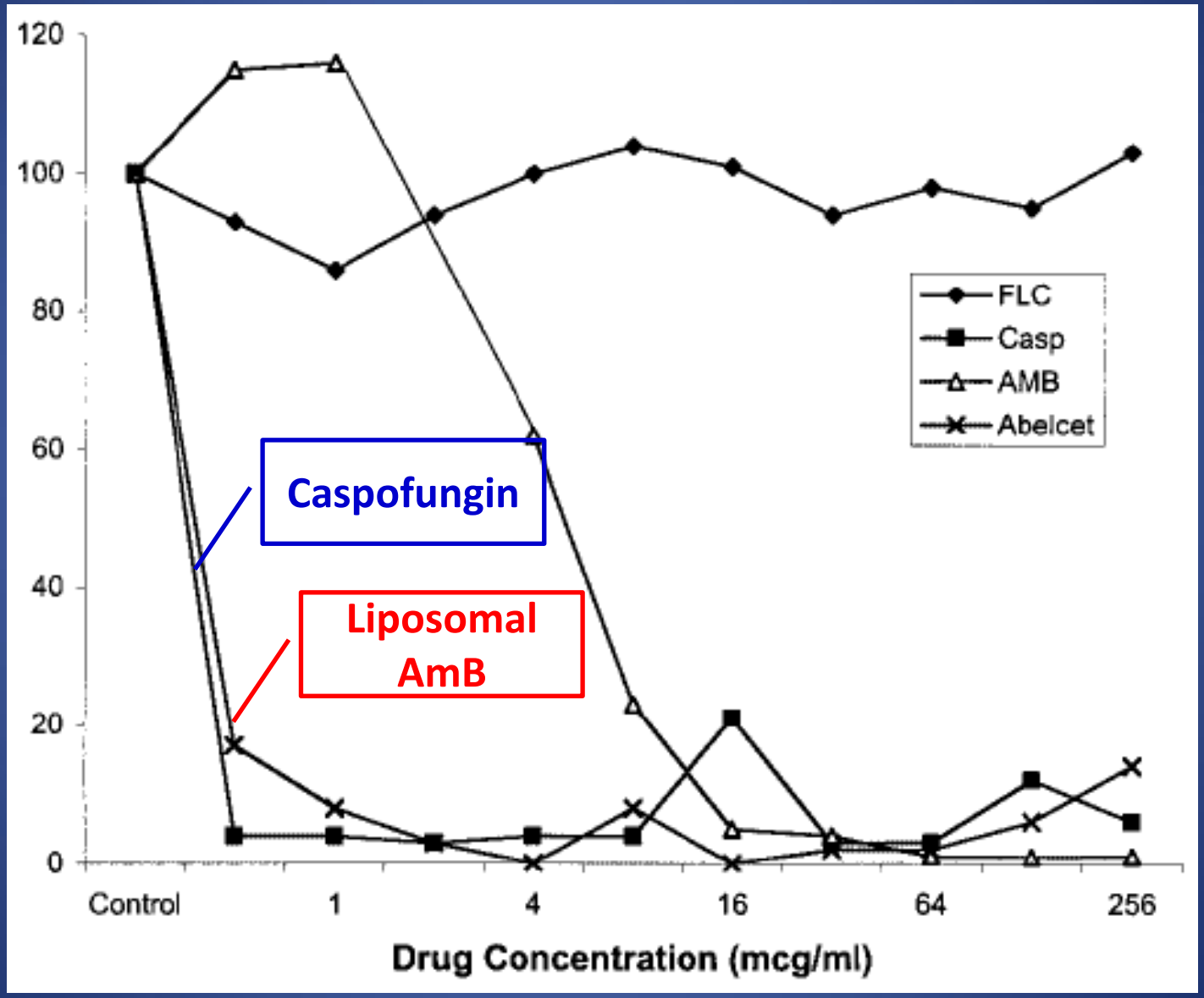
High vs. Low Biofilm Formation, Antifungal





Rajendran R et al. *Clin Microbiol Infect* 2016

Candins and Liposomal AmB Are Better!



Kuhn DM et al. *Antimicrob Agents Chemother* 2002

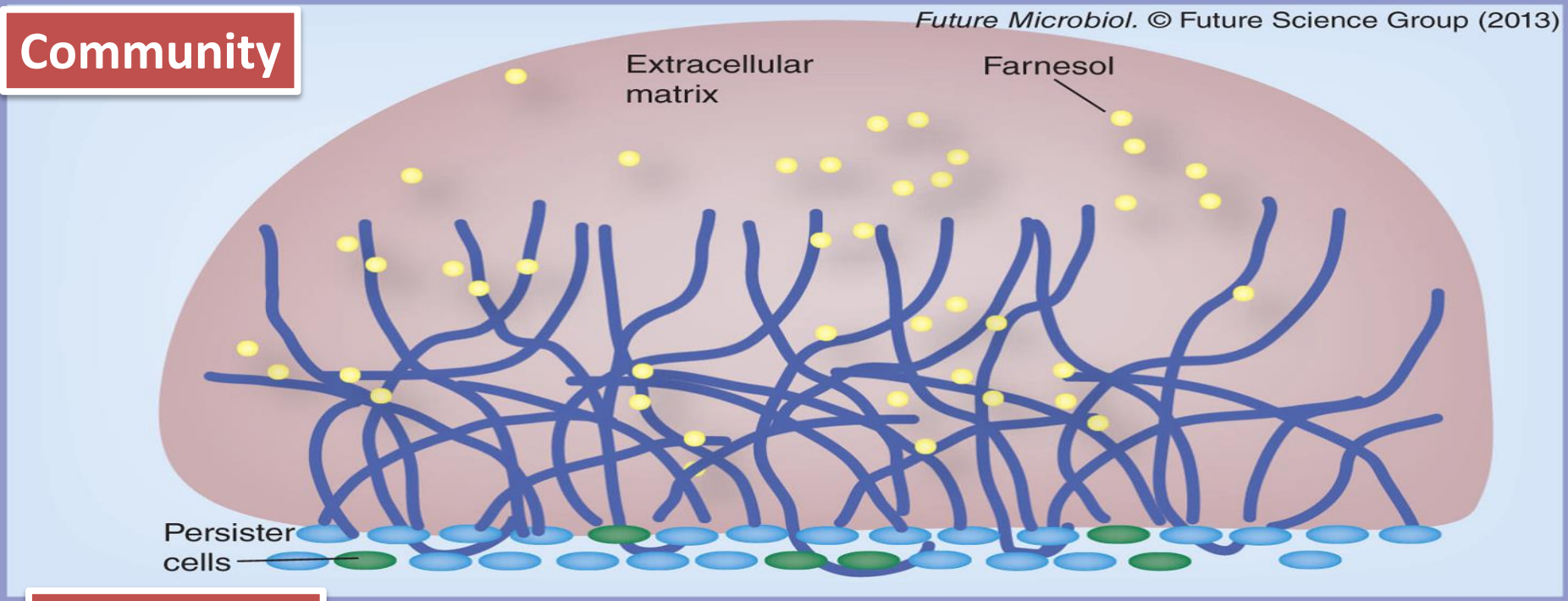
Biofilms Resistance

- Possible mechanisms
  1. Alteration in membrane sterol composition **(Early)**
  2. Overexpression of drug efflux pump during early phase of biofilm formation **(Intermediate & Mature)**
  3. Extra-cellular matrix retards the diffusion of drugs across biofilm, especially in a mixed-species biofilm

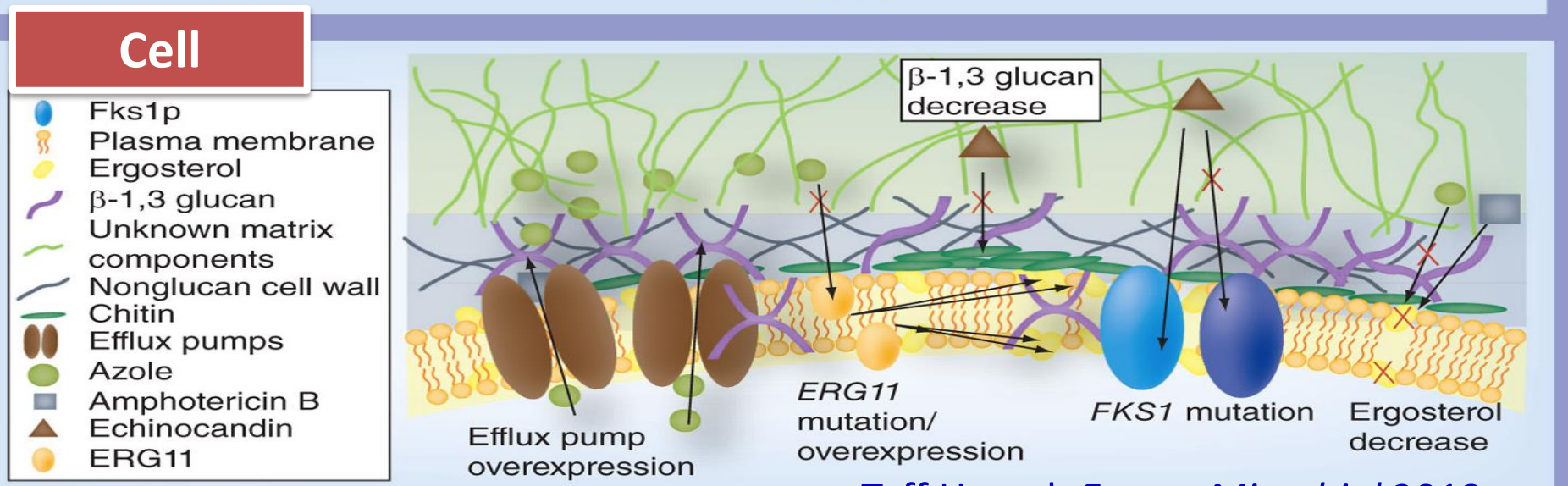
Mukherjee PK et al. *Drug Resistance Updates* 2004
Blankenship JR et al. *Curr Opin Microbiol* 2006

Candida Biofilm Resistance Mechanisms

Community



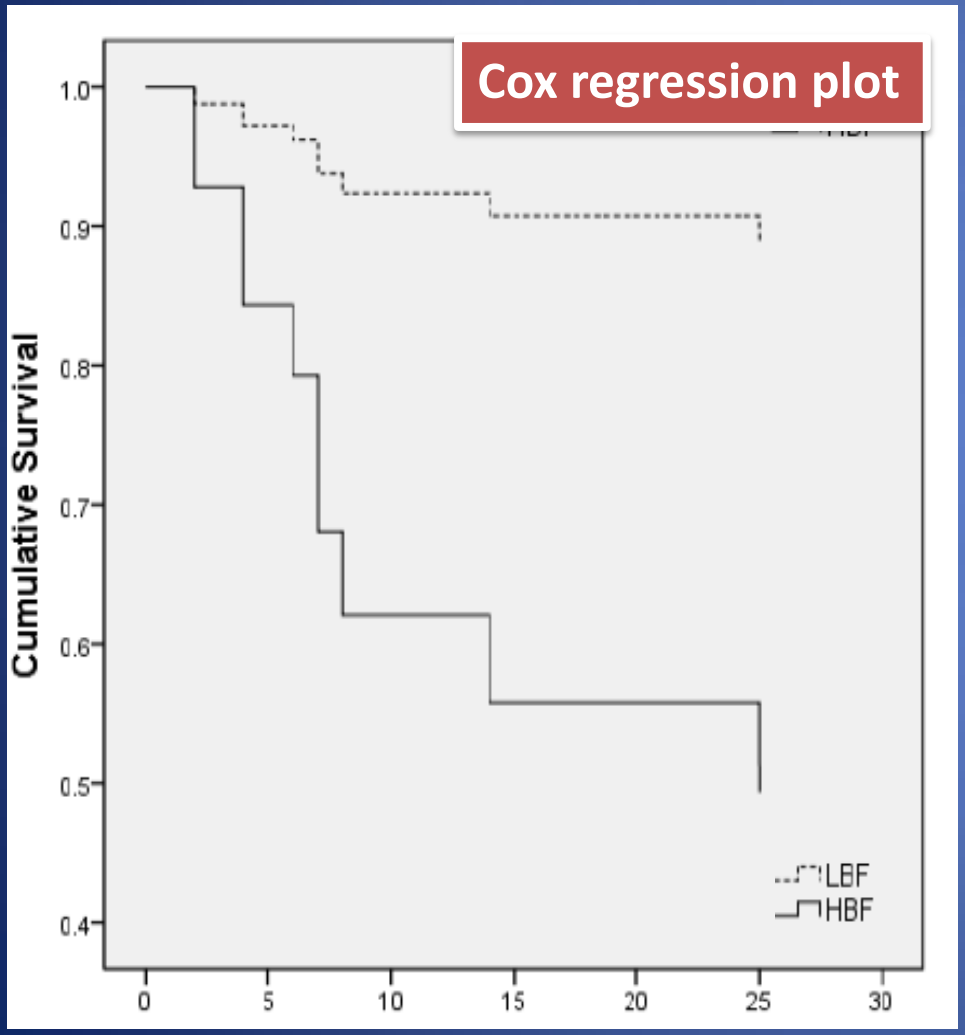
Cell



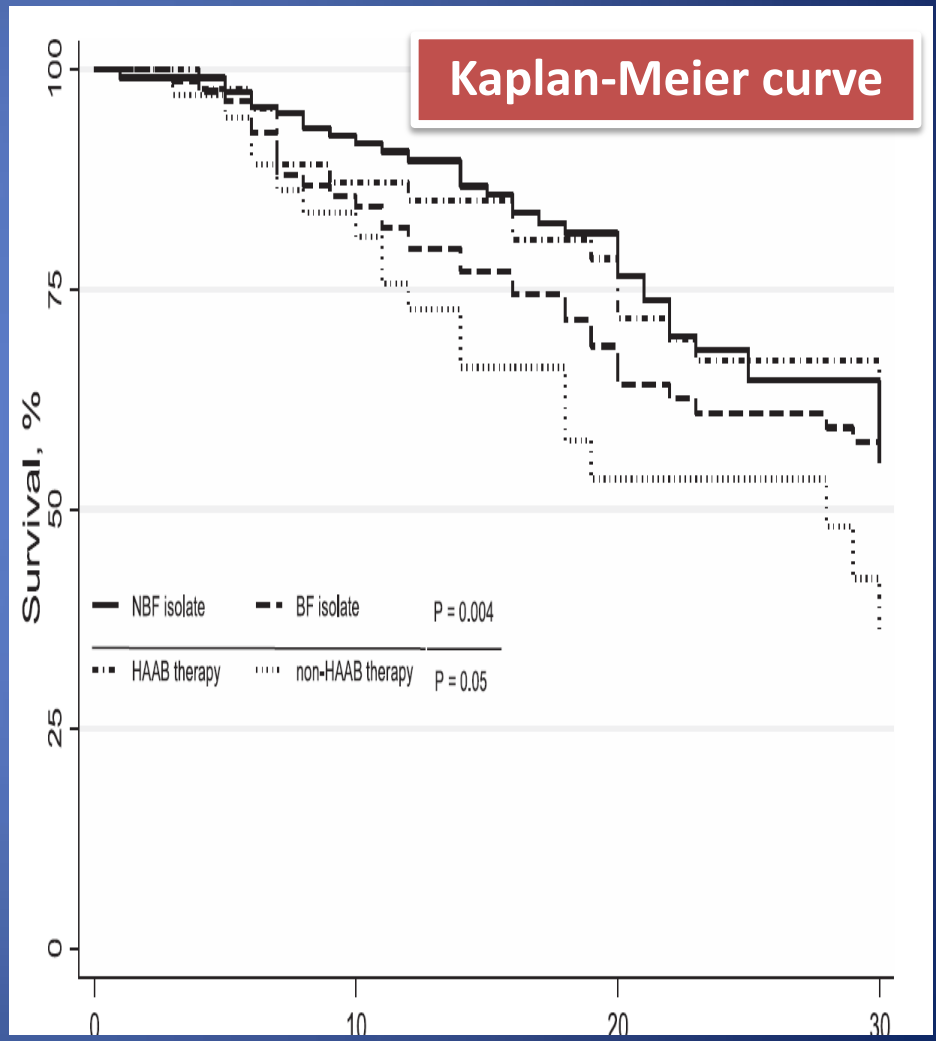
Taff H et al. *Future Microbiol* 2013

CLINICAL RELEVANCE & MANAGEMENT

Impact of Biofilm Formation & Antifungal on Patient Survival



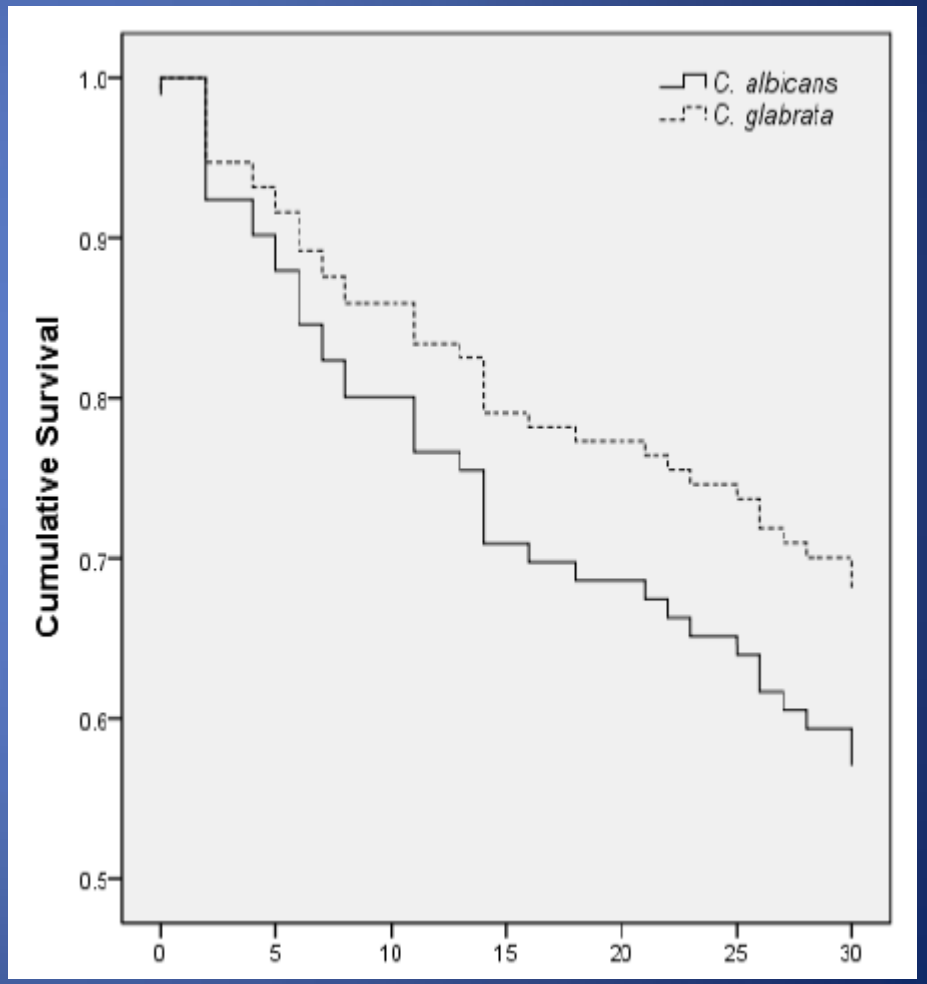
Rajendran R et al. *Clin Micrbiol Infect* 2016



Tumbarello M et al. *PLoS One* 2012

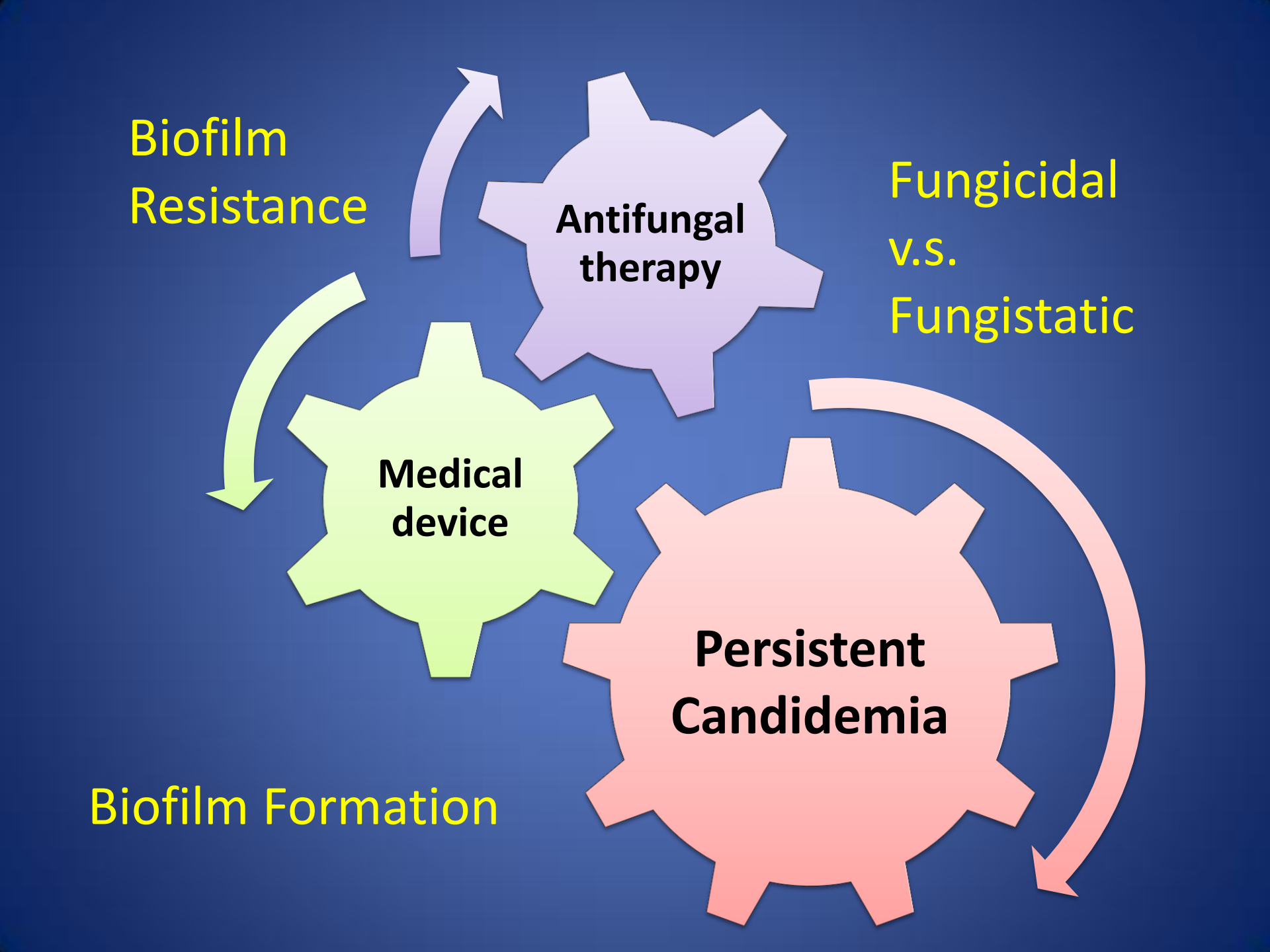
Species Difference, mainly on *C. albicans*

Candida species	OR (95% CI)	*P*[a]
C. albicans	3.90 (1.72–8.83)	<0.001
C. parapsilosis	4.16 (1.46–11.82)	0.003
C. tropicalis	0.88 (0.54–1.45)	0.62
C. glabrata	1.46 (0.32–6.62)	0.61
Other[b]		0.34
Total	2.76 (1.55–5.00)	<0.001

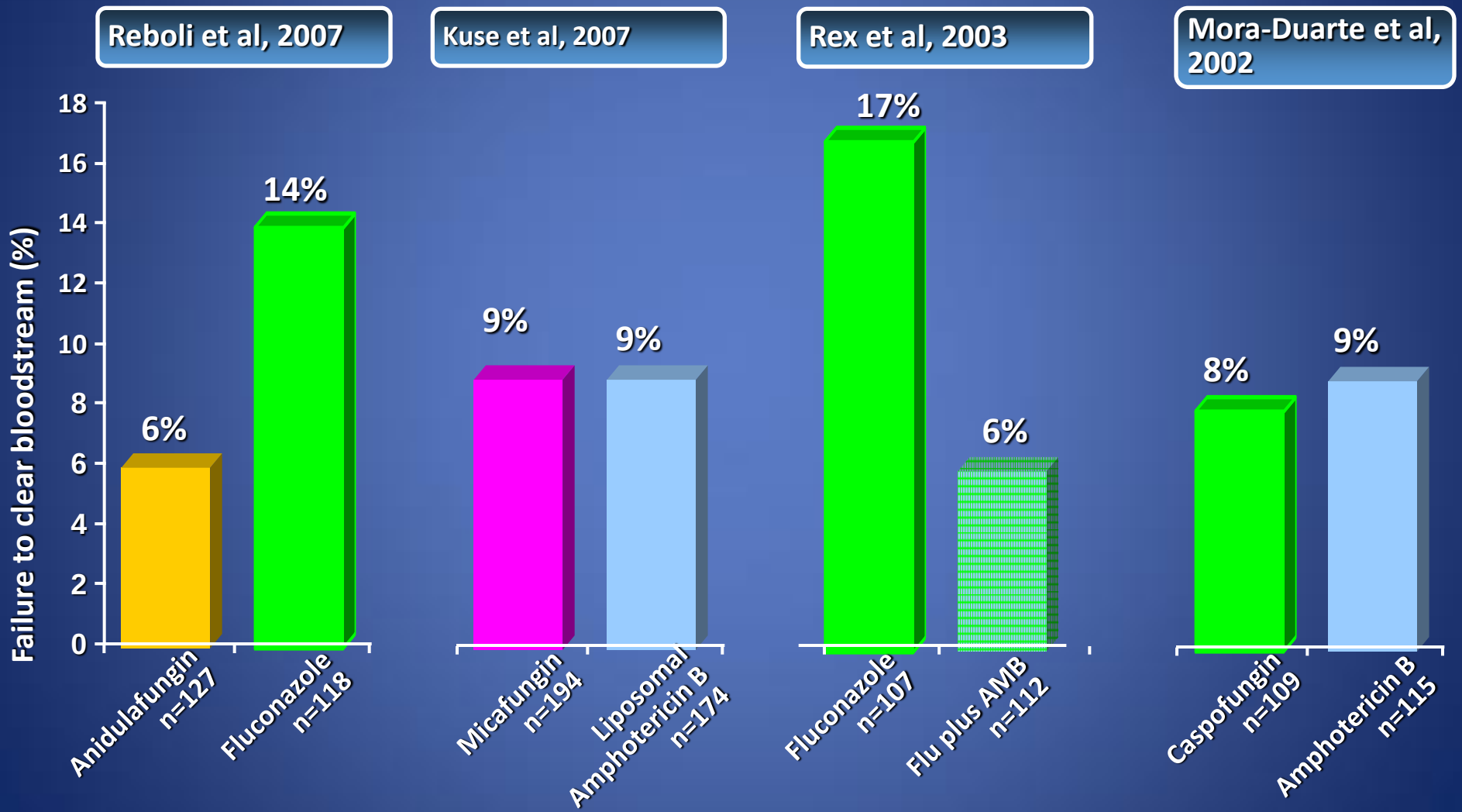


Tumbarello M et al. *J Clin Microbiol* 2007

Rajendran R et al. *Clin Micrbiol Infect* 2016


Biofilm Resistance
Antifungal therapy
Fungicidal v.s. Fungistatic
Medical device
Persistent Candidemia
Biofilm Formation

In published studies, end-of-treatment fungal persistence rates have ranged from 6% to 17%



Reboli AC et al. *N Engl J Med*. 2007;356:2472-2482.
Mora-Duarte J et al. *N Engl J Med*. 2002;347:2020-2029.

Kuse ER et al. Lancer Inf ect DIs. 2007;369:1519-1527.
Rex JH et al. *Clin Infect Dis*. 2003;36:1221-1228.

Etiologies of Persistence

1. Intravascular infection
   - Endocarditis
   - Suppurative thrombophlebitis
2. Metastatic sites
   - Osteoarticular infection
   - Endophthalmitis
3. Inserted medical device
   - Intravascular catheter (CVC, Hickman, etc.)
   - Prosthetic valves
   - Joint protheses
   - Pacemaker
4. Pharmacology
   - Adequate dosing
   - Drug resistance
5. Host immunity

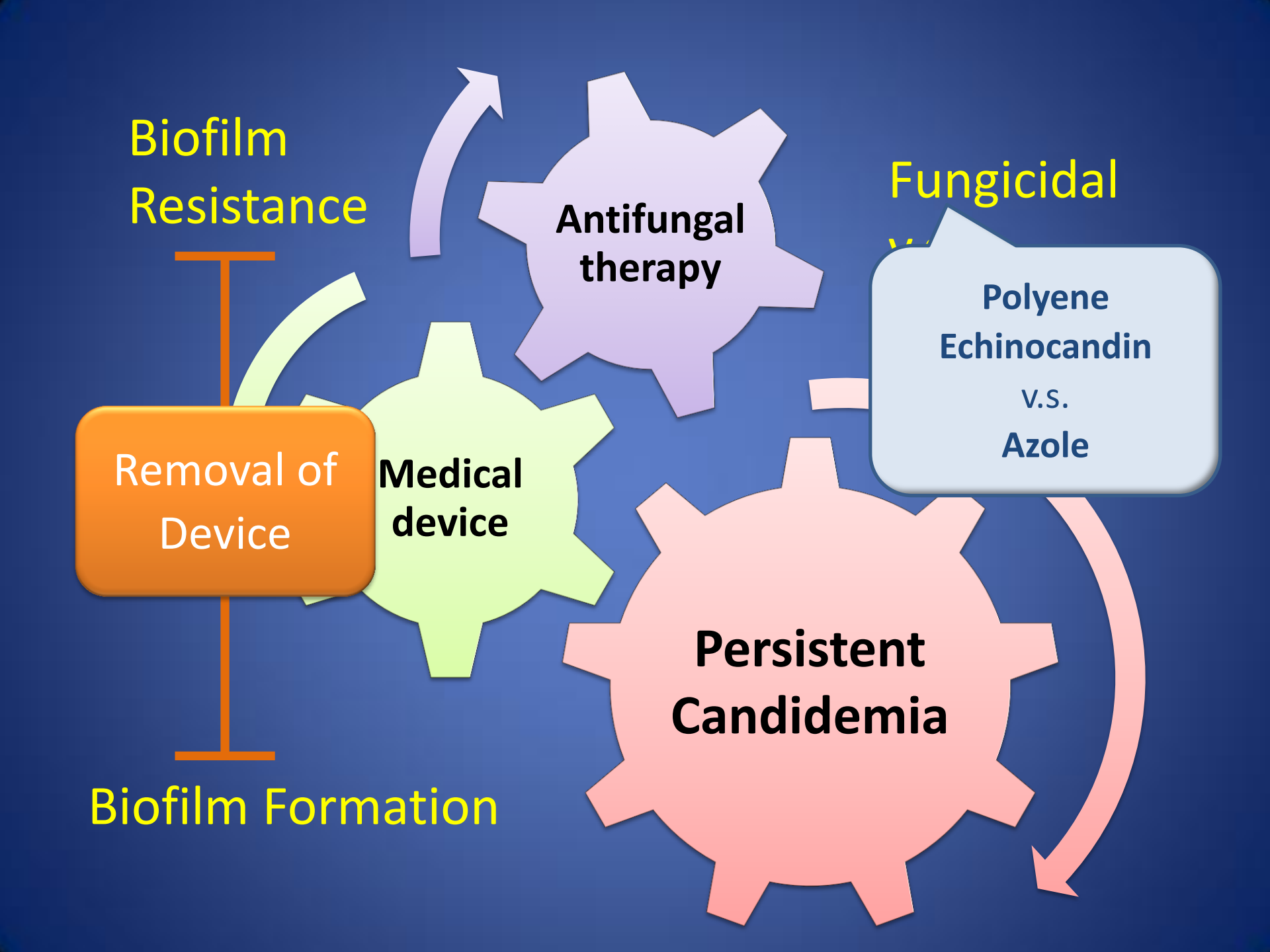
Biofilm Formation

How to Deal with Persistent Candidemia?

- Removal of intravascular devices if possible
- Finding out the possibility of other metastatic infection sites
- The susceptibility testing of the pathogen
- Change antifungal agents

  Azoles → polyenes or candins
- Ameliorating the immunosuppression status

Pappas et al. *Clin Infect Dis* 2004


Biofilm Resistance
Antifungal therapy
Fungicidal
Polyene
Echinocandin
v.s.
Azole
Removal of Device
Medical device
Persistent Candidemia
Biofilm Formation

Thanks! Any Comments?