Antifungal Resistance: Focus on *Candida* species

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Resistance Considerations

- Intrinsic resistance is the main driver of anti-fungal resistance
- Typically this happens by selection of resistant organisms as plasmid gene transfer doesn't occur in fungi
- Agricultural azole use could play a role
  - Comparison of fruit from organic vs. non-organic farms showed large difference in fluconazole MICs of *Candida*
  - Genetically related means of resistance found in clinical and soil sources of *Candida tropicalis*
  - Similar circumstances have led to MDR *Aspergillus* in the Netherlands
- High tolerance for large scale genetic alterations, in particular *C. glabrata*
  - Due to conditions in the host
  - Translocations, rearrangements, and new chromosomes

Arendrup, *Clinical Microbiology and Infection*, 2013; Yang, *PLoS One*, 2012; Verweij, *Lancet ID,* 2009; Perlin, *Curr Clin Micro Rpt*, 2015

Candida species Susceptibility Profile

Candida spp.	AMB*	FLUC	ITRA	VOR	Echino-candins
C. albicans	S	S	S	S	S
C. tropicals	S	S	S	S	S
C. parapsilosis	S	S	S	S	S/?
C. glabrata	S / NS	S^{DD} / R	S^{DD} / R	S / NS	S / R
C. krusei	S / NS	R	S^{DD} to R	S	S
C. lusitaniae	S / R	S	S	S	S

* No established breakpoints

S = susceptible; S^{DD} = susceptible-dose dependent; R = resistant; I = intermediate; NS, non-susceptible

Fluconazole – Mechanisms of Resistance Among *Candida* species

- Alterations of target enzyme
  - Point mutations in the ERG11 gene that encodes the P450 enzyme responsible for demethylation of lanosterol
  - More than 80 mutations identified, however only 12 have been associated with resistance
- Upregulation of target enzyme
- Efflux transporters
  - Pan-azole (ATP-binding cassette family)
  - Fluconazole specific (Major facilitator family)
- Alterations in sterol composition
  - Very uncommon
  - Tends to also cause Amphotericin B resistance

Akins, *Medical Mycology,* 2005; Sanglard, *Lancet Infectious Diseases*, 2002; Perlin, *Curr Clin Micro Rpt*, 2015

Fluconazole: Mechanisms of Resistance

- Risk factors for resistance
  - Prior exposure (Lortholary, 2011)
    - Decreased prevalence of *C. albicans*
    - Increased prevalence of *C. glabrata* and *C. krusei*
  - Suboptimal dosing (Shah, 2012; Pai 2007)
    - IDSA guidelines recommend 6-12mg/kg/day for candidemia
    - Less has been associated with increased resistance and mortality
  - Exposure to antibacterial agents (Ben-Ami 2012)
    - Association between exposure to bactrim, carbapenems, clindamycin, and colistin
    - Cephalosporins negatively associated
    - Metronidazole associated with *C. glabrata*
  - Collateral Damage
    - Among *C. glabrata* isolates, fluconazole exposure was also associated (trend, not quite significant) with voriconazole resistance (Chen 2012)

Geographic Variability of *Candida* spp.

TABLE 2. Geographic variation in frequency of common and uncommon species of *Candida*: ARTEMIS, 2001 to 2007[a]

Species	Species distribution (%) by region (total no. of isolates)[b]:					
	APAC (44,674)	EU (109,643)	AF/ME (8,259)	LAM (27,395)	NAM (11,682)	Total (201,653)
C. albicans	64.4	67.9	67.1	51.8	48.9	63.8
C. glabrata	12.6	11.3	8.8	7.4	21.1	11.6
C. tropicalis	11.7	4.9	6.6	13.2	7.3	7.7
C. parapsilosis	7.4	4.2	6.0	10.3	13.6	6.3
C. krusei	1.2	3.4	1.6	1.4	3.1	2.5
C. guilliermondii	0.4	0.5	0.1	2.2	0.5	0.7
C. inconspicua	<0.1	0.5		<0.1	<0.1	0.3
C. rugosa	0.4	<0.1	<0.1	1.2	<0.1	0.3
C. norvegensis	<0.1	0.2	<0.1	<0.1	0.2	0.1

[a] Includes all specimen types and all locations in hospitals from 133 institutions.
[b] Abbreviations: APAC, Asia-Pacific; EU, Europe; AF/ME, Africa-Middle East; LAM, Latin America; NAM, North America.

Pfaller MA et al. J Clin Micro 2010; 48: 1366-77

Trends in Fluconazole Resistance Among Candida Isolates 1997-2007

TABLE 5. Trends in *in vitro* resistance to fluconazole among *Candida* spp. as determined by CLSI disk diffusion testing over a 10.5-year period[a,b]

Species	1997–2000		2001–2004		2005–2007	
	No. of isolates tested	% R	No. of isolates tested	% R	No. of isolates tested	% R
C. albicans	39,152	0.9	71,027	1.4	57,598	1.4
C. glabrata	5,634	19.2	12,963	15.9	10,342	15.4
C. tropicalis	2,996	3.6	8,496	4.5	7,050	3.6
C. parapsilosis	2,633	2.5	7,783	3.5	5,005	3.6
C. krusei	1,207	65.8	2,840	77.5	2,239	79.3
C. guilliermondii	367	12.5	902	9.9	508	14.2
C. lusitaniae	276	2.9	674	4.3	559	6.6
C. kefyr	182	3.3	527	3.6	517	1.7
C. inconspicua	9	55.6	276	52.2	290	54.1
C. famata	123	17.1	375	12.5	247	6.9
C. rugosa	35	34.3	238	50.7	134	10.4
C. dubliniensis	1	0.0	113	2.7	197	2.0
C. norvegensis	11	54.5	135	36.3	113	46.0
C. lipolytica	7	0.0	80	37.5	50	14.0
C. sake			20	10.0	67	11.9
C. pelliculosa	1	0.0	47	0.0	40	15.0
C. apicola					57	1.8
C. zeylanoides	4	0.0	50	28.0	20	15.0
C. valida			9	66.7	12	58.3
C. intermedia			10	0.0	14	7.1
C. pulcherrima			6	0.0	8	0.0
C. haemulonii			6	0.0	3	33.3
C. stellatoidea					7	0.0
C. utilis					6	0.0
C. humicola			2	50.0	4	50.0
C. lambica					5	80.0
C. ciferrii					2	50.0
C. colliculosa					2	0.0
C. holmii					1	0.0
C. marina					1	0.0
C. sphaerica					1	0.0
Candida spp.[c]	2,591	10.5	6,186	8.2	3,558	10.1

[a] Includes all specimen types and all hospital locations in 141 institutions.
[b] % R, percent resistant (zone diameter, ≤14 mm).
[c] *Candida* species not otherwise identified.

Pfaller MA et al. J Clin Micro 2010; 48: 1366-77

Fluconazole and Voriconazole Susceptibility by Global Region

TABLE 7. Geographic variation in the *in vitro* susceptibilities of common and uncommon species of *Candida* to fluconazole and voriconazole, 2001 to 2007[a]

Species	Antifungal agent	APAC		EU		AF/ME		LAM		NAM	
		No. of isolates	% R	No. of isolates	% R	No. of isolates	% R	No. of isolates	% R	No. of isolates	% R
C. albicans	Fluconazole	28,781	0.9	74,408	1.3	5,539	0.6	14,178	2.1	5,718	5.1
	Voriconazole	27,827	0.8	72,873	1.1	5,502	0.3	13,711	1.7	5,681	3.6
C. glabrata	Fluconazole	5,629	13.0	12,439	16.3	728	16.2	2,039	15.1	2,470	19.5
	Voriconazole	5,515	8.2	12,288	9.8	705	8.1	2,000	11.3	2,460	14.6
C. tropicalis	Fluconazole	5,178	6.5	5,349	2.9	544	2.6	3,625	2.6	850	4.4
	Voriconazole	5,062	8.4	5,128	3.9	542	2.4	3,522	3.7	836	5.3
C. parapsilosis	Fluconazole	3,294	4.3	4,578	2.6	499	15.0	2,830	2.1	1,587	3.5
	Voriconazole	3,120	1.7	4,487	1.1	496	11.1	2,779	0.9	1,517	2.4
C. krusei	Fluconazole	532	73.5	3,678	80.8	134	72.4	370	66.8	361	74.0
	Voriconazole	516	5.0	3,637	7.7	134	4.5	351	14.0	363	5.5
C. guilliermondii	Fluconazole	178	13.5	567	13.8	12	8.3	590	9.0	63	7.9
	Voriconazole	175	10.9	558	6.1	12	0.0	567	3.7	63	4.8
C. inconspicua	Fluconazole	4	25.0	558	53.0			2	100.0	2	100.0
	Voriconazole	4	0.0	555	3.8			2	50.0	2	0.0
C. rugosa	Fluconazole	165	32.1	89	10.1	1	0.0	339	55.5	9	22.2
	Voriconazole	145	6.9	87	1.1	1	0.0	338	32.8	9	11.1
C. norvegensis	Fluconazole	7	14.3	204	49.0	1	0.0	13	0.0	21	0.0
	Voriconazole	7	0.0	203	4.9	1	0.0	13	0.0	21	0.0

[a] For definitions of abbreviations, see Table 2, footnote *b*.

Pfaller MA et al. J Clin Micro 2010; 48: 1366-77

Voriconazole Susceptibility of Fluconazole-resistant *Candida* Isolates

TABLE 4. *In vitro* susceptibilities of fluconazole-resistant isolates of *Candida* spp. to voriconazole as determined by CLSI disk diffusion testing[a]

Species	No. of isolates tested	% S	% SDD	% R
C. albicans	1,782	28.1	8.4	63.6
C. glabrata	3,550	19.1	21.7	59.2
C. tropicalis	629	17.0	15.3	67.7
C. parapsilosis	431	39.2	20.4	40.4
C. krusei	3,889	79.6	11.3	9.2
C. guilliermondii	157	43.9	16.6	39.5
C. lusitaniae	63	55.6	17.5	27.0
C. kefyr	27	66.7	7.4	25.9
C. inconspicua	297	83.8	10.1	6.1
C. famata	62	37.1	24.2	38.7
C. rugosa	242	28.1	21.5	50.4
C. dubliniensis	8	62.5	0.0	37.5
C. norvegensis	100	81.0	10.0	9.0
C. lipolytica	37	29.7	27.0	43.2
C. sake	9	44.4	11.1	44.4
C. pelliculosa	6	16.7	16.7	66.7
C. apicola	1	0.0	0.0	100.0
C. zeylanoides	15	46.7	26.7	26.7
C. valida	14	71.4	7.1	21.4
C. intermedia	1	100.0	0.0	0.0
C. haemulonii	1	0.0	0.0	100.0
C. humicola	3	0.0	33.3	66.7
C. lambica	4	25.0	50.0	25.0
C. ciferrii	1	0.0	100.0	0.0
Candida spp.[b]	850	47.6	14.6	37.8

[a] Isolates obtained from 133 institutions, 2001 to 2007. The zone diameters for voriconazole disk diffusion susceptibility categories were as follows: S, ≥17 mm; SDD, 14 to 16 mm; R, ≤13 mm.
[b] *Candida* species not otherwise identified.

Pfaller MA et al. J Clin Micro 2010; 48: 1366-77

Echinocandins

- The class was derived from the naturally occurring agent Pneumocandin
- Inhibit the synthesis of 1,3-beta-D-glucan
  - Synthesized by a transmembrane glucan synthase complex composed of FKS subunits 1, 2, and 3
  - Fibrillar and interwoven macromolecules that forms the fungal cell wall
- Reduce cell wall integrity → loss of control of the high turgor pressure of the yeast cell → membrane swelling and lysis
- Minimal differences between the three agents (micafungin, caspofungin, and anidulafungin)

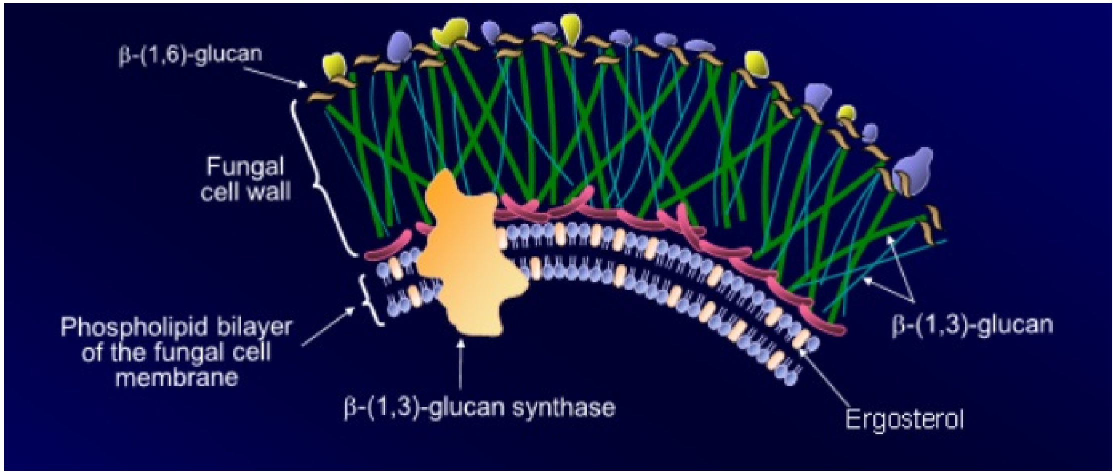
Principles and Practice of Infectious Diseases,8th edition, chapter 39; Fromtling, 1989; Kurtz 1994

Echinocandins

- Treatment of choice for initial therapy in candidemia (Pappas 2016)
- Fungicidal against *Candida* species, limited activity against filamentous fungi, no activity against *Cryptococcus*
- Minimal drug-drug interactions
- Minimal adverse reactions due to the lack of an analogous pathway in mammals
- Intravenous only

Echinocandins: Mechanisms of Resistance

- FKS mutations
  - Intrinsic - *C. parapsilosis* and *C. guilliermondii* have altered FKS1 genetic sequences
  - Acquired
    - Point mutations in the genetic sequences of FKS1 have been identified in clinically resistant isolates of *C. albicans, C. tropicalis, C. krusei,* and *C. glabrata*
    - Only *C. glabrata* has had mutations identified in FKS2



Beyda, *The Annals of Pharmacotherapy*, 2012; Perlin, *Curr Clin Micro Rpt*, 2015; http://howmed.net/pharmacology/anti-fungal-drugs-azoles-echinocandins-allylamines-and-flucytosine/

Echinocandins: Mechanisms of Resistance

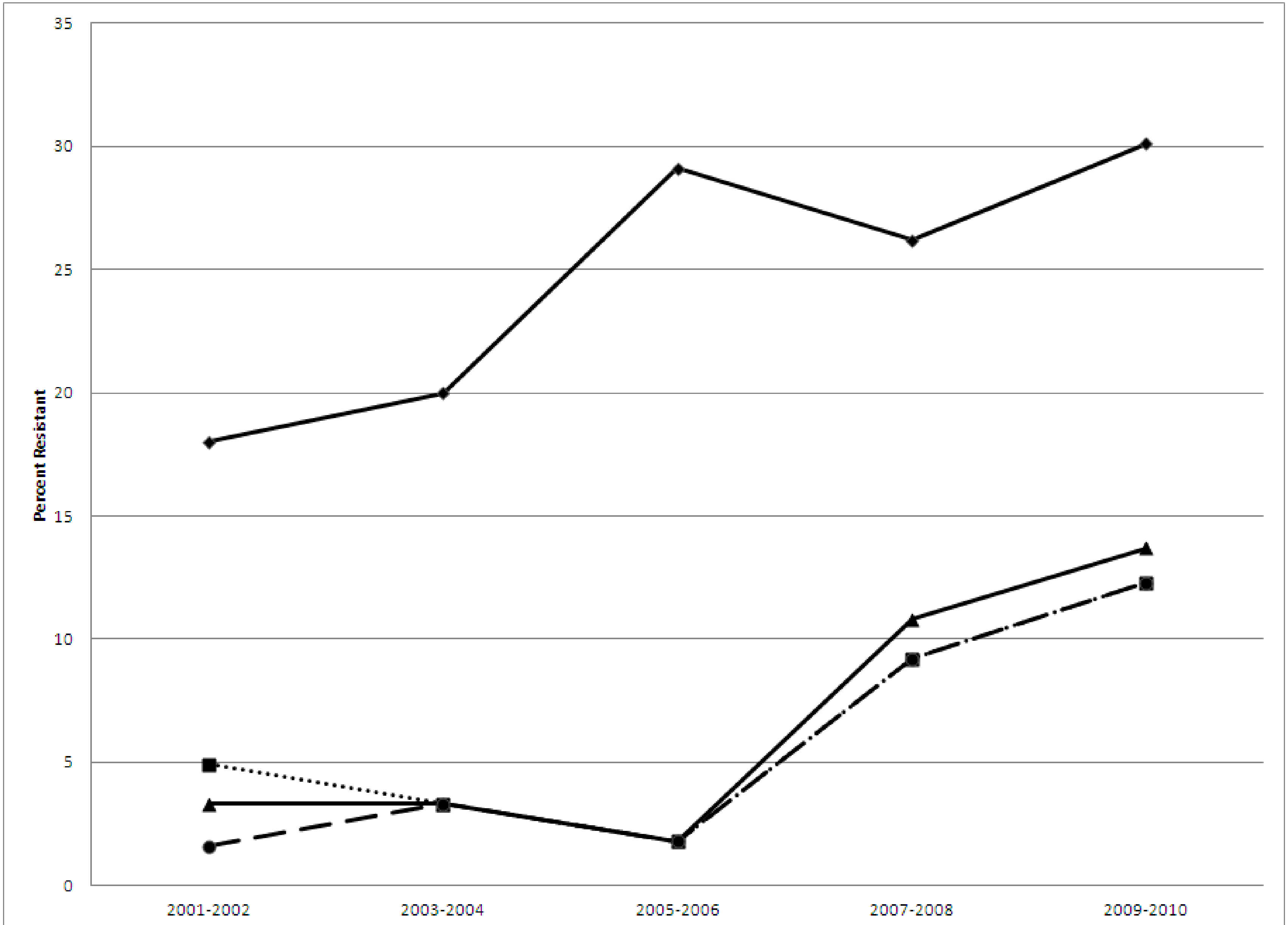
- Defining resistance
- Clinical breakpoints initially established in 2008
  - >/= 2mcg/mL defined as resistant
  - Mostly due to the lack of clinical evidence of failure
- Updated in 2011 due to increasing reports of clinical failure in isolates defined as sensitive
  - For albicans, tropicalis, and krusei: >0.25 mcg/mL for all 3 agents
  - For glabrata: >0.06mcg/mL (micafungin) or >0.12mcg/mL (anidulafungin, caspofungin)
  - For parapsilosis and guilliermondii: >2mcg/mL for all 3 agents

Pfaller, *J Clin Microbiol 2008*; Pfaller, *Drug Resistance Updates*, 2011

Clinical Importance

- *Candida glabrata* is more common in the setting of fluconazole usage and is inherently more resistant to fluconazole
- Fluconazole resistance, whether proven or suspected, leads to increased usage of echinocandins
- *C. glabrata* displays the most echinocandin resistance
- Unfortunately, this means the organism developing the most echinocandin resistance is already resistant to azoles
- Risk factors for echinocandin resistance (MIC or FKS mutation)
  - Echinocandin exposure
  - Breakthrough infection, Solid Organ Transplant

Alexander, *CID,* 2013; Beyda, *CID*, 2014



Alexander et al CID 2013; 56: 1724-32

Clinical Importance

- Duke study
- Multiple patients treated with an echinocandin responded to therapy
- Amphotericin associated with increased risk of mortality
- Univariable analysis:
  - FKS mutation associated with decreased 10 day treatment success (80% vs. 94.4%), but not 30 day treatment success, 10 day mortality, or 30 day mortality
  - Resistant MIC associated with increased risk of 30 day mortality
- On full multivariable model neither FKS mutation nor resistant MIC were predictive of mortality

Alexander, CID, 2013; Pappas, CID, 2009

TABLE 5 Association of *FKS* mutations, prior echinocandin exposure, and echinocandin MICs with clinical failure

Predictor variable	No. of successes (*n* = 44)	No. of failures (*n* = 22)	*P* value	Odds ratio	95% CI	% PPV[a]	% NPV[b]
Presence of *FKS* mutation	1	9	0.0001	29.7	3.44–257.5	90 (9/10)	77 (43/56)
Prior echinocandin exposure	8	13	0.002	6.50	2.07–20.4	62 (13/21)	80 (36/45)
Caspofungin MIC of >0.5 μg/ml (BMD-RPMI)	6	7	0.10	2.96	0.85–10.3	54 (7/13)	72 (38/52)
Caspofungin MIC of >0.06 μg/ml (BMD-AM3)	2	8	0.002	12.0	2.27–63.4	80 (8/10)	75 (42/56)
Caspofungin MIC of >0.25 μg/ml (YeastOne)	6	9	0.03	4.39	1.31–14.7	60 (9/15)	75 (38/51)
Caspofungin MIC of >0.25 μg/ml (Etest)	3	11	0.0001	13.7	3.24–57.7	79 (11/14)	79 (41/52)

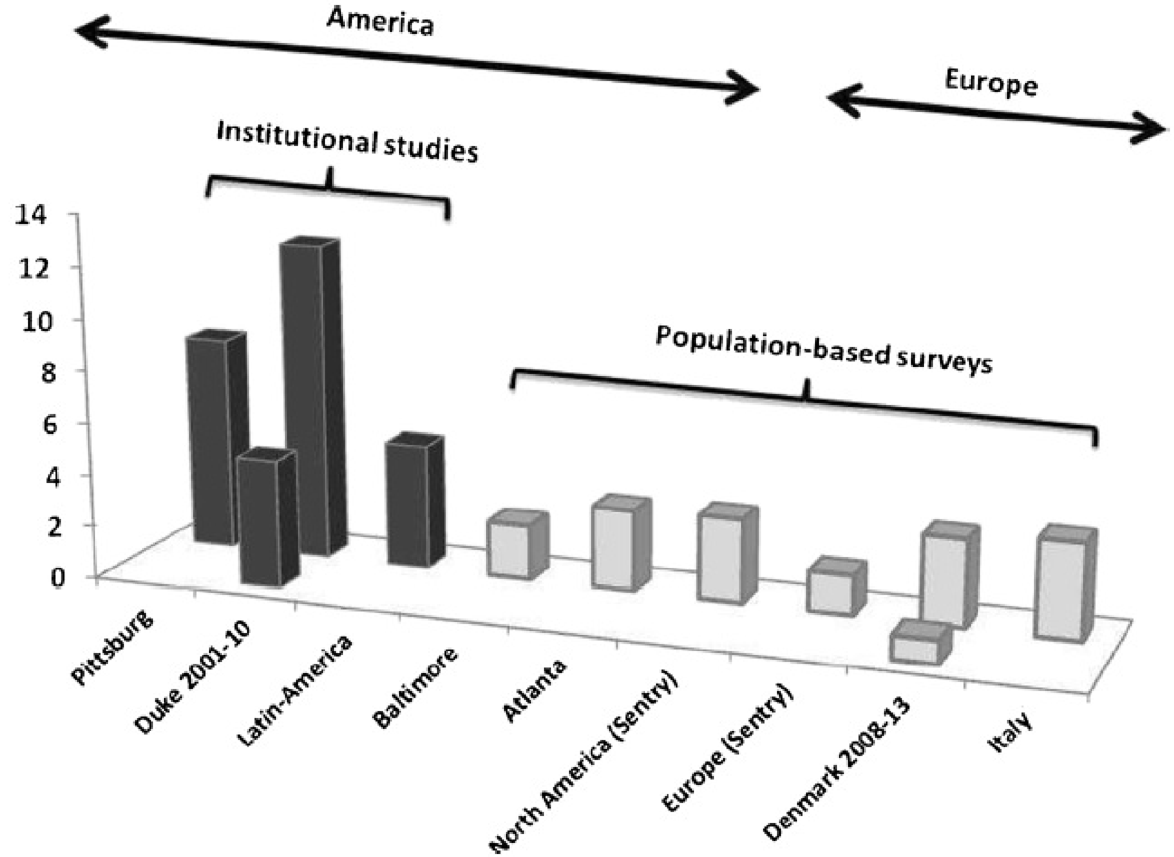
[a] Positive predictive value (PPV) is the percentage of positive tests associated with failure.

[b] Negative predictive value (NPV) is the percentage of negative tests associated with success.

Shields et al. Antimicrob Agent Chemo 2013; 57: 3528-35.

Echinocandin Susceptibility

Fig. 1 Echinocandin resistance in *C. glabrata* in Europe and America. Resistance rate varies among different studies. The rate reported from institutional studies is higher than that from population-based surveys, where only the initial blood isolate is included to avoid biasing the data set. Adapted from Arendrup et al. [14]



Arendrup, *Curr Opin Infect Dis*, 2014

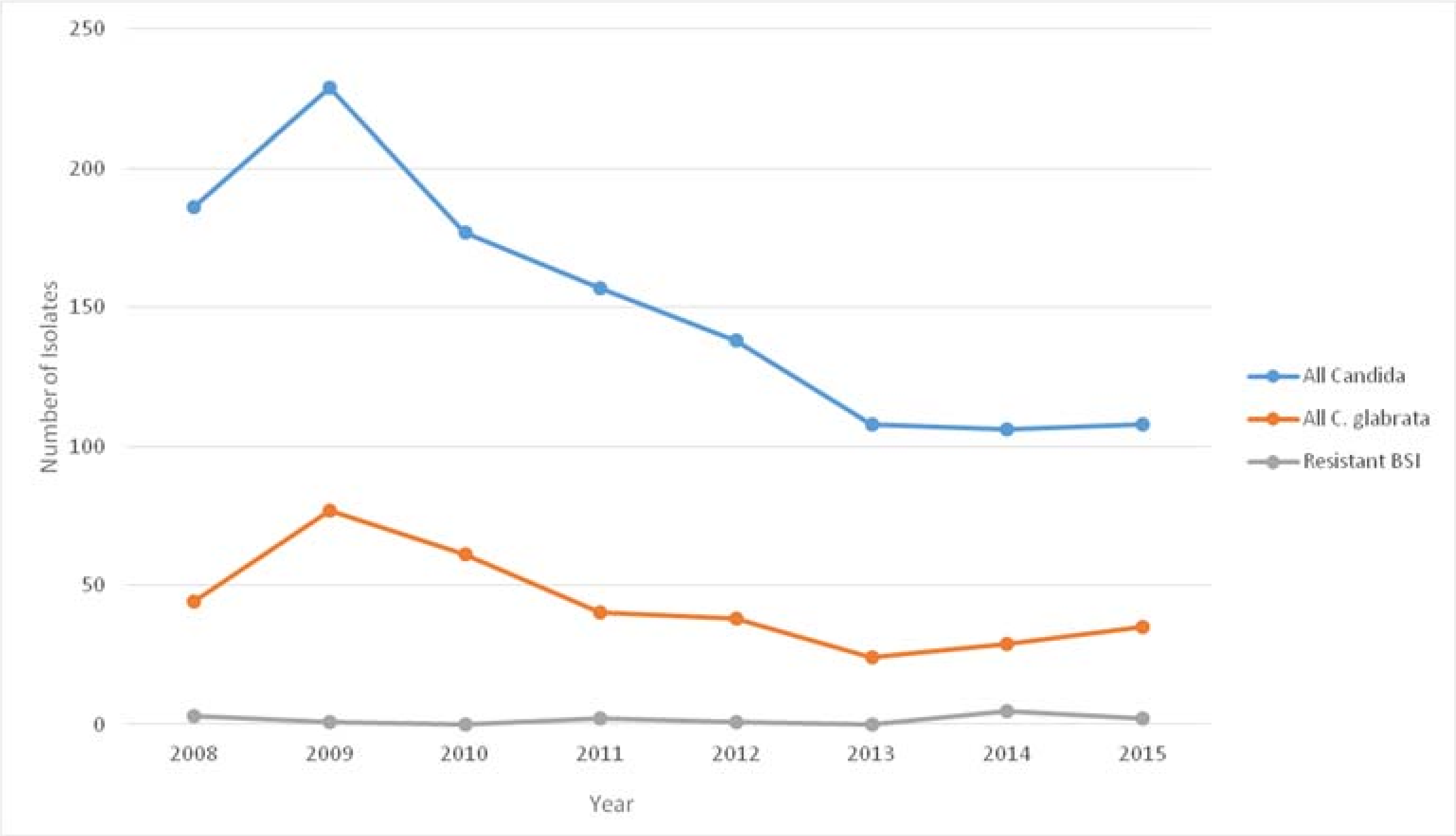
UAB Micafungin Susceptibility

- All stored *Candida* isolates from 2005-15
  - Blood and various body fluids
  - 3,876 isolates
    - 1,921 *C. albicans*
    - 832 *C. glabrata*
    - 508 *C. parapsilosis*
    - 405 *C. tropicalis*
    - 88 *C. krusei*
  - 33 total isolates with ↑ MIC or FKS mutation
    - 1st isolated identified in 2007
    - 15 bloodstream infections, 18 from other sites (primarily intra-abdominal fluid)

UAB Micafungin Susceptibility

- Bloodstream infections
  - 12 *C. glabrata,* 2 *C. tropicalis,* 1 *C. lusitaniae*
  - 30-day mortality was 60%
  - UAB Candidemia mortality 2015-2016: ~35%
- Non-bloodstream infections
  - Intra-abdominal infections - 8 cases, 3 deaths
  - Urine – 5 cases

Candida fungemia over time



Year	2008	2009	2010	2011	2012	2013	2014	2015	Total
% total candidemia	1.6%	0.4%	0.0%	1.3%	0.7%	0.0%	4.7%	1.9%	1.2%

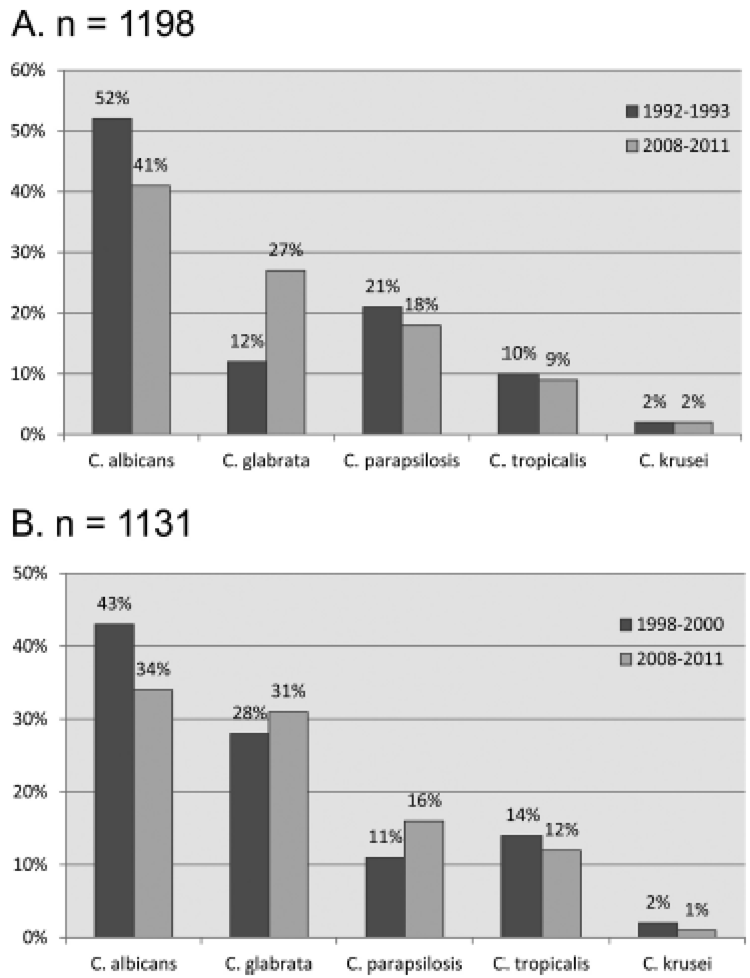


FIG 1 Differences in species distribution between past (1992 to 1993) and present (2008 to 2011) surveillance in Atlanta (A) and Baltimore (B).

Lockhart et al. J Clin Micro 2012; 50: 3435-42

TABLE 4 *In vitro* susceptibilities of *Candida* surveillance isolates from 2008 to 2011 to selected antifungal agents in Atlanta and Baltimore

Species and antifungal agent	Susceptibility profile of Atlanta isolates: No. of isolates[a]	MIC$_{50}$ (μg/ml)	MIC$_{90}$ (μg/ml)	% Resistant isolates	Susceptibility profile Baltimore isolates: No. of isolates[a]	MIC$_{50}$ (μg/ml)	MIC$_{90}$ (μg/ml)	% Resistant isolates
All species	1,141				1,068			
Fluconazole		1	16	8.2		1	16	6.5
Voriconazole		0.06	0.5	1.1		0.06	0.5	0.9
Caspofungin		0.06	0.25	1.0		0.06	0.25	1.0
Anidulafungin		0.03	1	1.0		0.03	1	1.0
Micafungin		0.03	1	1.0		0.03	1	1.0
C. albicans	489				388			
Fluconazole		0.5	2	2.2		0.5	1	2.3
Voriconazole		0.03	0.125	0.6		0.03	0.125	1.3
Caspofungin		0.03	0.06	0.6		0.03	0.06	0.3
Anidulafungin		0.015	0.06	0.4		0.03	0.06	0.3
Micafungin		0.03	0.03	0.4		0.015	0.03	0.3
C. glabrata	318				352			
Fluconazole		8	64	13.2		8	64	10.8
Voriconazole		0.25	2			0.25	1	
Caspofungin		0.06	0.125	2.5		0.06	0.125	2.3
Anidulafungin		0.06	0.125	3.1		0.06	0.125	2.3
Micafungin		0.015	0.03	2.8		0.015	0.03	2.5

Lockhart et al. J Clin Micro 2012; 50: 3435-42

Candida: Emerging Resistance Issues

C. glabrata

Azoles (10-25% of all isolates)

Echinocandins (3-15% of all isolates)

Azole and echinocandin co-resistance (10-20% of azole-R isolates)

C. parapsilosis

Echinocandins (elevated MICs, intrinsic)

Azoles (~4% acquired)

C. krusei

Inherently fluconazole resistant

C. auris

>90% isolates R to fluconazole

ECH R is less common and geographically/clonally determined

AmB R is least common, pan-resistant isolates reported, esp in India/Pakistan

Rare species

Intrinsic resistance to azoles and echinocandins

C. guilliermondii, C. rugosa

Pfaller et al. J Clin Microbiol 2007;45:1735-45

Ευχαριστώ πολύ!