Antifúngicos: Mecanismos de ação e de Resistência

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Classificação das micoses

Micose	Tecido	Espécies
Superficial	Extrato córneo do tecido epitelial, pelo e cabelo	*Malassezia* spp. *Hortaea werneckii* *Piedraia hortae* *Trichosporon* spp.
Cutâneo	Porções queratinizadas da pele, pelo e cabelo	*Trichophyton* spp. *Microsporum* spp. *Epidermophyton floccosum*
Subcutâneo	Derme, músculos e tecido conjuntivo	*Sporothrix* spp. *Fonsecaea pedrosoi*
Sistêmico endêmico	Inicia-se com uma infecção pulmunar podendo atingir qualquer órgão	*Paracoccidioides* spp. *Histoplasma capsulatum* *Coccidioides* spp.
Sistêmico	Qualquer tecido	*Candida* spp. *Cryptococcus* spp. *Aspergillus* spp. *Fusarium* spp. *Rhizopus* spp., *Mucor* spp.

Lista de patógenos fúngicos prioritários da OMS para orientar pesquisa, desenvolvimento e ação de saúde pública

25 de outubro, 2022.
https://www.who.int/publications/i/item/9789240060241

Histórico – antifúngicos

1900

1903

KI

1940

1939

Griseofulvina isolada

1949

Nistatina isolada

1950

1956

AMB isolada

1960

1963

5-FC atividade antifúngica

1970

1970s

Primeiros **azóis** (imidazóis)

no mercado

1980

1981

Cetoconazol

1987

Formulação lipídica p/ AMB

1988

Equinocandina

(produtos semi-sintético)

1990

1990 e 1992

Triazóis - 1ª. geração

Fluconazol
Itraconazol

Alilamina

Terbinafina

2000...

2002 - atual

Triazóis – 2ª. geração

2002-Voriconazol
2006-Posaconazol

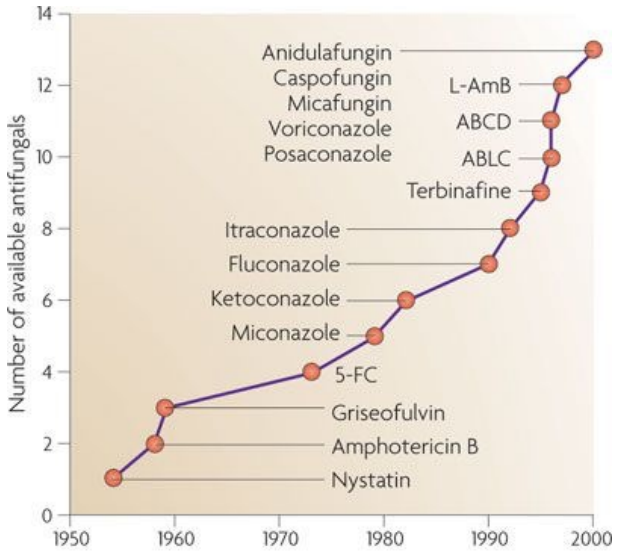
2015 - Isavuconazol

Equinocandinas

2002-Caspofungina

2005- Micafungina

2006- Anidulafungina

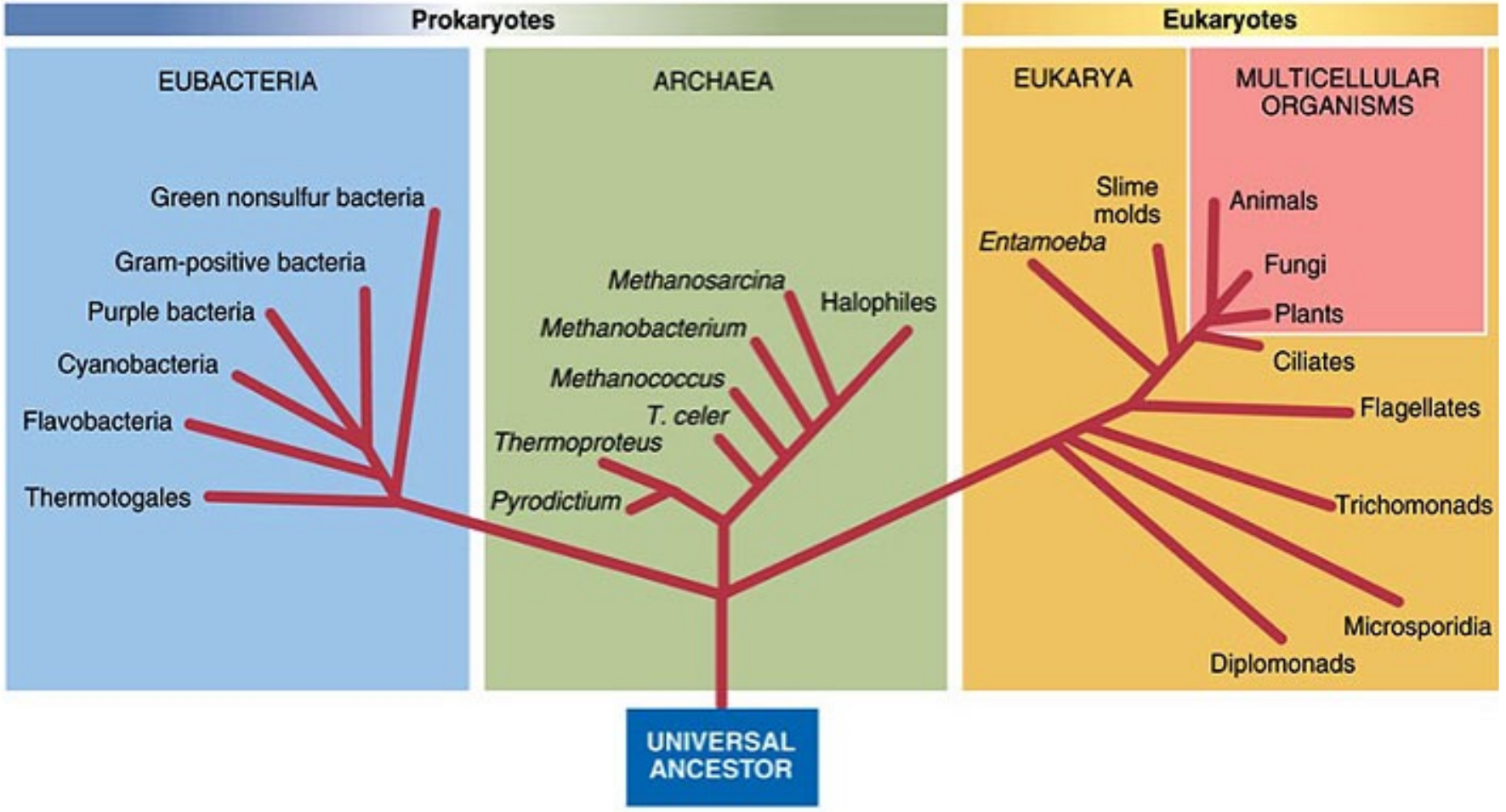


Ostrosky-Zeichner et al.
Nature Reviews Drug Discovery (9): 719-727, 2010.

Fluconazole has been on WHO's Essential Medicines List for many years, amphotericin B and flucytosine were listed in 2013, and itraconazole and voriconazole were added in 2017.

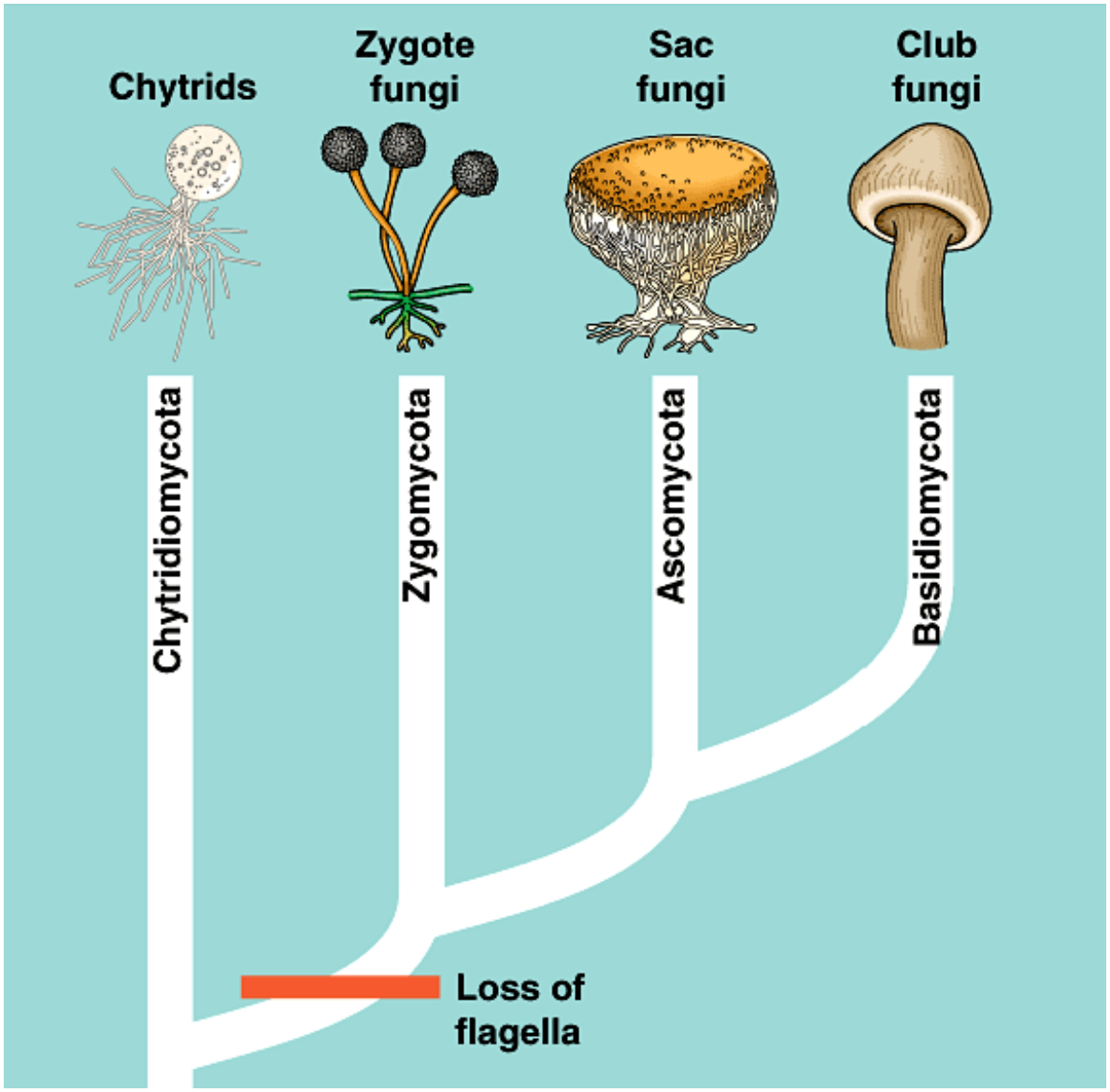
WHO. WHO model lists of essential medicines. http://www.who.int/medicines/publications/essentialmedicines/en/ (accessed April 5, 2017).

Domínio dos seres vivos

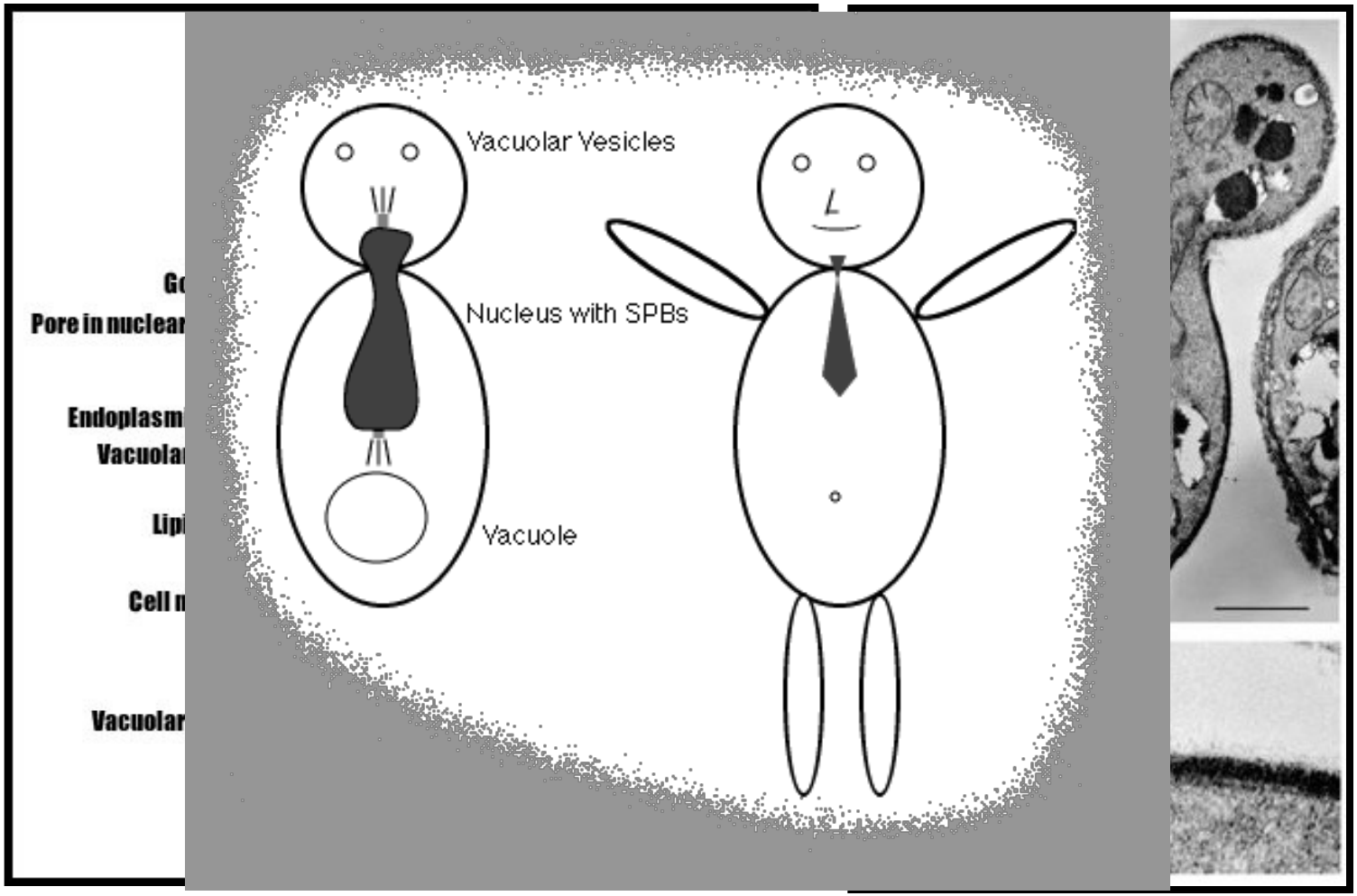


http://vsites.unb.br/ib/cel/microbiologia/intromicro/intromicro.html

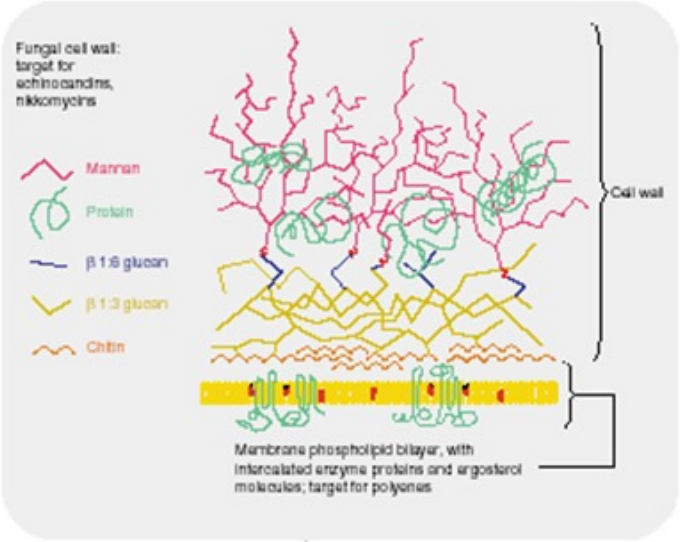
Filos


Chytrids
Zygote fungi
Sac fungi
Club fungi
Chytridiomycota
Zygomycota
Ascomycota
Basidiomycota
Loss of flagella

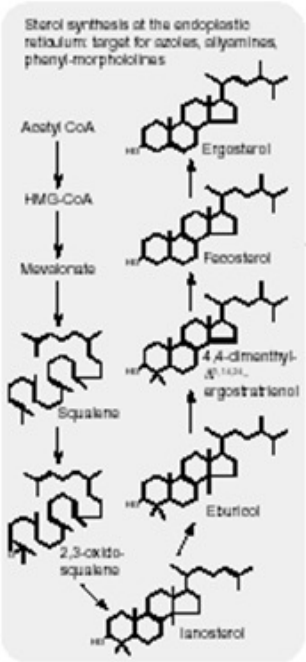
A célula fúngica



Alvos para a quimioterapia antifúngica

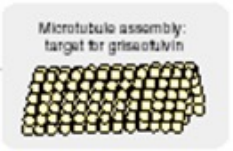


Equinocandinas



Poliênicos
Azóis

5-Fluorocitosina



Griseofulvina

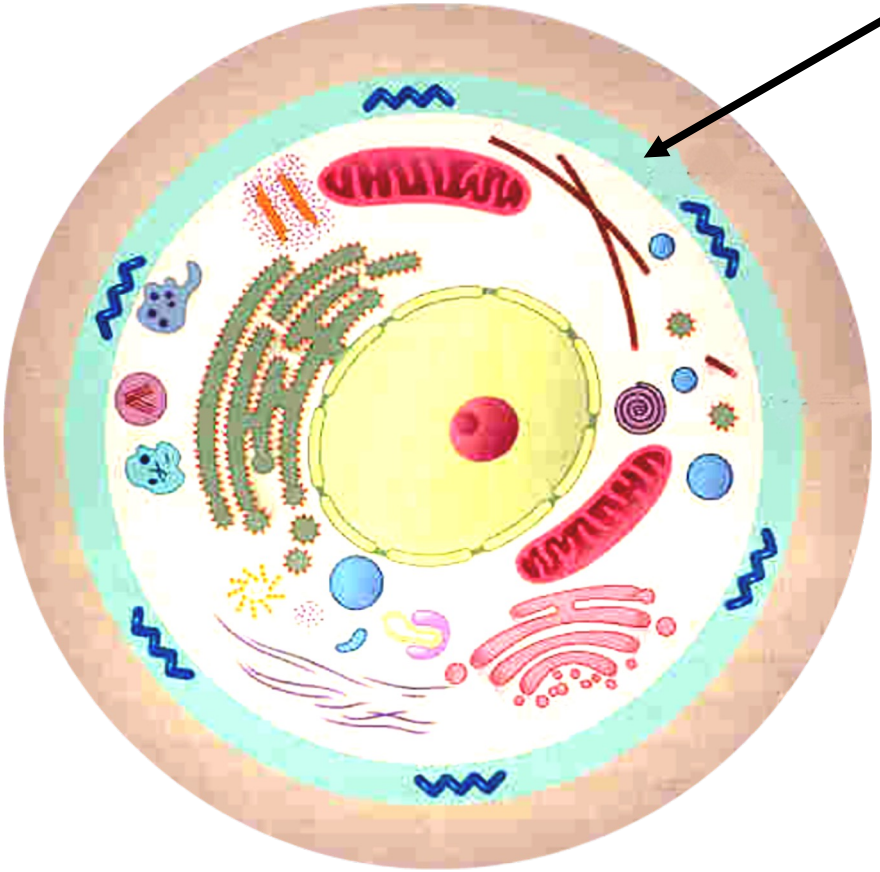
Odds *et al.* TRENDS in Microbiology, 11: 272-279, 2003.

O antifúngico ideal...

- Toxicidade seletiva $\Rightarrow \downarrow$ efeitos colaterais
- Amplo espectro de ação
- Não permita a seleção de amostras resistentes
- Não ser alérgeno (Efeito antigênico)
- Solúvel em água
- Boa estabilidade
- Boa farmacocinética (Absorção, distribuição, metabolismo e excreção)
- Baixo custo

Antifúngicos e Mecanismos de ação

Antifúngicos que agem na membrana celular

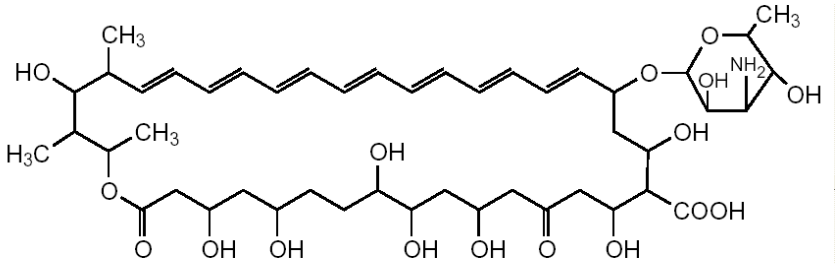


Membrana celular

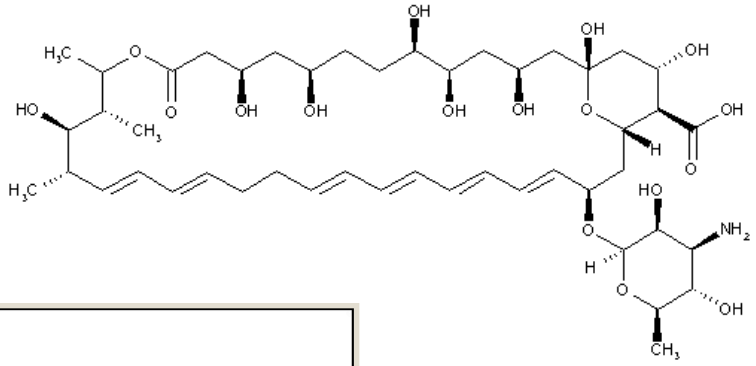
- Poliênicos
  - Anfotericina B e formulações lipídicas
  - Nistatina
- Azóis
  - Cetoconazol, Miconazol,
  - Fluconazol, Itraconazol
  - Voriconazol, Posaconazol, Isavuconazol
  - Albaconazol, Ravuconazol,
- Alilamina (Terbinafina)
- Derivados morfolínicos (Amorolfina)

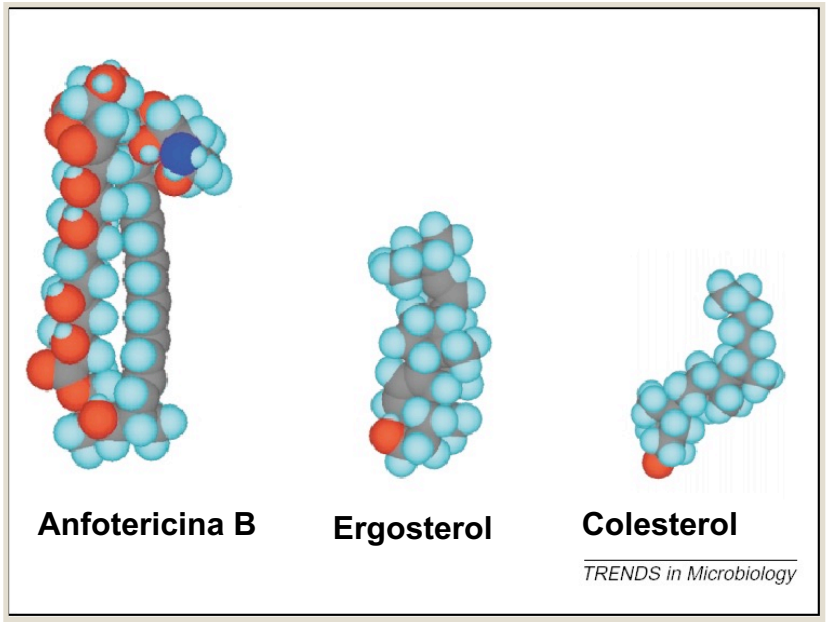
Ação sobre o ergosterol- agentes poliênicos

Anfotericina B

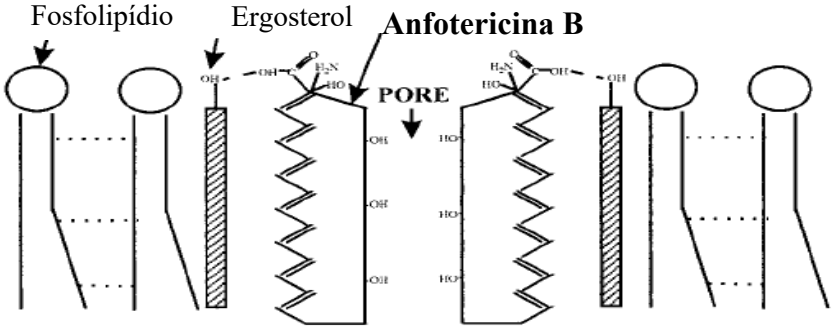


Nistatina





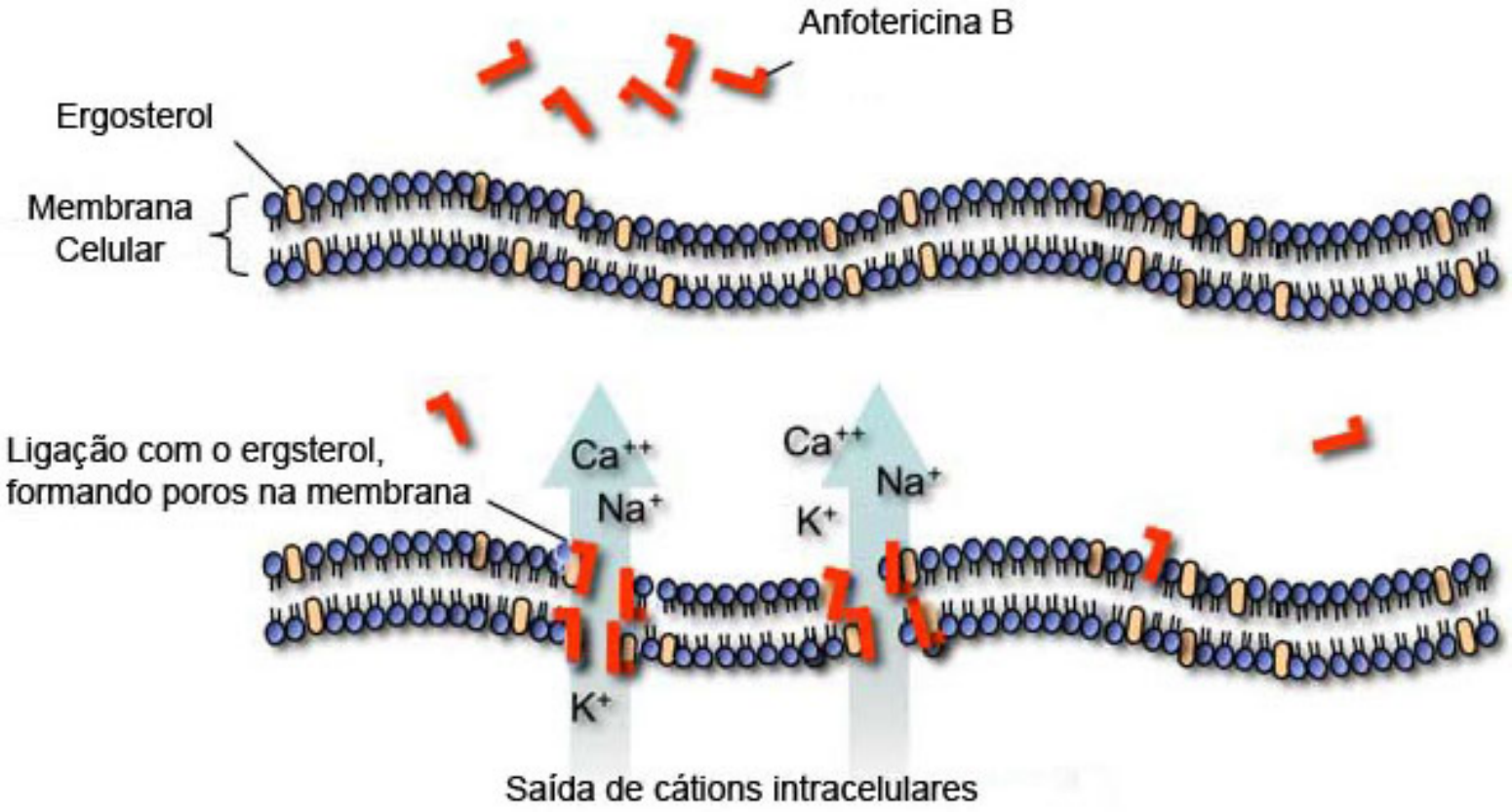
Odds *et al.* TRENDS in Microbiology, 11: 272-279, 2003.



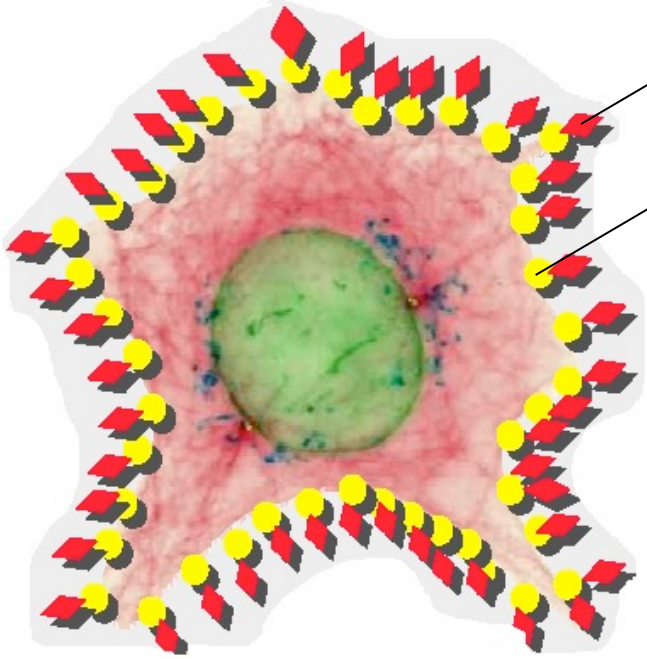
Meio aquoso

Bicamada lipídica

Adaptado de White,1998.



Interações com células de mamíferos



Anfotericina B/Nistatina

Colesterol

Nefrotoxicidade

Hepatotoxicidade

Cardiotoxicidade

Anemia hemolítica

Nistatina

- 1º poliênico (1949)
- Isolado de *Streptomyces noursei*
- Uso somente tópico – suspensão, pastilhas, pomadas, cremes
- Tratamento de dermatófitos e infecções mucocutâneas por *Candida*
- Má absorção mucocutânea
- Altamente tóxico

Anfotericina B desoxicolato

• Isolado de *Streptomyces nodosus* (1956)

- Desvantagem:
Muito tóxica, < nistatina

- Vantagens
Amplo espectro de ação
Fungicida
Baixo custo

espectro de ação:
- *Candida* spp. (exceto *C. lusitaniae*)
- *Cryptococcus* spp.
- Fungos dimórficos
- *Aspergillus* spp., *Fusarium* spp.
- Fungos negros, Zigomicetos

Não tem ação para:
- *Trichosporon* spp.
- *Fusarium* spp.

•Formulação Tópica e Endovenosa
•Efeitos adversos:
Agudos - febre, calafrio, vômito, náusea, cefaléia
Crônicos - Nefrotoxicidade (50% dos pacientes), anemia, efeito neurotóxico.

Nistatina - Uso tópico e altamente tóxico
Anfotericina B desoxicolato - tópico e IV

Nistatina lipossomal (Nyotran) – estudos até +/-2005

Anfotericina B lipossomal (L-AMB, Ambissome)
Anfotericina B dispersão coloidal (ABCD – Amphocil ou Amphotec)
Anfotericina B Complexo lipídico (ABLC, Abelcet)

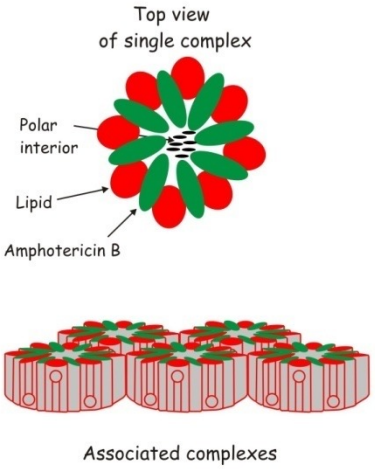
Eficácia semelhante a AMB desoxicolato

↓↓ Efeitos colaterais

Formulações lipídicas da Anfotericina B

Abelcet ® ABLC

Complexo lipídico



Ribbon-like particles

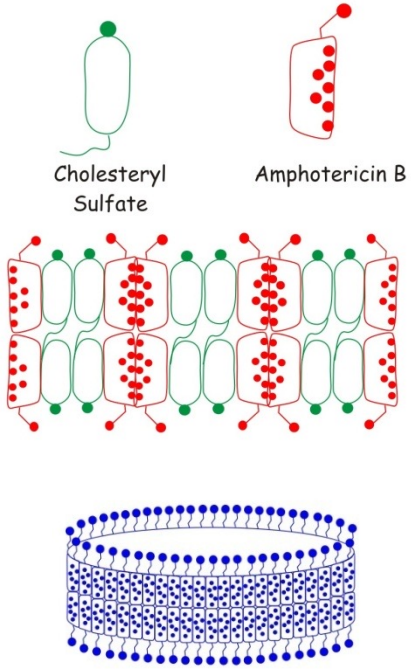
Carrier lipids: DMPC, DMPG

Particle size (µm): 1.6-11

DMPC-Dimyristoyl phospitidylcholine
DMPG- Dimyristoyl phospitidylcglycerol

Amphotec ® ABCD

Dispersão coloidal



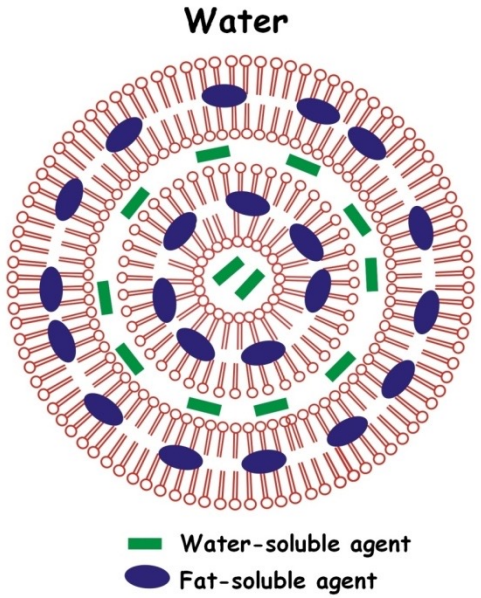
Disk-like particles

Carrier lipids: Cholesteryl sulfate

Particle size (µm): 0.12-0.14

Ambisome ® L-AMB

AMB lipossomal



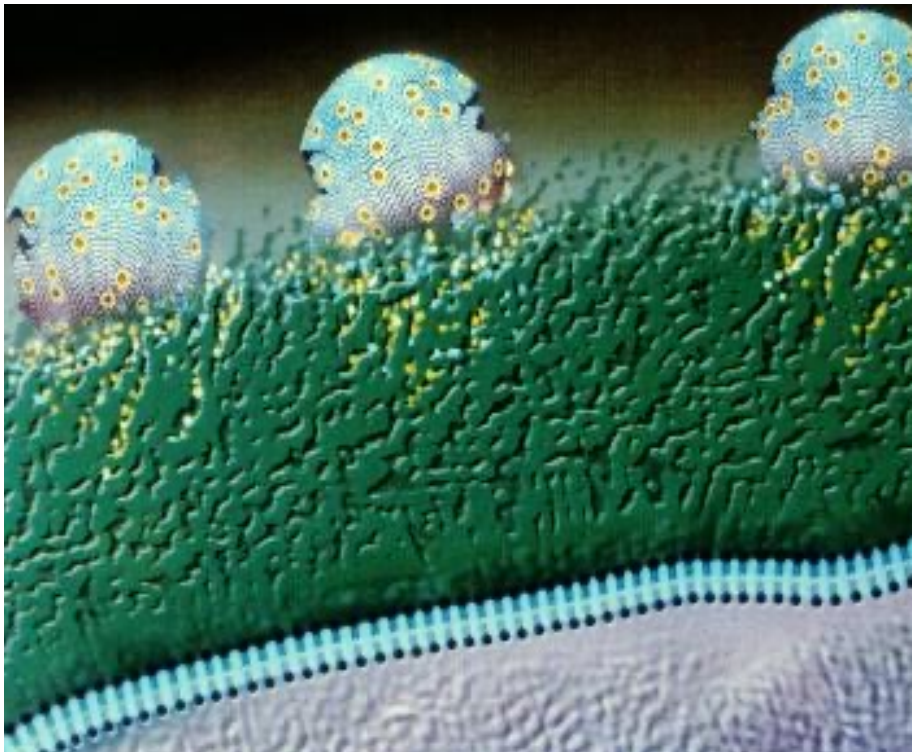
Unilaminar liposome

Carrier lipids: HSPC, DSPG, cholesterol

Particle size (µm) : 0.08

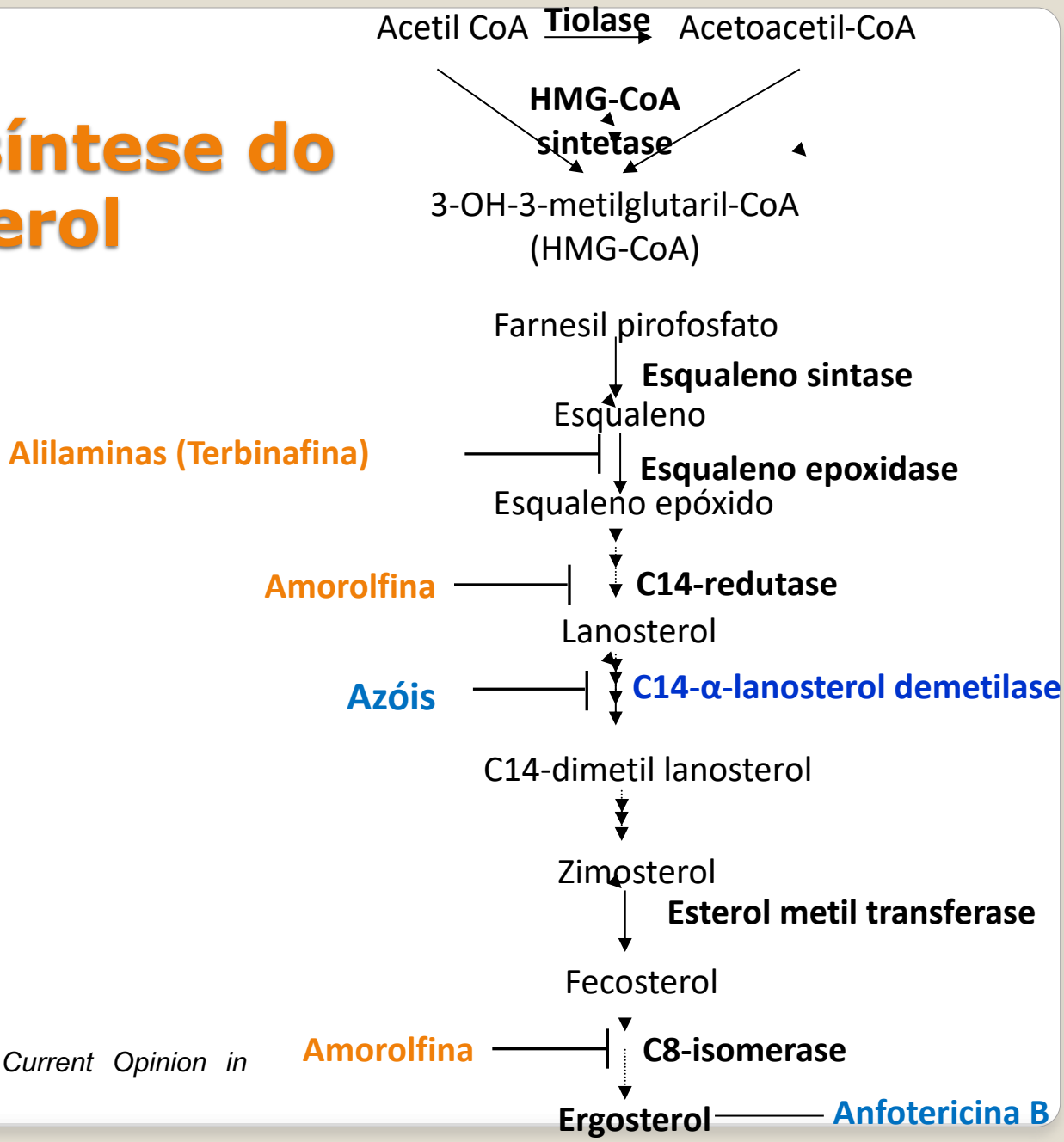
HSPC-Hydrogenated soy phosphatidylcholine
DSPG-Distearoyl phosphitidylcholine

AMB lipossomal



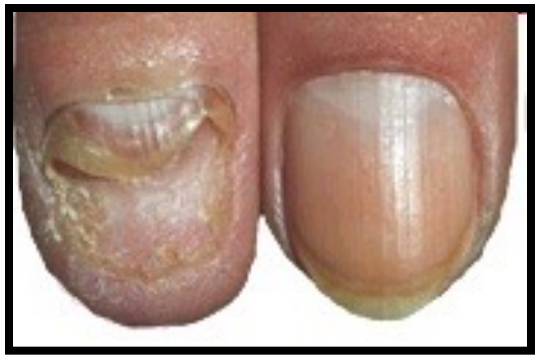
- Maior afinidade pela célula fúngica
- Menor toxicidade, por impedir liberação nas células do hospedeiro
- Permite maior dosagem e tempo de tratamento
- Custo muito elevado (~100x a mais que a AMB desoxicolato)

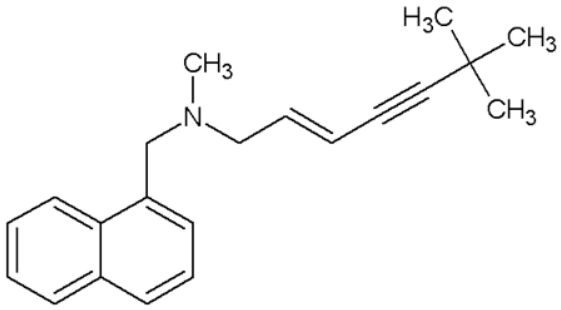
Inibição da síntese do ergosterol



Adaptado de Shea e Del Poeta. *Current Opinion in Microbiology*, 9: 352-358, 2006.

Alilaminas (ex. terbinafina)





- Inibe a enzima **esqualeno epoxidase**

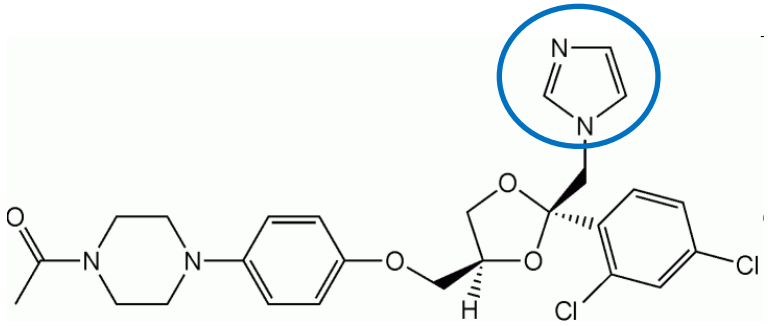
  Efeito antifúngico devido o acúmulo de esqualeno e falta de ergosterol
- Onicomicoses causadas por **dermatófitos**

Efetivo em 90 % dos casos

- Uso tópico (1% em creme) e oral
- Acumula na pele, unha e tecido adiposo
- Reações adversas: diarréia, náuseas, erupções cutâneas, urticária e fotossensibilidade.

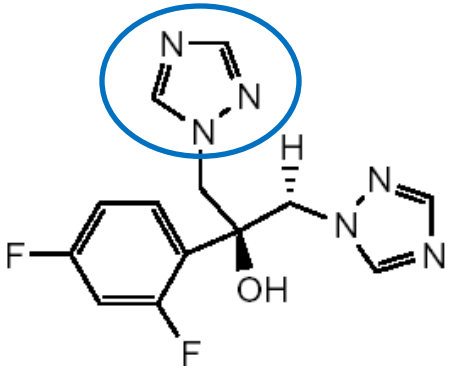
Azóis: imidazóis e trizóis

- Imidazóis
  - 1ª. Geração
  - 2ª. Geração



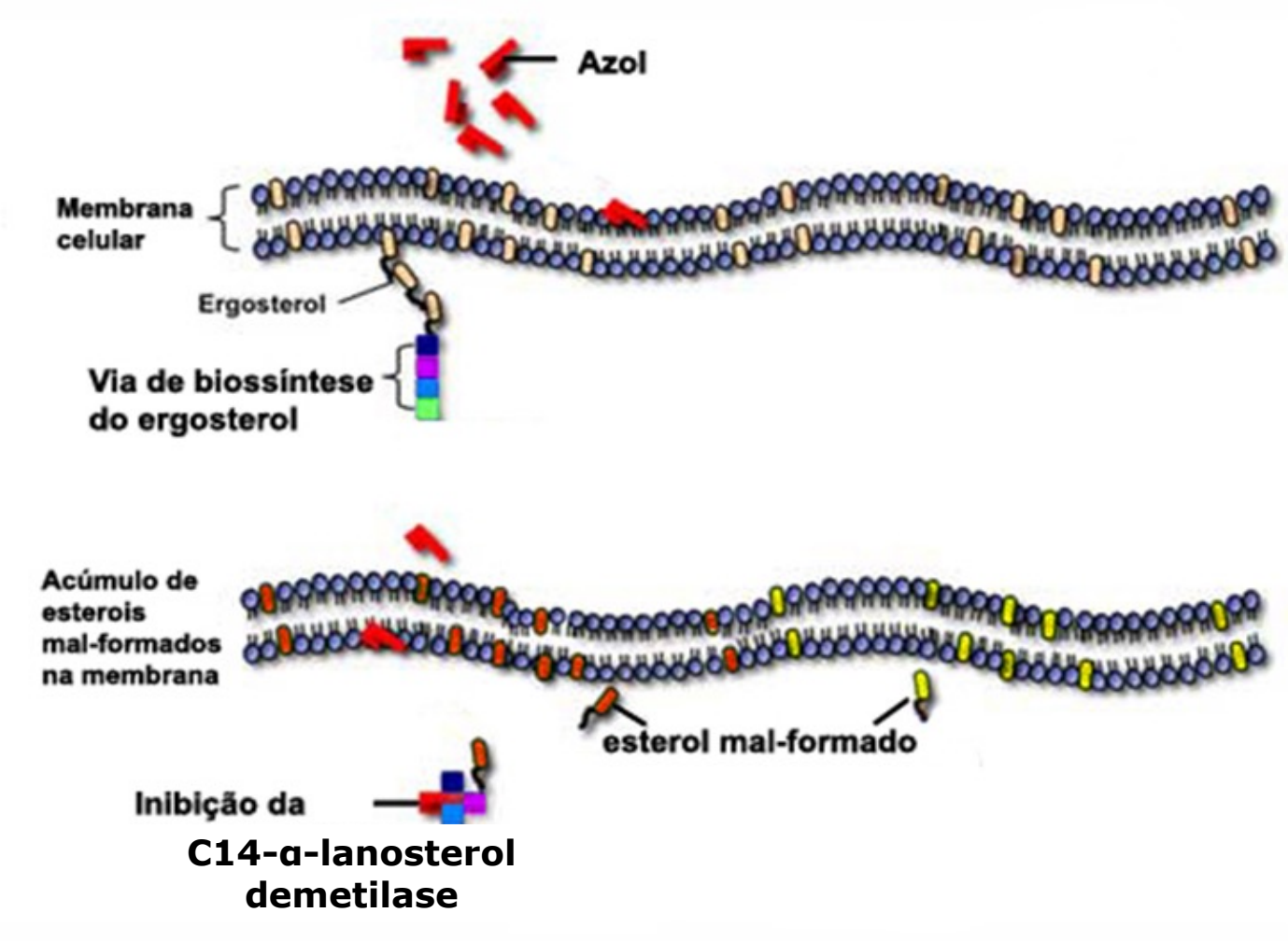
cetoconazol

- Triazóis
  - 1ª. Geração
  - 2ª. Geração
  - 3ª. Geração



Fluconazol

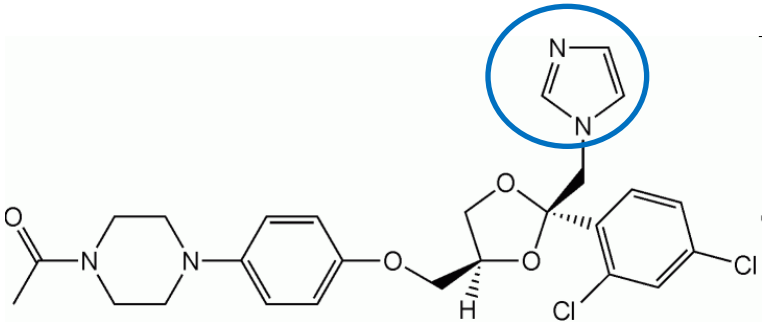
Azóis: imidazóis e trizóis



Agentes azólicos: imidazóis

- Econazol
- Miconazol
- Clotrimazol

} Tópico



cetoconazol

- Cetoconazol (tópico e sistêmico)
  - (Nizoral, Jassen Pharmaceutica)

São Fungistáticos

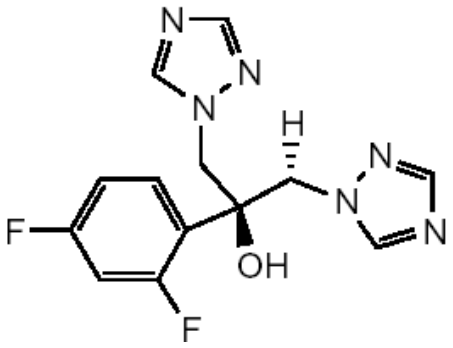
Dermatofitoses, candidíasis cutânea/mucocutânea

Uso tópico (creme, loção, shampoo) e sistêmico (comprimido)

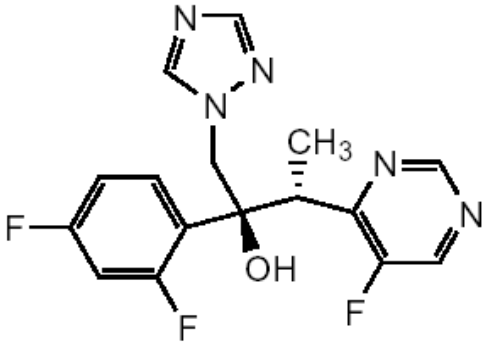
Absorção do cetoconazol – pH ácido

São hepatotóxicos, altera níveis de testosterona e cortisol

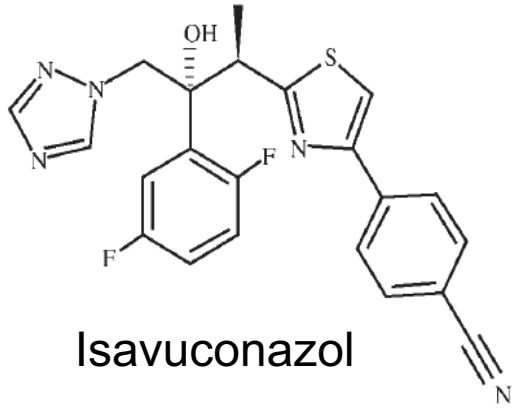
Agentes azólicos: triazóis



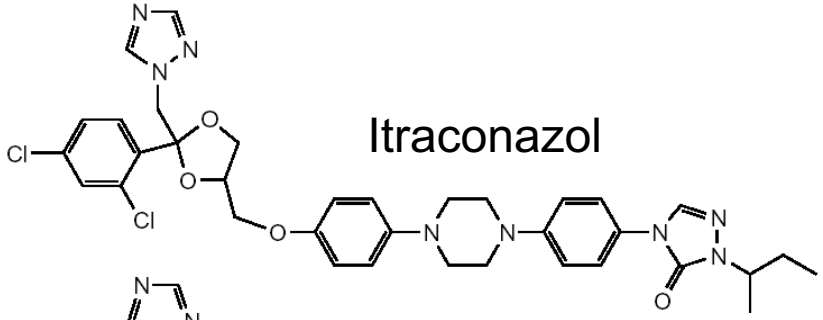
Fluconazol



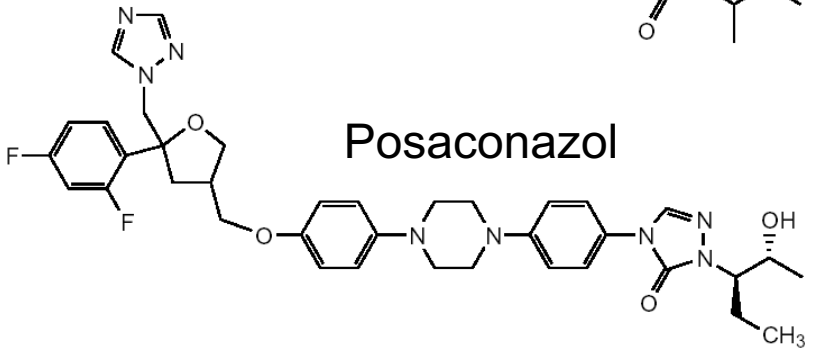
Voriconazol



Isavuconazol



Itraconazol



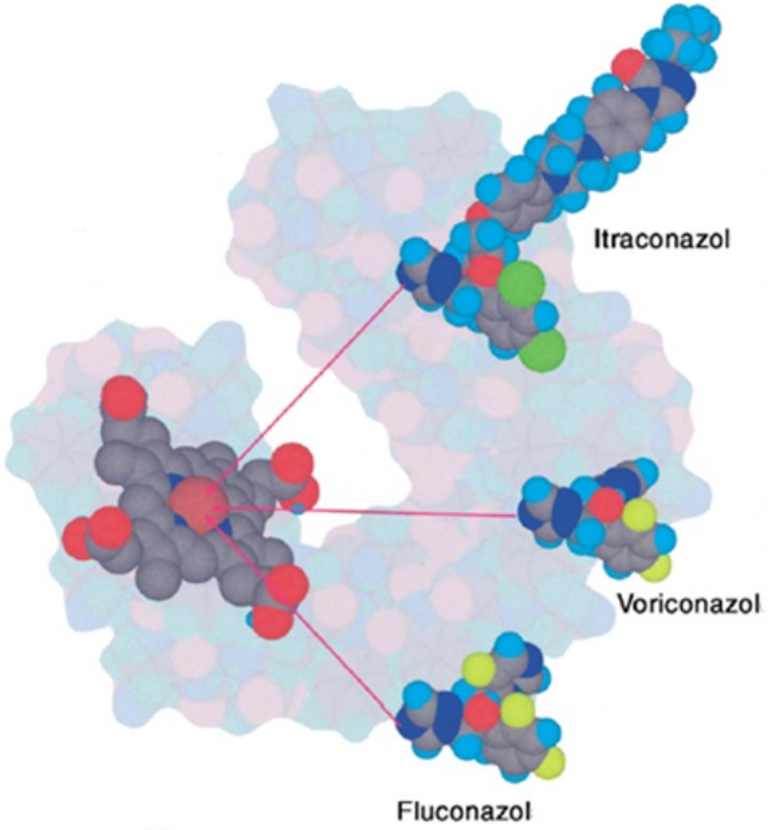
Posaconazol

No tratamento de Infecções sistêmicas.

Alta afinidade pela C14-α demetilase – ↓efeitos colaterais

-São mais específicos para a enzima do fungo
-Redução dos efeitos colaterias

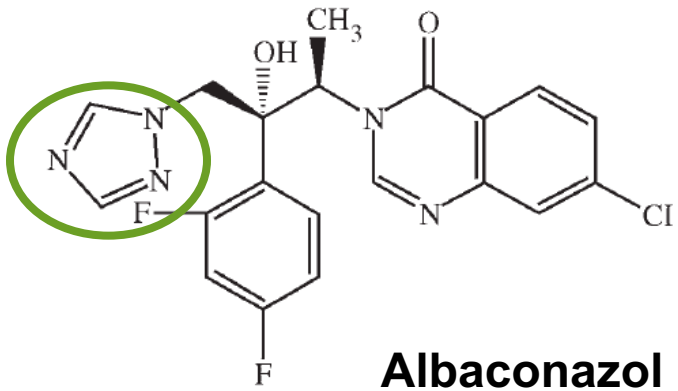
-Espectro de ação ampliado: leveduras, dermatófitos, fungos filamentosos e fungos dimórficos



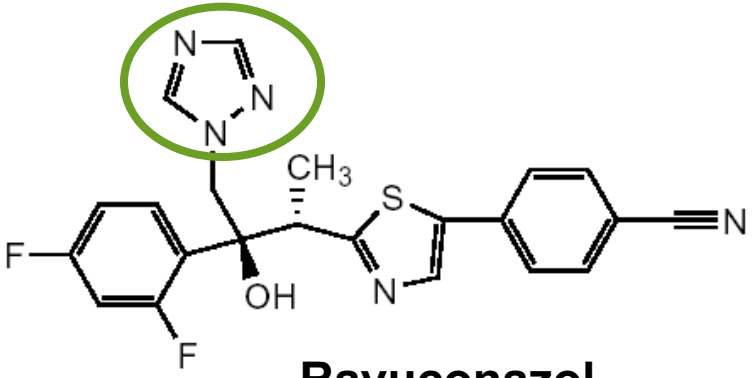
Odds *et al.* TRENDS in Microbiology, 11: 272-279, 2003.

	Fluconazol (Pfizer)	Voriconazol (Pfizer)	Itraconazol (Janssen Pharmaceuticals)	Posaconazol (Schering-Plough)
Indicação	-Candidíase (*C. krusei* e *C. glabrata* baixa susceptibilidade) -Criptococose. -Profilaxia	Amplo espectro, exceto Zigomicetos	- Paracoccidiodomicose (PCM) - Esporotricose Candidíase Criptococose	- Candidíase esofágica, - Infecção fúngica refratária e profilaxia
Efeito antifúngico	Fungistático	-Fungistático: *Candida* spp. e *Cryptococcus* spp. -Fungicida: *Aspergillus* spp.	Fungistático	Fungicida para: - *Candida* spp., - *Cryptococcus* spp., -*Aspergillus* spp.
Características relevantes	-Bem tolerada -Possível hepatotoxicidade	Farmacocinética semelhante ao fluconazol	- pH estomacal – influencia na absorção - Não passa pela barreira hematoencefálica	- pH estomacal – influencia na absorção - Não passa pela barreira hematoencefálica

Novos triazóis



Albaconazol

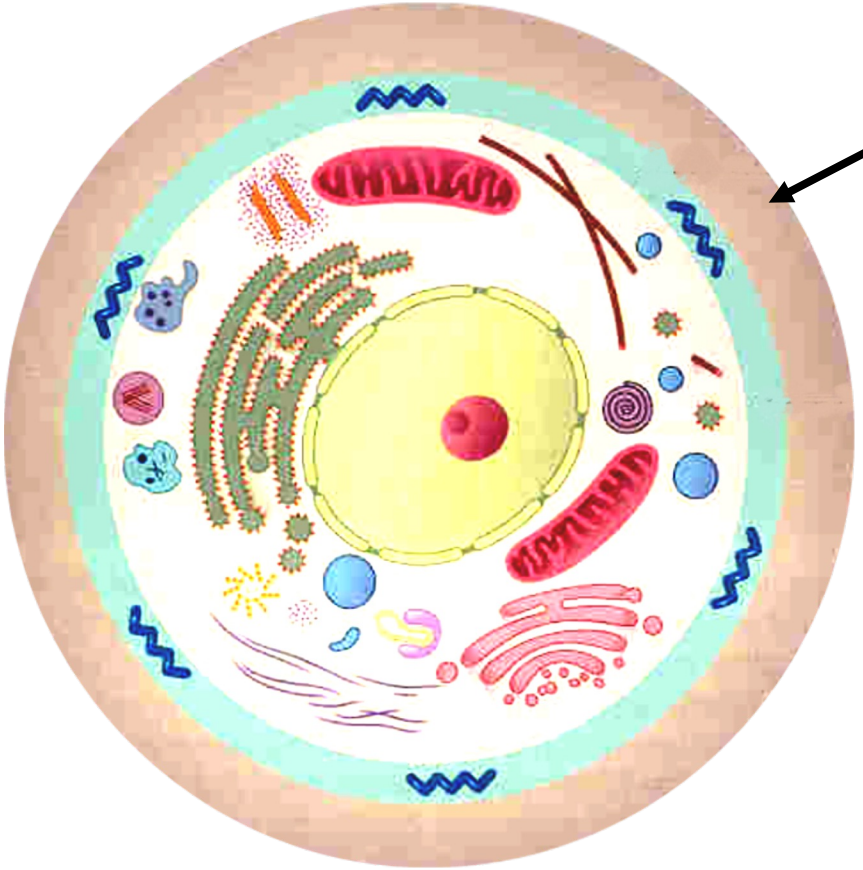


Ravuconazol

Espectro de ação dos triazóis

	Candida	*Cryptococcus*	*Aspergillus*	*Fusarium*	*Zigomicetos*
Flu	++	+++	-	-	-
Itra	++	++	+++	-	-
Vori	+++	+++	+++	++	-
Posa	+++	+++	+++	++	++
Isav	+++	+++	+++	+	+

Antifúngicos que agem na síntese da parede celular

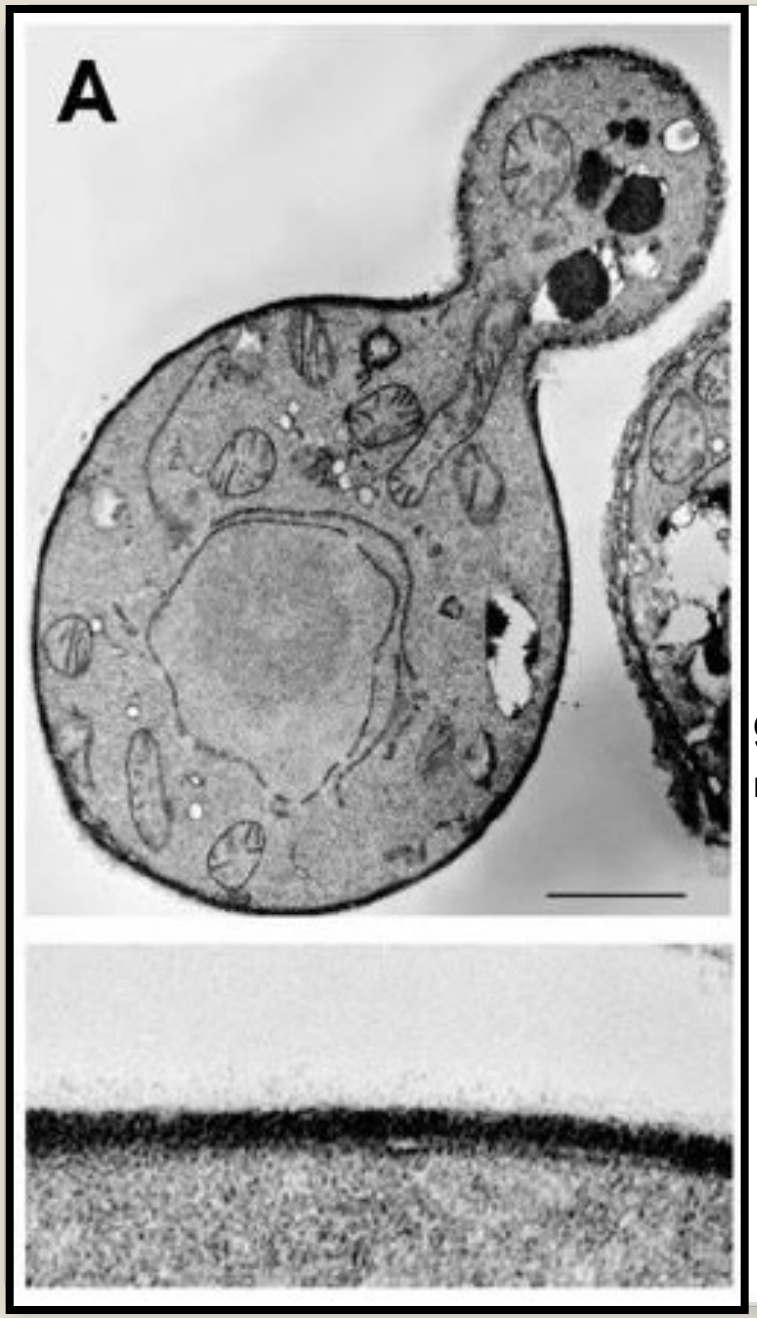


Parede celular

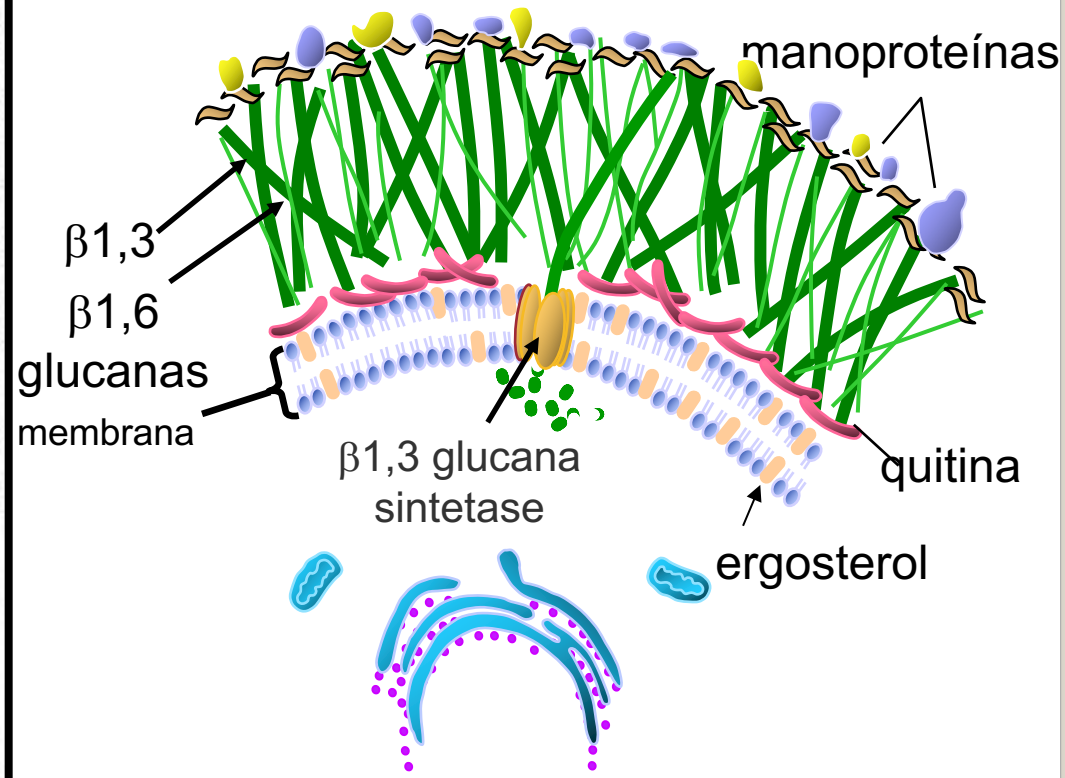
- Equinocandinas

- Caspofungina
- Micafungina
- Anidulafungina
- Aminocandina*
- Rezafungina*

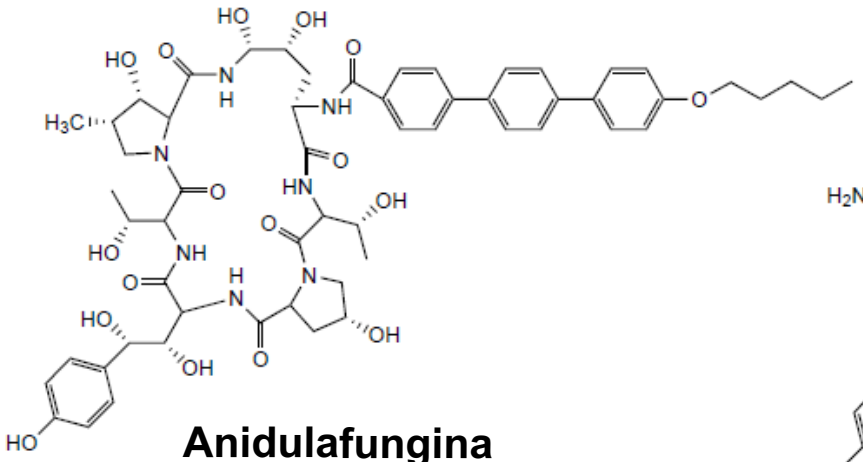
*em fase clínica de estudo



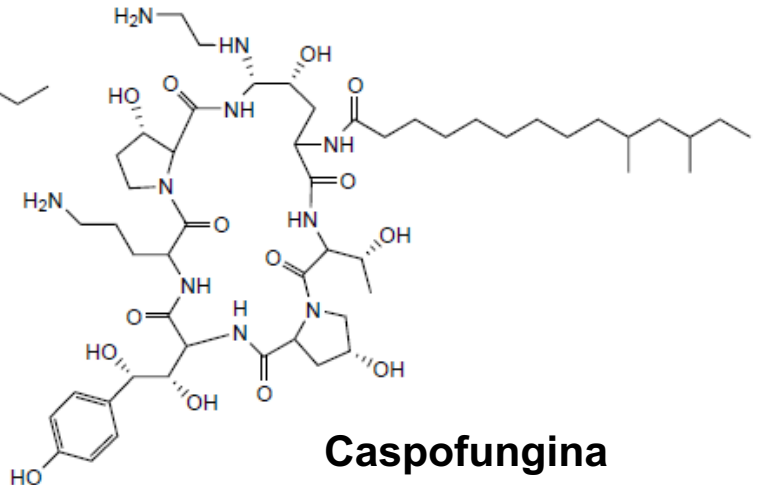
Parede celular fúngica



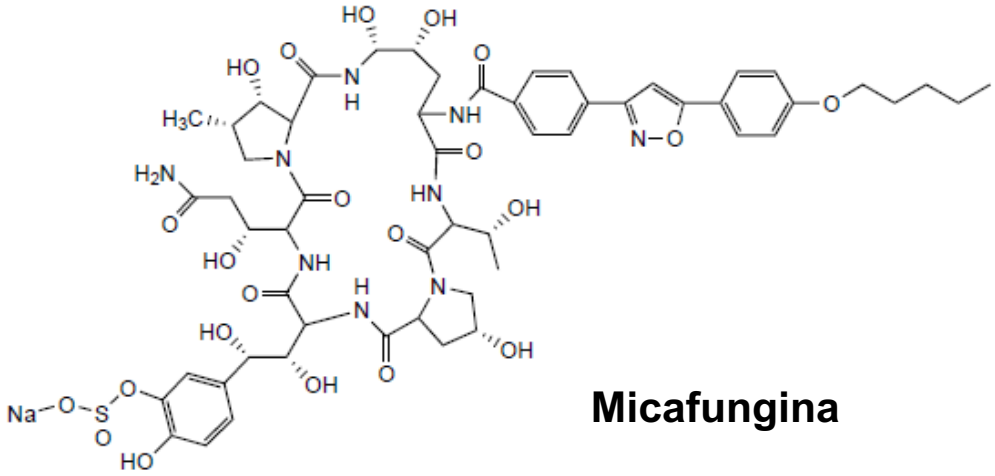
Agentes lipopeptídio: peptídio cíclico + cadeia de ácido graxo



Anidulafungina

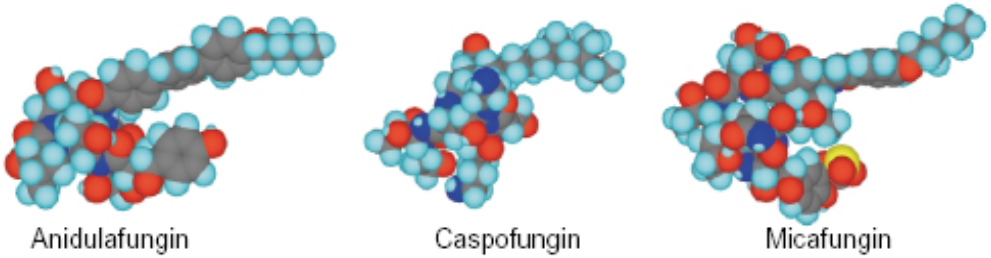


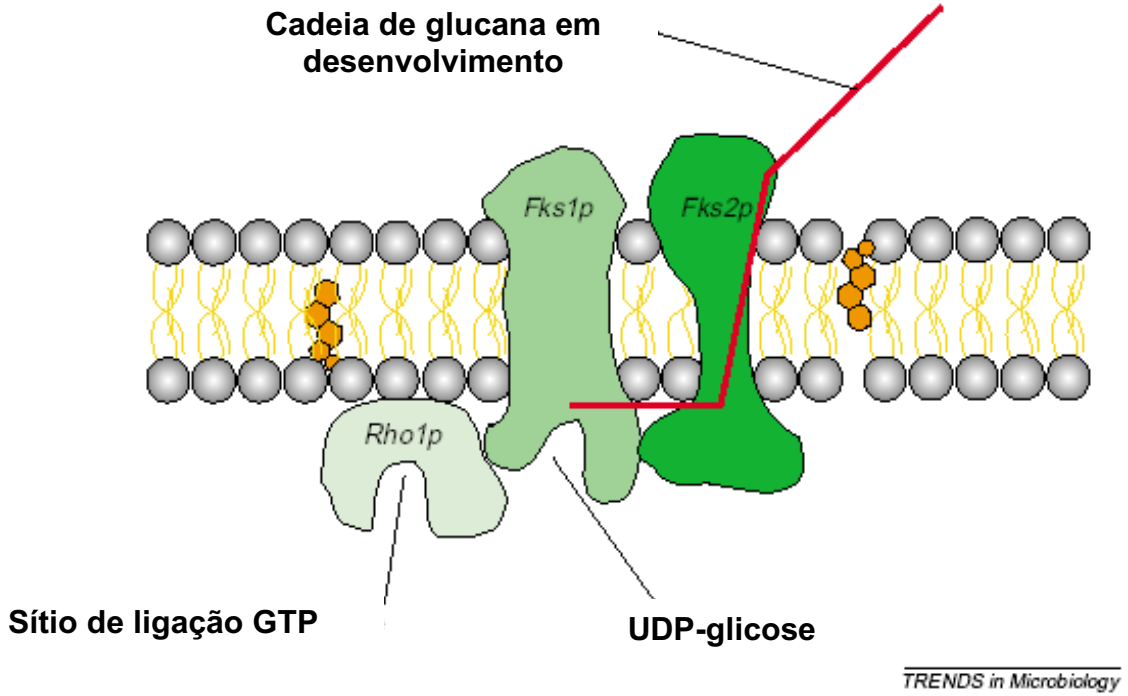
Caspofungina



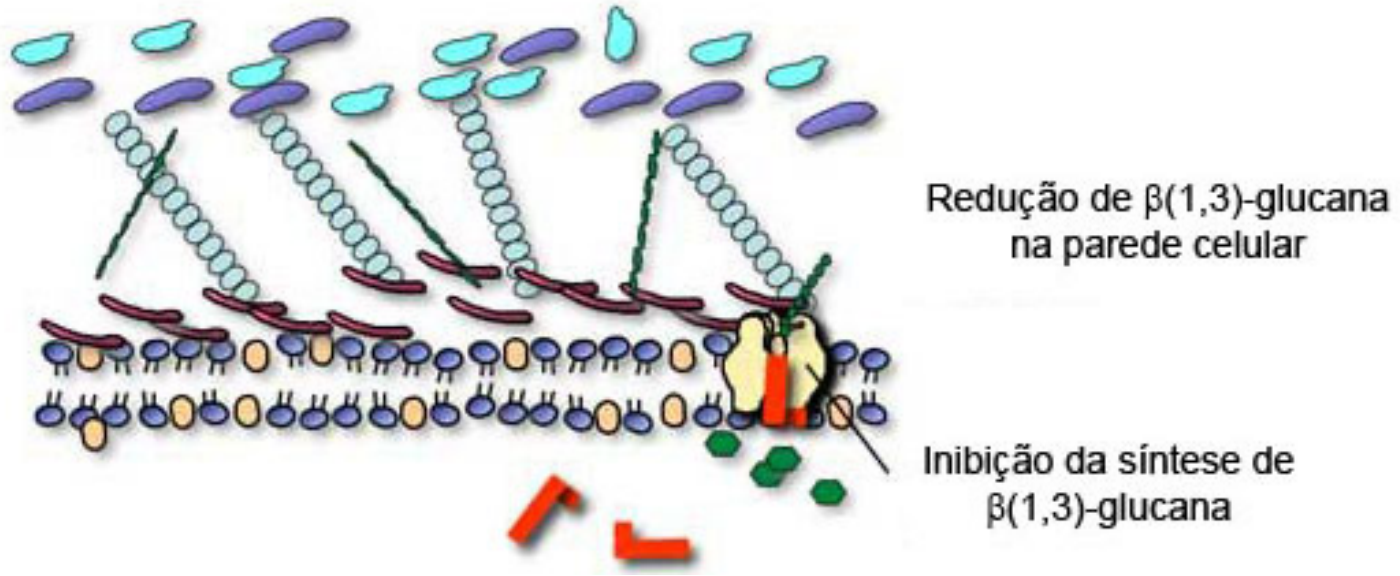
Micafungina

Inibidores não-competitivos





Odds *et al.* TRENDS in Microbiology, 11: 272-279, 2003.



Perda da integridade da parede celular

Fragilidade Osmótica

Efeito fungicida

Ativos contra:

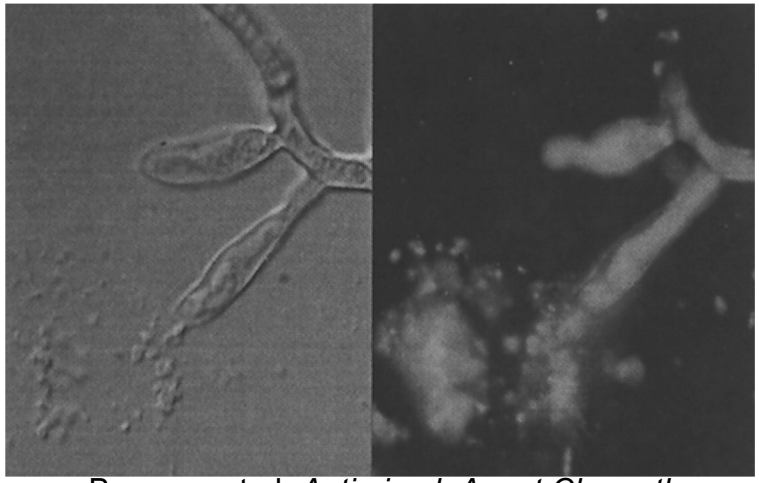
Candida spp. - fungicida

Aspergillus spp. - fungistático

Indicações:

Candidíase invasiva e candidemia, Aspergilose invasiva refratária a outros antifúngicos

Aspergillus



Bowman et al. *Antimicrob Agent Chemother* 2002;46:3001-12

Via de administração IV uma vez por dia.

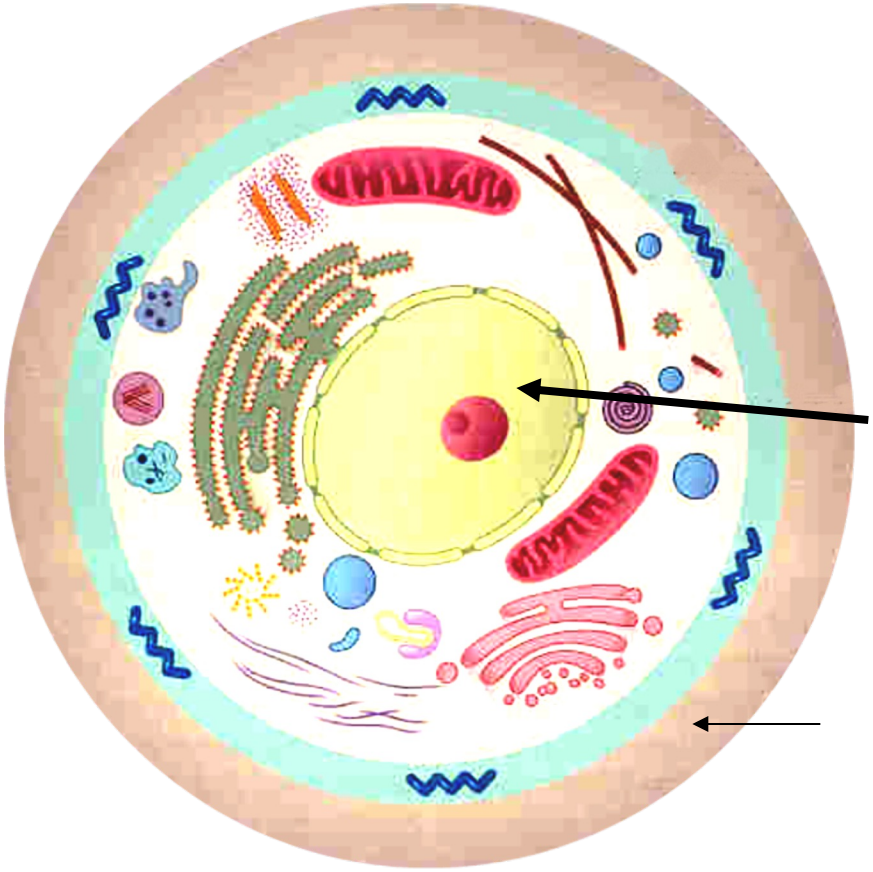
Pouca interação com outras drogas

Efeitos adversos: gastrointestinais

Não apresenta resistência cruzada os agentes azólicos

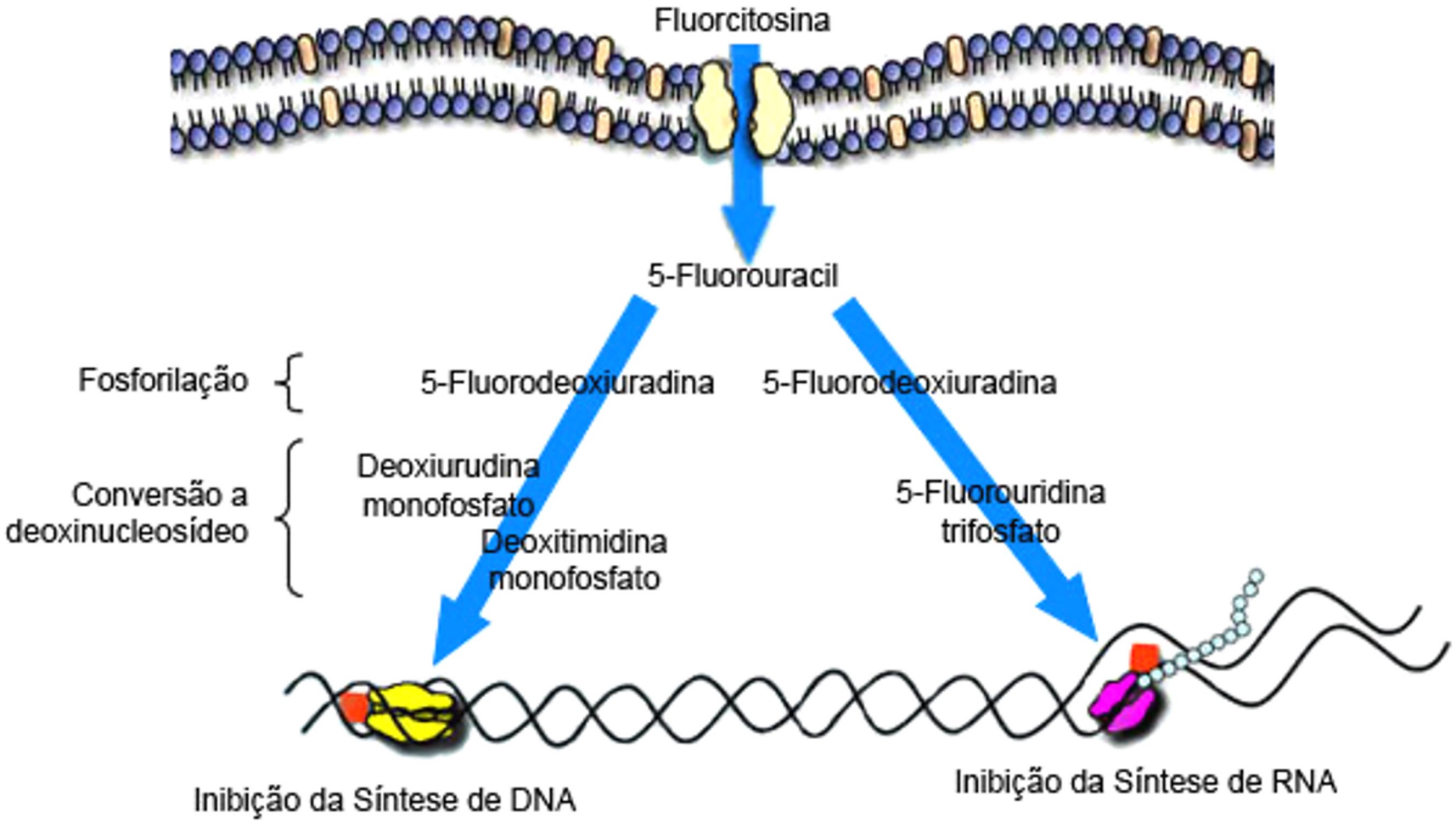
Caspofungina, Anidulafungina e Micafungina - São similares - eficácia e segurança

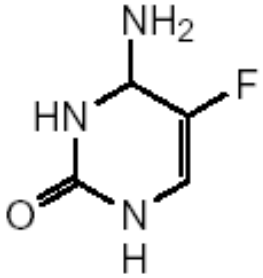
Antifúngico que inibe a síntese de ácidos nucléicos



Síntese DNA/RNA

- **Análogos de Pirimidina**
  - **Flucitosina ou 5-fluorocitosina (5-FC)**


Fluorcitosina
5-Fluorouracil
Fosforilação
5-Fluorodeoxiuradina
5-Fluorodeoxiuradina
Conversão a deoxinucleosídeo
Deoxiurudina monofosfato
Deoxitimidina monofosfato
5-Fluorouridina trifosfato
Inibição da Síntese de DNA
Inibição da Síntese de RNA



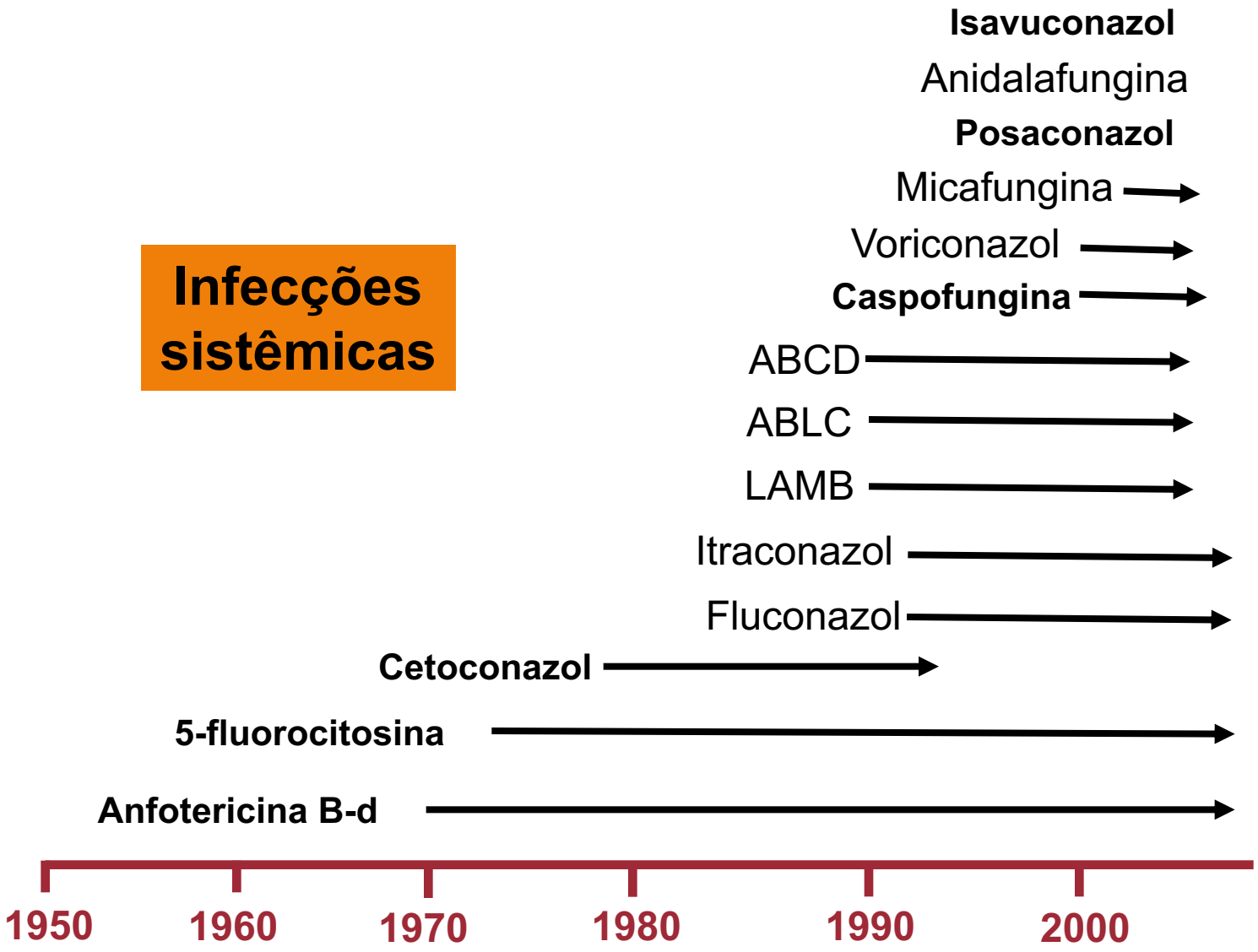
5-fluorocitosina

Sintetizada em 1957 como agente antitumoral

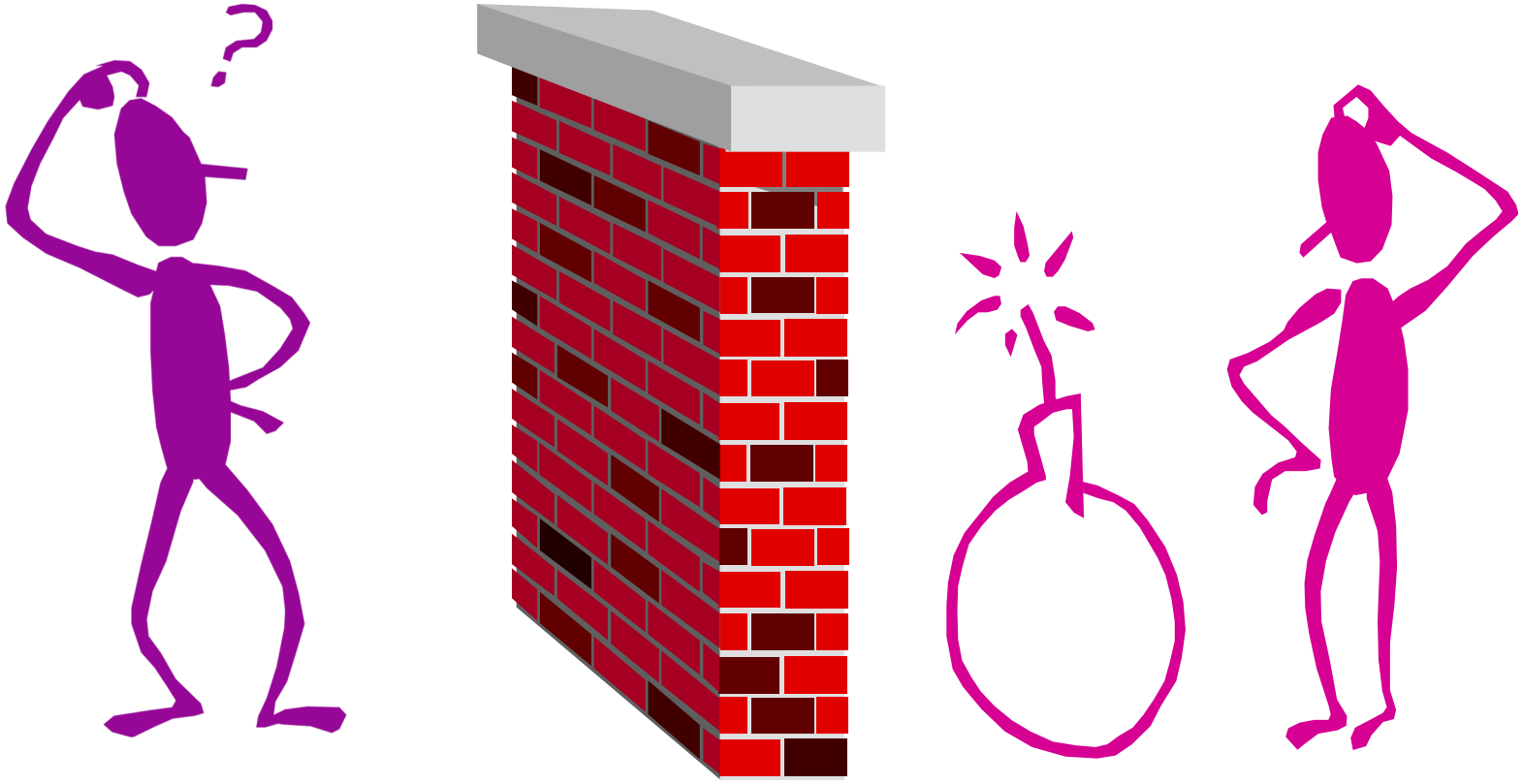
1962 – atividade antifúngica

- Administração oral e intravenosa
- Espectro de ação restrito (*C. neoformans e Candida* sp.)
  Monoterapia Limitada
  Usada em combinação com Anfotericina B e fluconazol
- Resistência desenvolvida rapidamente durante o tratamento
- Resistência: Mutação da citosina permease e citosina deaminase

Evolução dos antifúngicos sistêmicos



Resistência aos agentes antifúngicos



Resistência e sensibilidade aos antifúngicos

- **Resistência:** capacidade do micro-organismo em crescer em concentrações plasmáticas da droga.
- **Sensibilidade:** quando não crescem nessas concentrações.
- **CIM**: menor concentração do antifúngico necessária para inibir o crescimento fúngico (μg/mL)

O que pode levar à resistência ?

“ O antimicrobiano não induz à resistência”

O que ocorre é a seleção natural de microrganismos resistentes ao antimicrobiano”

“A resistência microbiana reflete o princípio evolutivo de que os organismos se adaptam geneticamente a mudanças no seu meio ambiente”

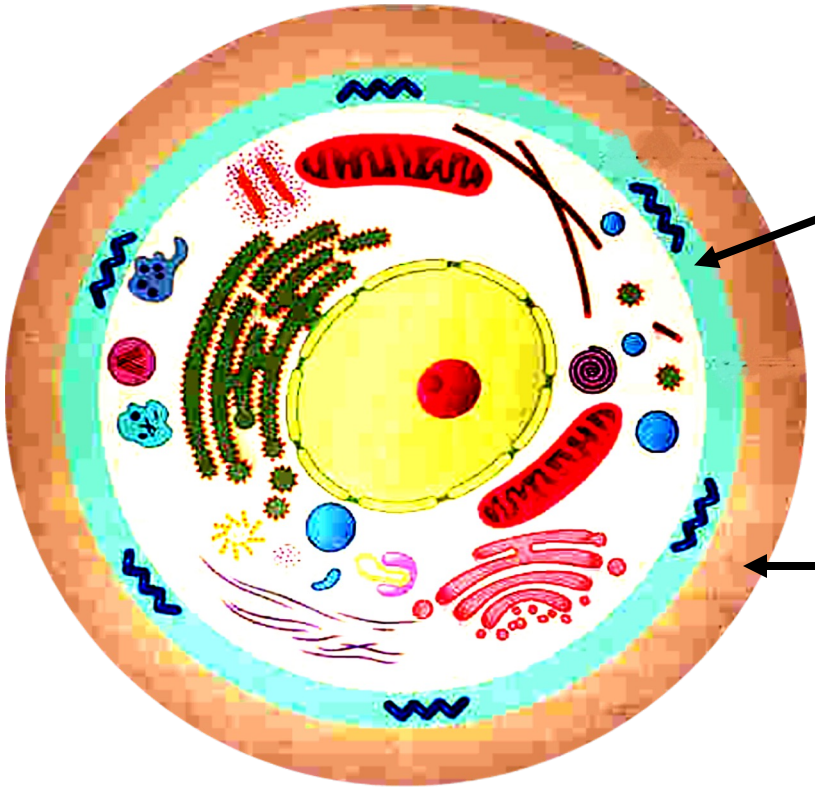
- **Resistência clínica**
  - Local da infecção
  - Diagnóstico errado da doença
  - Tratamento errado
  - Farmacocinética desfavorável
  - Dificuldade do paciente de se aderir ao tratamento
- **Resistência microbiológica** – relacionada com características do fungo
  - Primária = Intrínsica (*C. krusei* ao fluconazol)
  - Secundária = Extrínsica (*C. albicans* ao fluconazol)

Como minimizar o desenvolvimento de resistência?

- Diagnóstico correto e tratamento adequado
- Evitar o uso indiscriminado de antifúngicos
- Utilizar dosagens adequadas e suficientes
- Utilizar associação de drogas
- Mudar tão logo de antifúngico quando se observa que o fungo possui sinais de resistência
- Fazer teste de sensibilidade aos antifúngicos, quando necessário

Mecanismos de resistência aos antifúngicos

Antifúngicos



Membrana plasmática
Poliênicos
Azóis

Parede celular
Equinocandinas

Mecanismos de resistência aos agentes poliênicos

- Diminuição da quantidade de ergosterol
- Acúmulo de outro esterol, diferente de ergosterol, com baixa afinidade pelos poliênicos.

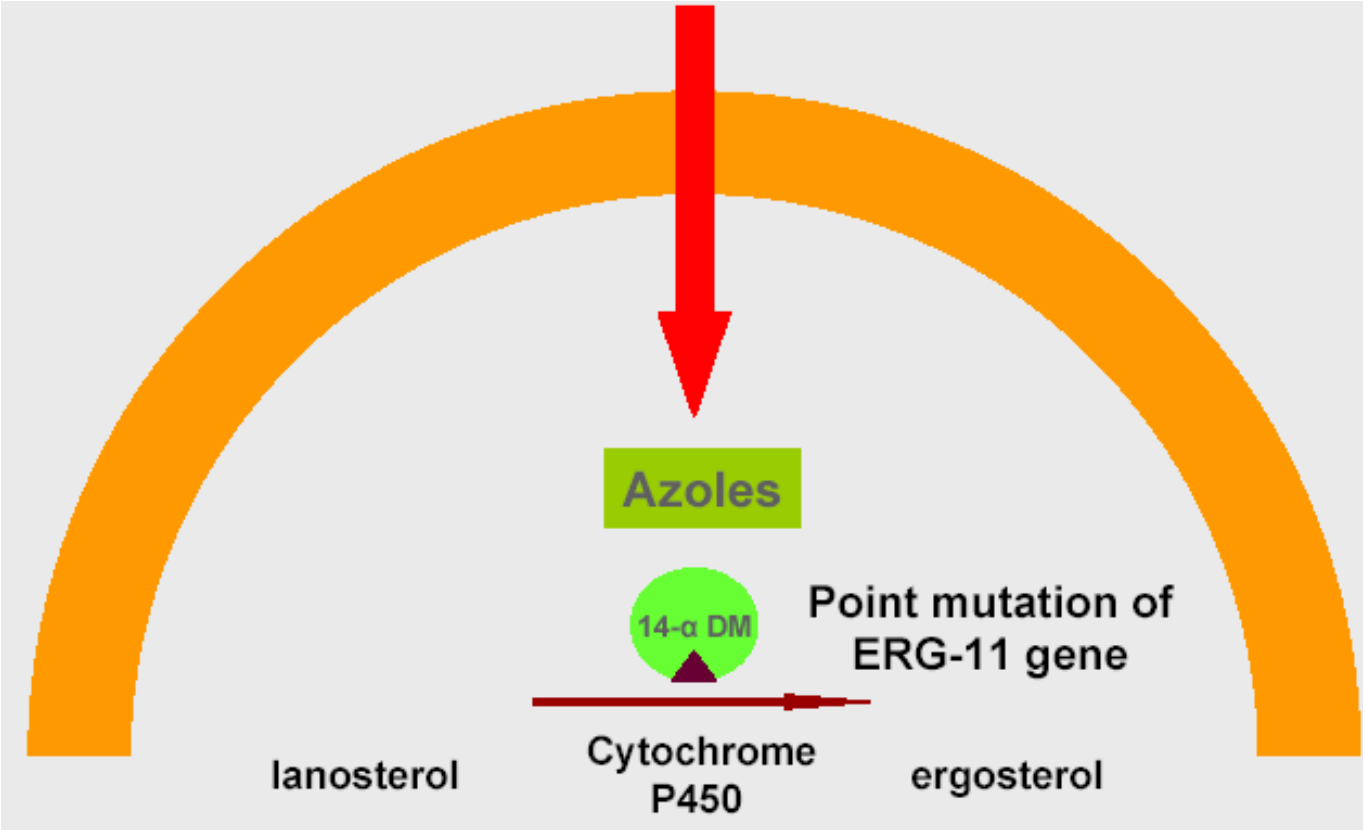
Resistência intrínsica: *C. lusitaniae*
Resistência secundária: *C. albicans*, *C. glabrata* e *C. krusei*

Resistência é rara.

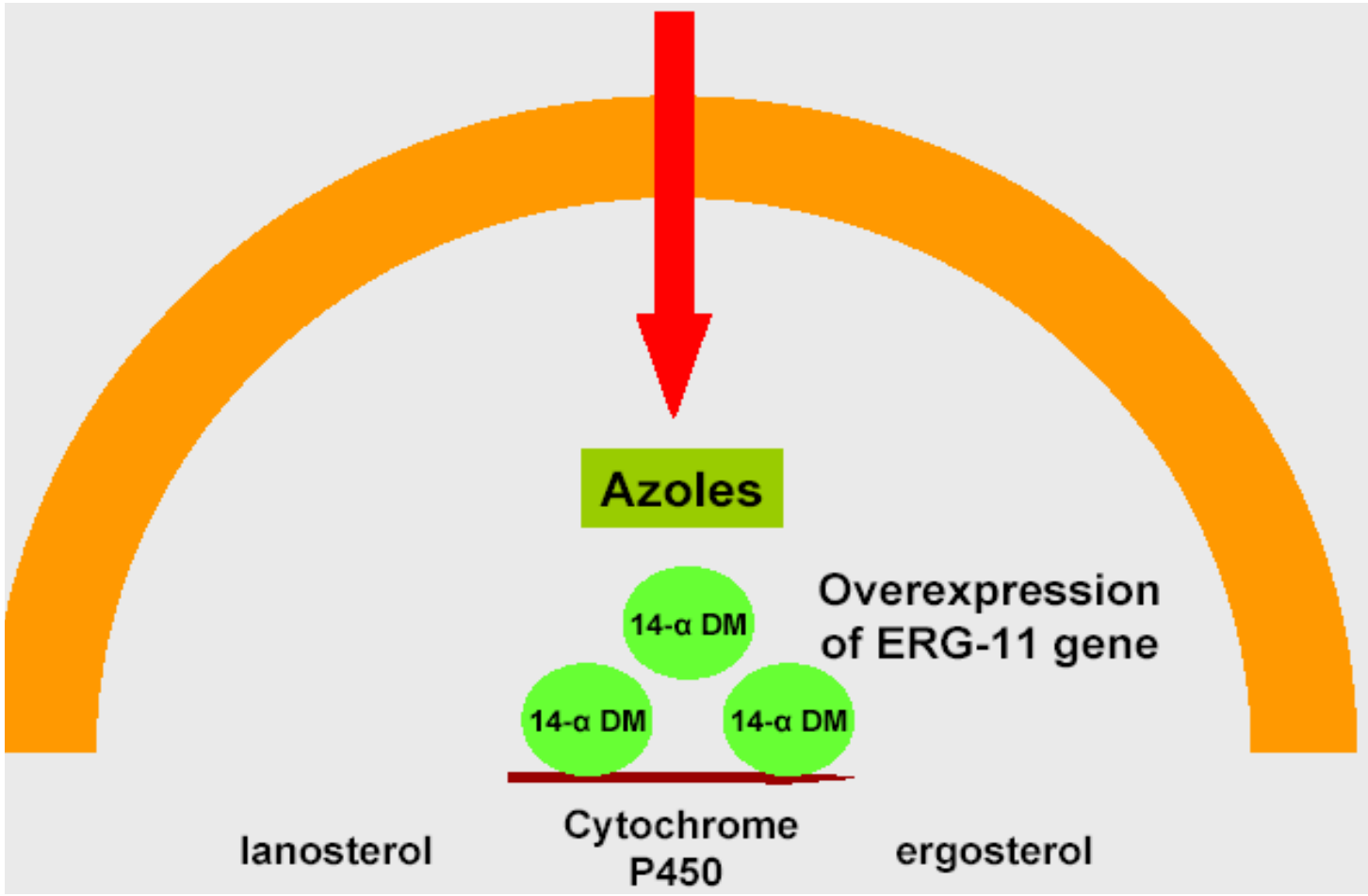
Resistência *Candida* spp. CIM>1µg/mL

Mecanismos de resistência aos azóis

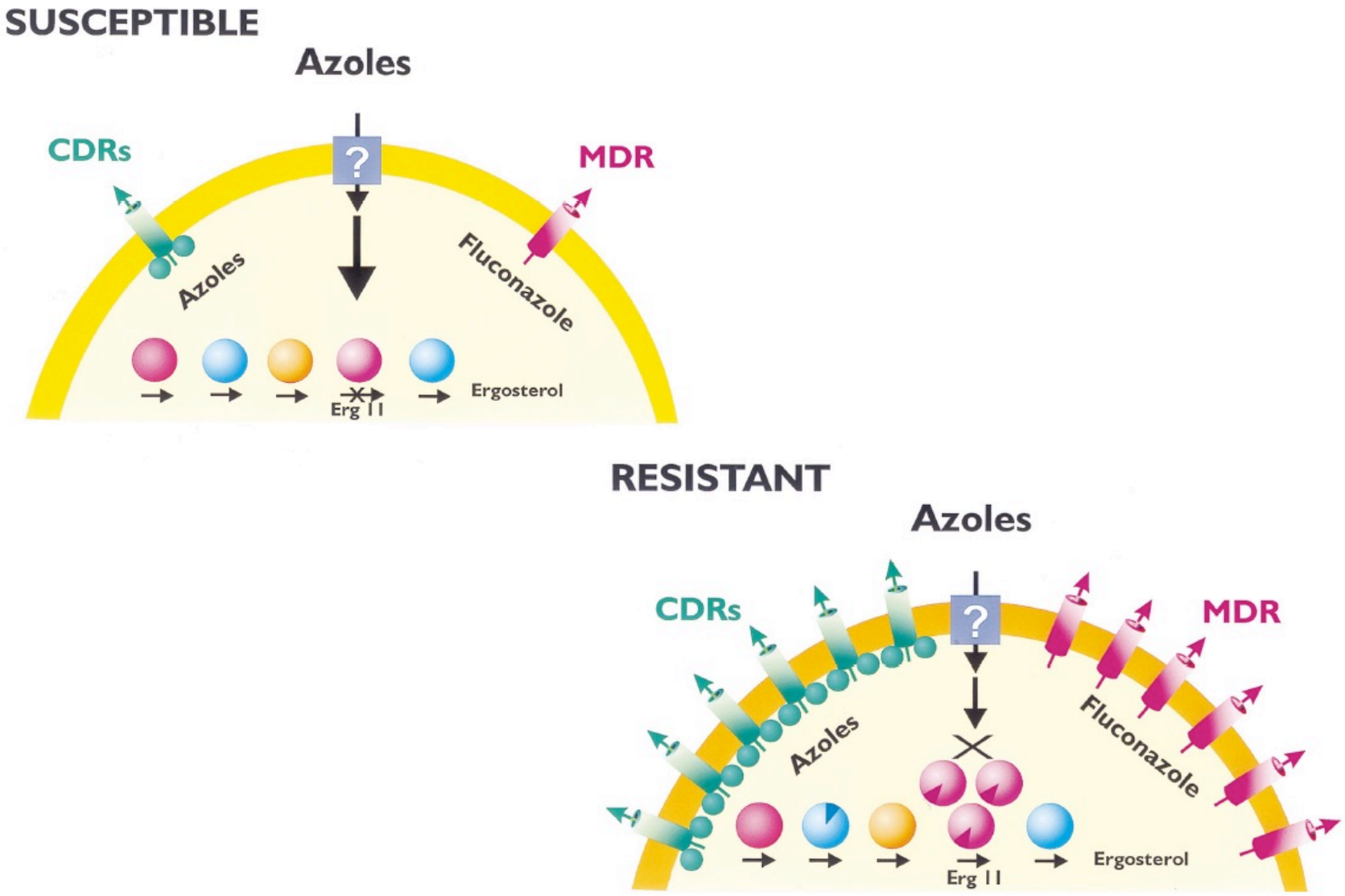
Mutação do gene *ERG 11* (C14-α- lanosterol demetilase)



Aumento da expressão da C14-α- lanosterol demetilase



Aumento de bombas de efluxo



Resistência às equinocandinas

- *C. parapsilosis* – menor suscetibilidade
- Aumento dos casos de resistência - *C. glabrata*
- Mecanismos:
  - Mutação no gene FKS1p (β 1,3 glucana sintetase)
  - Bomba de efluxo

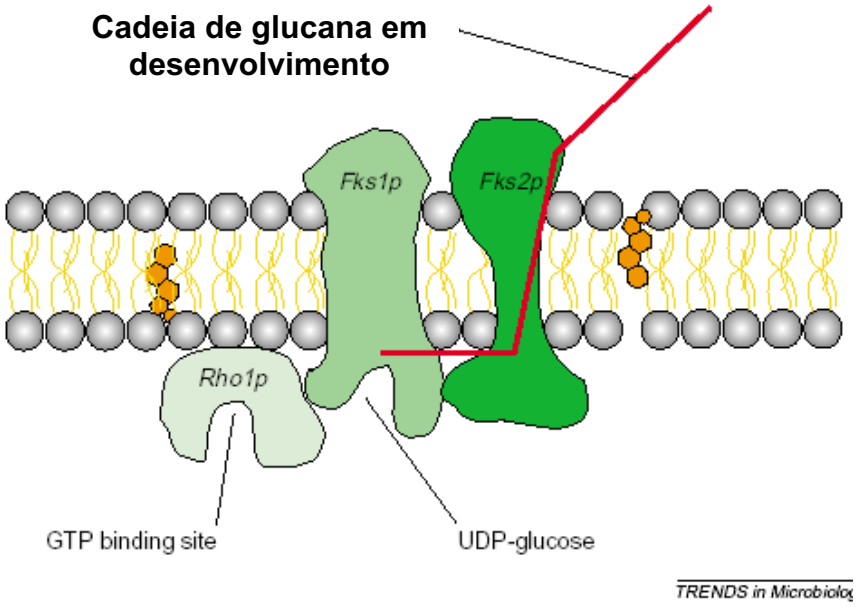


TABLE 2 Frequency of antifungal resistance among clinical isolates of *Candida* spp. by geographic region in the 2010-2011 SENTRY Surveillance Program

Candida species	Antifungal agent	No. of isolates (% resistant[a] to each antifungal agent) by region				
		North America	Europe	Latin America	Asia-Pacific	Total
C. albicans	Anidulafungin	503 (0.0)	552 (0.0)	186 (0.0)	164 (0.0)	1,405 (0.0)
	Caspofungin	503 (0.0)	552 (0.4)	186 (0.0)	164 (0.6)	1,405 (0.2)
	Micafungin	503 (0.0)	552 (0.4)	186 (0.0)	164 (0.0)	1,405 (0.1)
	Fluconazole	503 (0.6)	552 (0.2)	186 (0.0)	164 (0.6)	1,405 (0.4)
	Posaconazole[c]	503 (0.6)	552 (8.3)	186 (6.5)	164 (0.6)	1,405 (4.4)
	Voriconazole	503 (0.6)	552 (0.2)	186 (0.0)	164 (0.6)	1,405 (0.4)
C. glabrata	Anidulafungin	306 (1.6)	175 (1.7)	38 (0.0)	52 (3.8)	571 (1.8)
	Caspofungin	306 (1.6)	175 (1.7)	38 (0.0)	52 (1.9)	571 (1.6)
	Micafungin	306 (1.3)	175 (1.1)	38 (0.0)	52 (1.9)	571 (1.2)
	Fluconazole	306 (9.8)	175 (6.3)	38 (5.3)	52 (13.5)	571 (8.8)
	Posaconazole[c]	306 (3.3)	175 (2.9)	38 (5.3)	52 (5.8)	571 (3.5)
	Voriconazole[b]	306 (11.1)	175 (9.1)	38 (7.9)	52 (13.5)	571 (10.5)
C. parapsilosis	Anidulafungin	173 (1.2)	195 (0.0)	104 (1.0)	93 (0.0)	565 (0.5)
	Caspofungin	173 (0.0)	195 (0.0)	104 (0.0)	93 (0.0)	565 (0.0)
	Micafungin	173 (0.0)	195 (0.0)	104 (0.0)	93 (0.0)	565 (0.0)
	Fluconazole	173 (0.0)	195 (3.1)	104 (1.0)	93 (5.4)	565 (2.1)
	Posaconazole[c]	173 (1.2)	195 (0.5)	104 (3.9)	93 (6.5)	565 (2.3)
	Voriconazole	173 (0.0)	195 (0.0)	104 (0.0)	93 (1.1)	565 (0.2)
C. tropicalis	Anidulafungin	109 (0.0)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.0)
	Caspofungin	109 (0.0)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.0)
	Micafungin	109 (0.0)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.0)
	Fluconazole	109 (2.7)	89 (1.1)	76 (0.0)	44 (0.0)	318 (1.3)
	Posaconazole[c]	109 (7.3)	89 (7.9)	76 (2.6)	44 (0.0)	318 (5.3)
	Voriconazole	109 (0.9)	89 (0.0)	76 (0.0)	44 (0.0)	318 (0.3)
C. krusei	Anidulafungin	40 (0.0)	28 (0.0)	8 (0.0)	3 (0.0)	79 (0.0)
	Caspofungin	40 (0.0)	28 (0.0)	8 (0.0)	3 (0.0)	79 (0.0)
	Micafungin	40 (0.0)	28 (0.0)	8 (0.0)	3 (0.0)	79 (0.0)
	Posaconazole[c]	40 (17.5)	28 (7.1)	8 (25.0)	3 (33.3)	79 (15.2)
	Voriconazole	40 (2.5)	28 (0.0)	8 (0.0)	3 (0.0)	79 (1.3)

[a] Resistance is defined as an MIC of >0.5 μg/ml for anidulafungin, caspofungin, and micafungin against *C. albicans*, *C. tropicalis*, and *C. krusei*, an MIC of >4 μg/ml against *C. parapsilosis*, an MIC of >0.25 μg/ml for anidulafungin and caspofungin, and an MIC of >0.12 μg/ml for micafungin against *C. glabrata*; an MIC of >4 μg/ml for fluconazole against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, an MIC of >32 μg/ml against *C. glabrata*; and an MIC of >0.5 μg/ml for voriconazole against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.

[b] In lieu of clinical breakpoints for voriconazole against *C. glabrata*, the epidemiological cutoff value (ECV) of >0.5 μg/ml was used to identify non-wild-type (non-WT) isolates.

[c] Posaconazole ECVs were used to identify non-WT isolates of *C. albicans* (ECV > 0.06 μg/ml), *C. glabrata* (ECV > 2 μg/ml), *C. parapsilosis* (ECV > 0.25 μg/ml), *C. tropicalis* (ECV > 0.12 μg/ml), and *C. krusei* (ECV > 0.5 μg/ml).

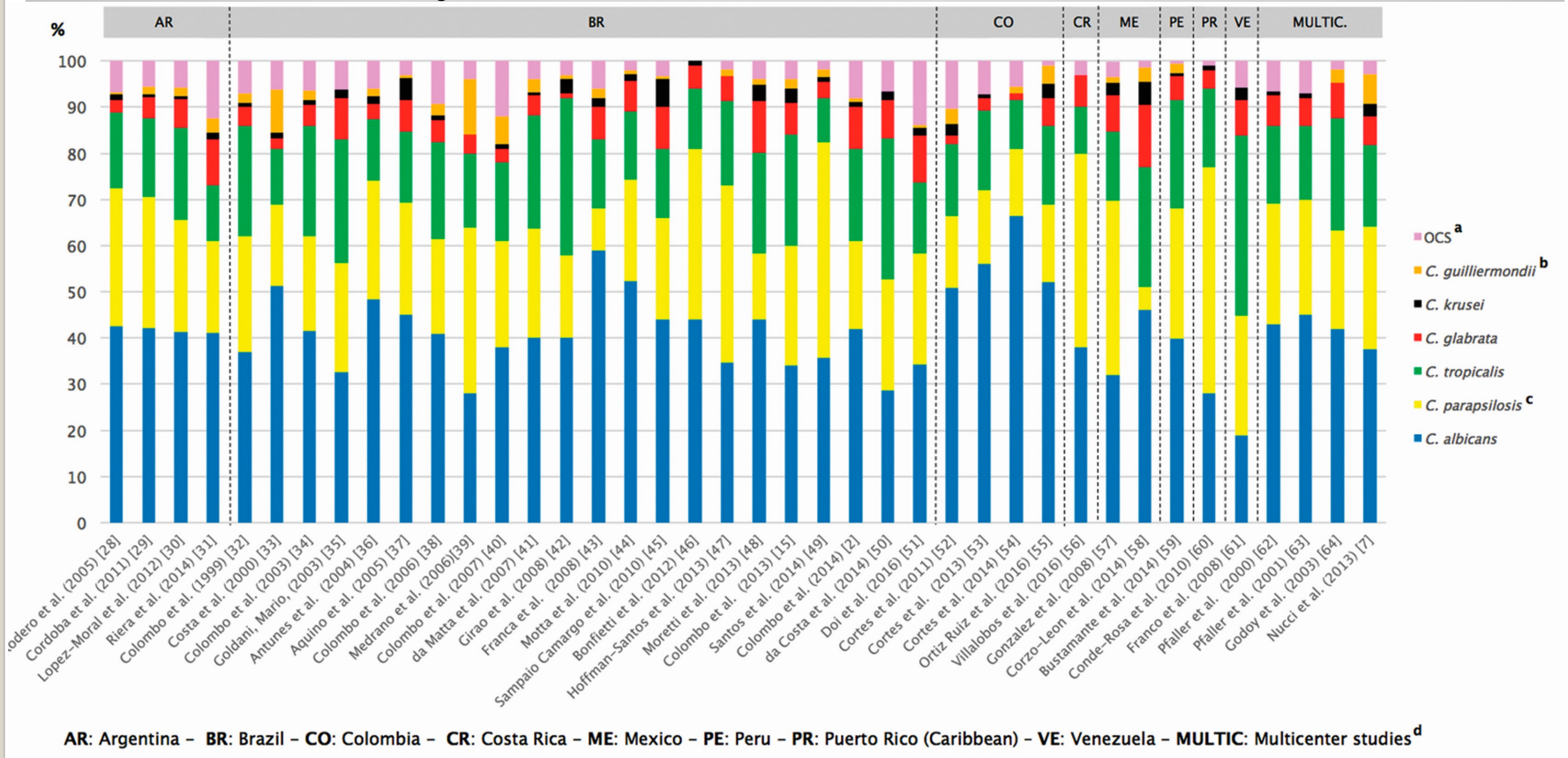
Pfaller. Journal of Clinical Microbiology 51(8): 2571–2581, 2013.

Brasil

Ca: 0.4% a 1.2%
Cp: 0.5% a 2.3%
Ct: 0 a 2.6%

FCZ-R

Da Matta et al., 2017. J. Fungi 2017, 3, 24



AR: Argentina – **BR**: Brazil – **CO**: Colombia – **CR**: Costa Rica – **ME**: Mexico – **PE**: Peru – **PR**: Puerto Rico (Caribbean) – **VE**: Venezuela – **MULTIC**: Multicenter studies [d]

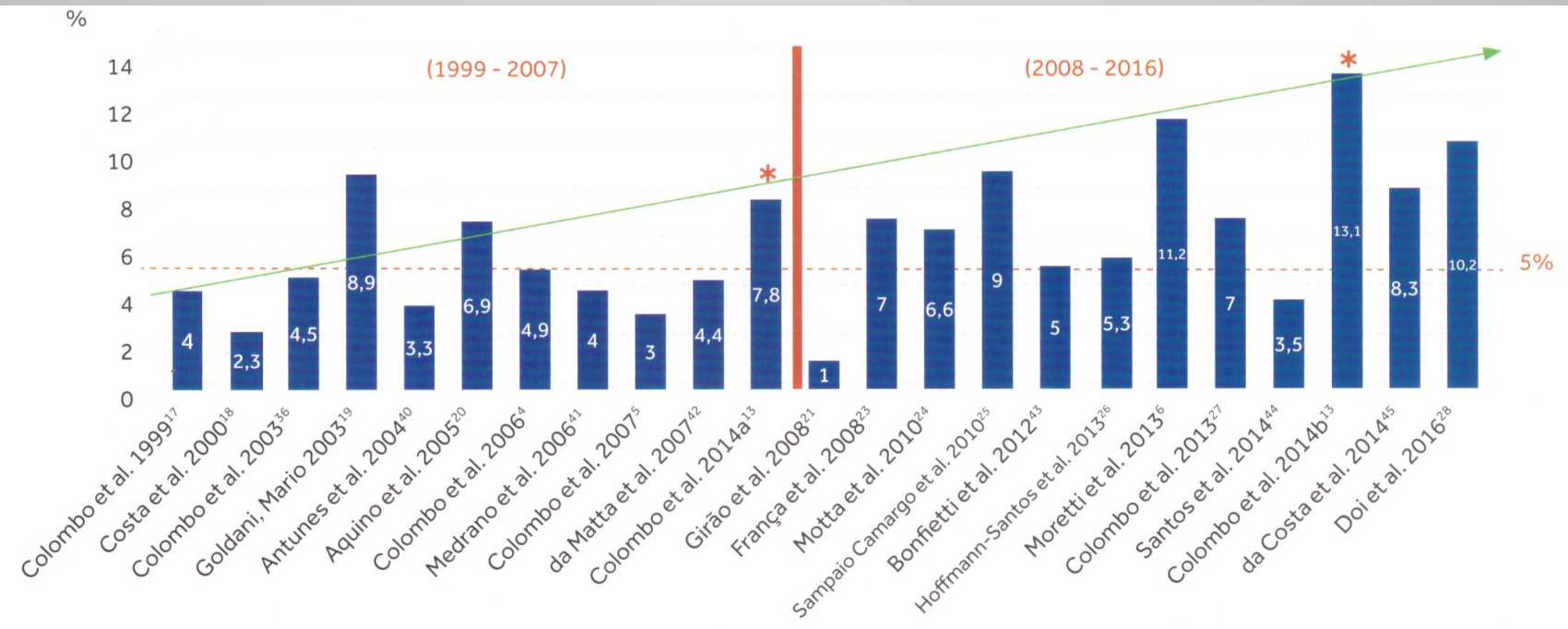


Figura 2. Taxa de prevalência de isolamento de *C. glabrata* em 4.320 episódios de candidemia documentados em hospitais do Brasil ao longo do período de 1999 a 2016. *No estudo de Colombo et al., 2014[13] as coletas de hemoculturas envolveram dois períodos distintos (2003-2007) e (2008-2012). Adaptado de da Matta et al., 2017[3]

Perlin et al. Lancet Infect Dis 2017; 17: e383–92

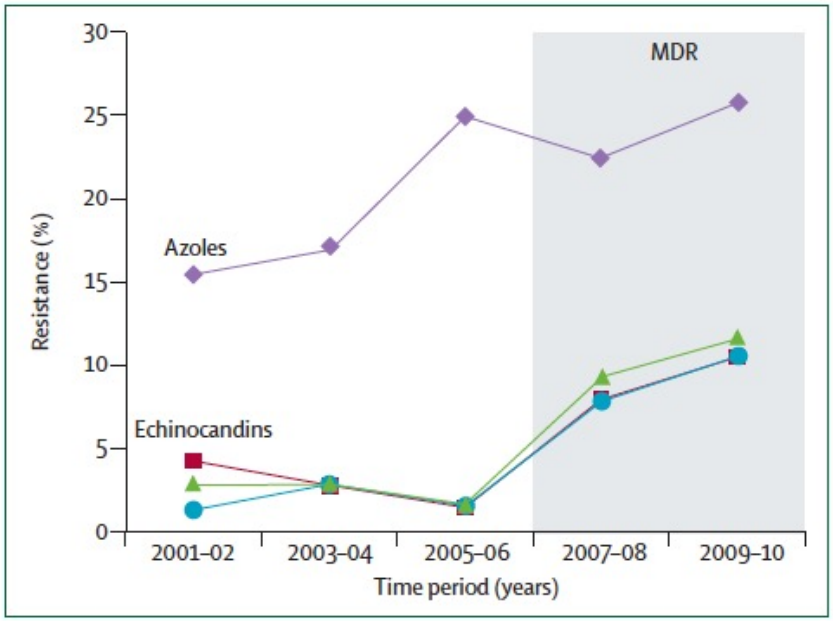
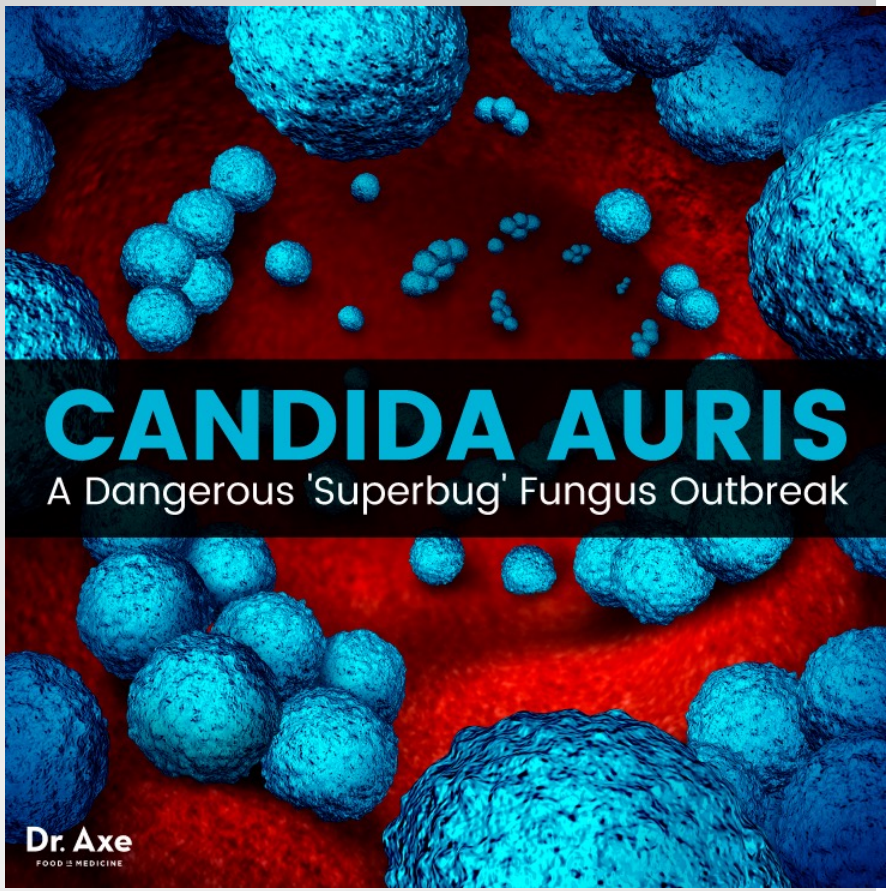


***Figure 3*: Parallel rise in azole and echinocandin resistance in *Candida glabrata* bloodstream isolates over a 10-year period, showing emergence of multidrug-resistant strains**

The grey-shaded box shows the time of emergence of substantial multidrug resistance. The three echinocandin-class drugs are shown: red, anidulafungin; green, caspofungin; blue, micafungin (adapted from Alexander et al[73]). MDR=multidrug resistance.

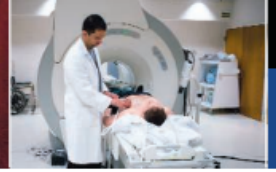


Uso de antifúngicos como conservantes de alimentos, cosméticos e na produção animal
Uso de agentes azólicos na agricultura

Exemplos: epoxiconazol, triadimenol, propiconazol, protioconazol, meticonazol, ciproconazol, tebuconazol, flusilazol, paclobutrazol, entre outros.

Uso no controle de pragas, principalmente causadas por fitopatógenos (ex.: *Septoria tritici* e *Gibberella zeae*)

Existe relação entre a utilização de fungicidas na agricultura na seleção de fungos patógenos humanos resistentes aos agentes azólicos?



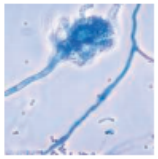
Special Report:
Science and the Stimulus

1176

INFECTIOUS DISEASES

Farm Fungicides Linked to Resistance in a Human Pathogen

A team of Dutch researchers has reignited a debate on the agricultural use of fungicides with a review in the December issue of *The Lancet Infectious Diseases*. The authors maintain that the massive use of fungicides to protect European orchards, vineyards, and grain fields may be contributing to resistance against drugs used to treat people with life-threatening infections of *Aspergillus fumigatus*. Although the overuse of antibiotics in animal husbandry is known to have caused resistance in the human population, this would be the first time a similar link is found between farm use of fungicides and human health.



Deadly mold. Invasive *Aspergillus fumigatus* infections can be fatal.

If true, the authors warn ominously, that "confronts us with a major challenge with worldwide dimensions." But Herbert Hof, director of the Institute for Medical Microbiology at the University of Heidelberg in Germany, accuses them of crying wolf, saying the paper amounts to "publicity seeking" by frightening the public "in the way horror films do." The group does have its supporters, however. David Denning of the University of Manchester, who heads the United Kingdom's National Aspergillosis Centre, says "they have a very strong case." The leader of the team, Paul Verweij of Radboud University Nijmegen Medical Centre in the Netherlands, concedes that they haven't yet clinched the case, but he says enough evidence has accumulated to issue a warning.

A. fumigatus causes infections, sometimes fatal, primarily in people with compromised immune systems and certain diseases, such as chronic obstructive pulmonary disease. Patients are thought to become infected when they inhale spores of the fungus, which are ubiquitous in soil. Drugs of a class called azoles are doctors' mainstay, and resistance has long been known to crop up in individual patients. The mutations in the fungus that cause the resistance usually differ from one patient to the next; in a paper published in July, for instance, Denning's team reported finding 18 different mutations in an *Aspergillus* gene called *cyp51A* in 30 patients in the United Kingdom.

But Verweij's team has found something strange in resistant *Aspergillus* strains in the Netherlands: In 94% of the isolates from his own hospital and 69% of those from other Dutch hospitals, the resistance was caused by a single pair of mutations—a point mutation in *cyp51A* and a so-called tandem repeat in the gene's promoter. To Verweij, that similarity points to a new scenario: that all the patients breathed in spores that were already resistant. That's why he believes there's an environmental cause.

Azoles are used to ward off a range of plant pathogens and are applied on 50% of Europe's grain and grape acreage, says plant pathologist Gert Kema of Wageningen University in the Netherlands, a co-author of the paper. Much smaller amounts are used in the United States, where farming is less-intensive and spraying is less cost-effective. But the compounds are popular in other parts of the world as well, he says.

The risk that heavy agricultural use of azoles might lead to resistance problems in people has been debated for years. In 2002, an expert panel for the European Commission concluded that it was unlikely. But since then, the evidence has been building, Verweij says. The resistant fungus found in patients is also resistant to certain agricultural fungicides, which is suggestive of a link. And in a paper published in June, his group showed that resistant *Aspergillus* could be isolated from soil in flower beds close to hospitals and in commercial compost, leaves, and seeds bought at a garden center. Thirteen of 15 of these environmental samples also had the two mutations seen in clinical isolates.

In an e-mail to *Science*, Hof called the authors "prejudiced" and said resistance in fungi is unlikely to become a major public health problem because unlike bacteria, fungi don't swap resistance genes. Dominique Sanglard of the University Hospital in Lausanne, Switzerland, on the other hand, says the Dutch researchers seem to be on to something real, although many questions remain. A key step is to show that one or more of the azole fungicides—at least 30 of them are on the market—can actually trigger the mutations in *A. fumigatus* seen in hospitals, says Verweij; that study is already under way.

And what if the link is proven? Verweij says a ban on certain fungicides could be an option. The team has been talking to several fungicide producers, and "they aren't very keen on studying this further," he says. A spokesperson for Syngenta, a major azole fungicide producer, says that resistance may have arisen in other ways—such as azole use in cosmetics—and that the company is "not convinced" of a causal link. —MARTIN ENSERINK

Farmers' friend. Azoles are used to protect a wide variety of crops from fungi.

CREDITS (TOP TO BOTTOM): CDC; HENRY ARDEN/GETTY IMAGES

Uso abusivo de azóis na agricultura pode levar a seleção de cepas de fungos resistentes.

Problema de saúde pública.

E se essa relação for provada

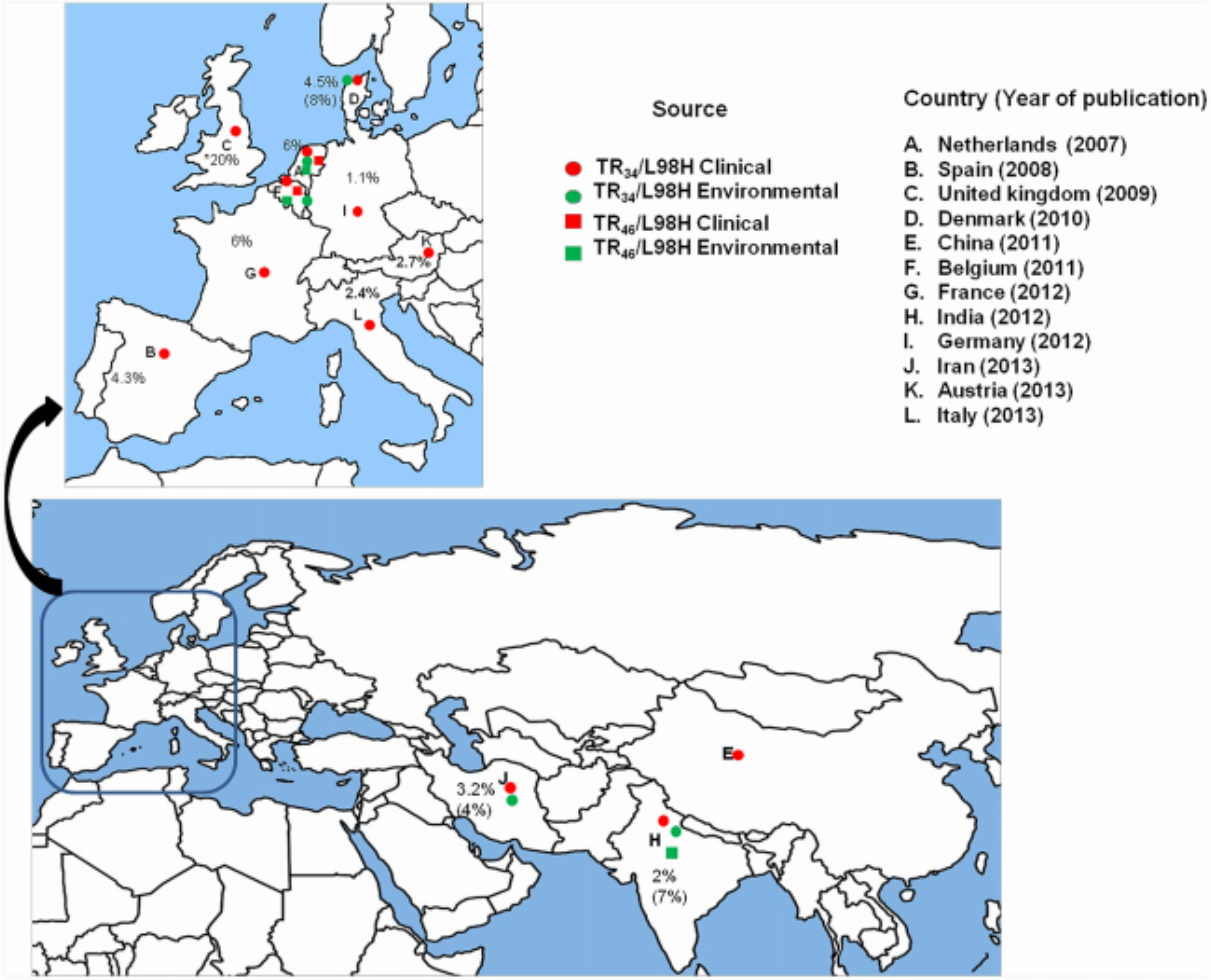


Figure 2. A global map depicting geographic distribution of multi-triazole-resistant clinical (red) and environmental (green) *Aspergillus fumigatus* strains carrying the TR_{34}/L98H (circle) and the TR_{46}/Y121F/T289A mutations (square). Countrywide prevalence rates (%) of *A. fumigatus* carrying TR_{34}/L98H are presented excepting the United Kingdom, where overall azole resistance is illustrated. The percent in parentheses denotes environmental prevalence rates.
doi:10.1371/journal.ppat.1003633.g002

Chowdhary et al. Emergence of AzoleResistant *Aspergillus fumigatus* Strains due to Agricultural Azole Use Creates an Increasing Threat to Human Health. PLoS Pathog 9(10): e1003633, 2013.
Gisi. Assessment of selection and resistance risk for demethylation inhibitor fungicides in Aspergillus fumigatus in agriculture and medicine: a critical review. Pest Manag Sci; 70: 352–364, 2014.
Berger S, et al. Azole Resistance in Aspergillus fumigatus: A Consequence of Antifungal Use in Agriculture? Front. Microbiol. 8:1024, 2017.

Como saber se um microrganismo é resistente ou sensível a uma droga?

Comitês

- Organizações internacionais, interdisciplinares e educacionais que promovem o desenvolvimento e a ampla utilização de normas e procedimentos laboratoriais padronizados.

1. Padronização das técnicas
2. Critérios para interpretação dos resultados
3. Parâmetros para o controle qualidade

- Testes de sensibilidade aos antifúngicos “in vitro”
  - Método de difusão em ágar
  - Método de diluição em caldo
    - Macrodiluição
    - Microdiluição
  - Metodologia do E-teste
  - Métodos automatizados

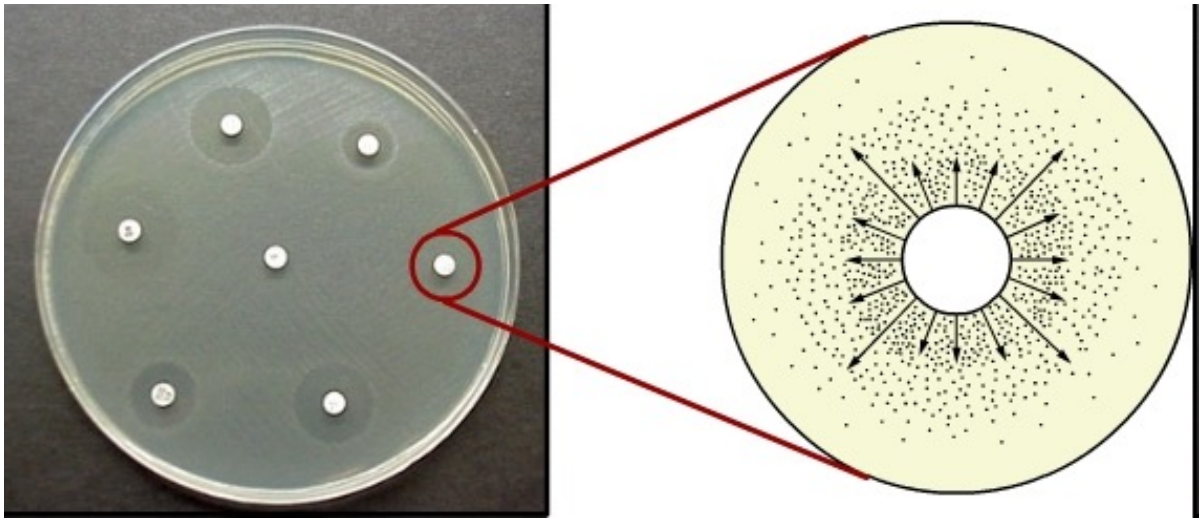
Difusão em ágar

Técnica de difusão em ágar– Documento **44-A2** (CLSI, 2008).

Candida spp.

1. **Suspensão fúngica: 1-5 x 10^6 UFC/mL**
2. **Ágar Muller-Hinton**

2. Discos de papel impregnados com antifúngicos:
Fluconazol – 40 µg



E teste® - bioMérieux

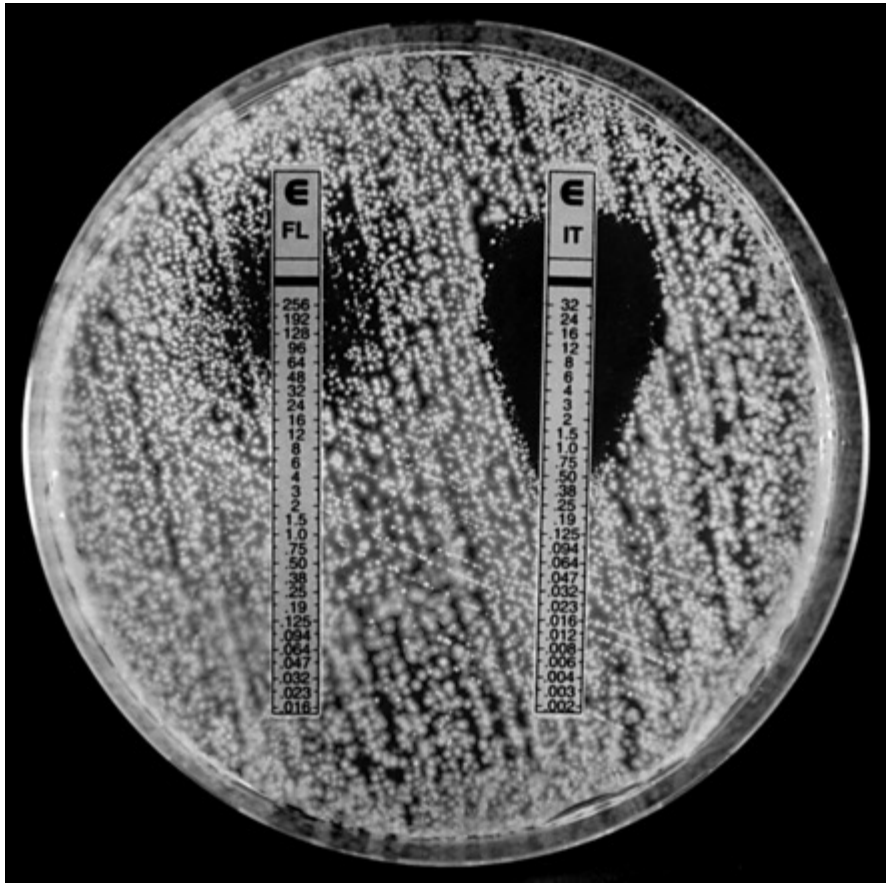
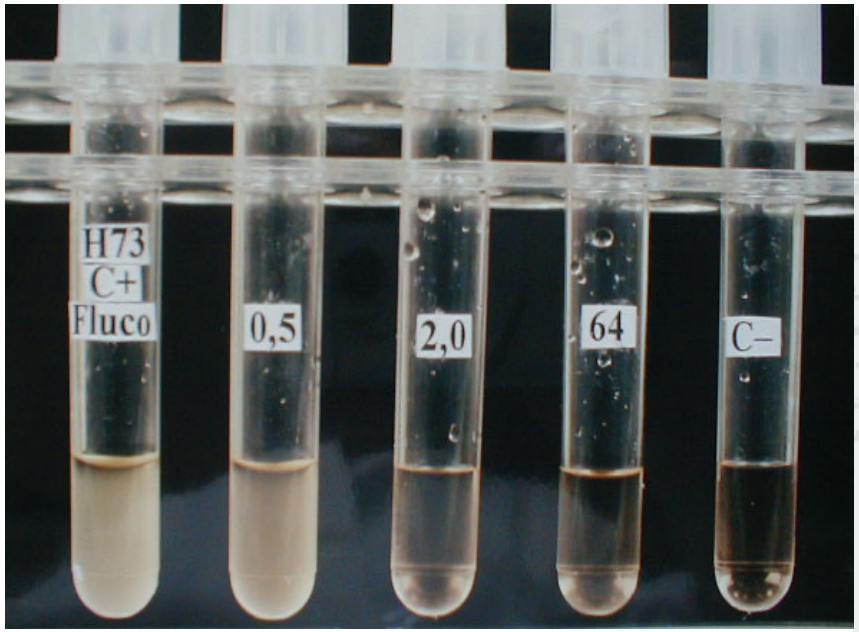


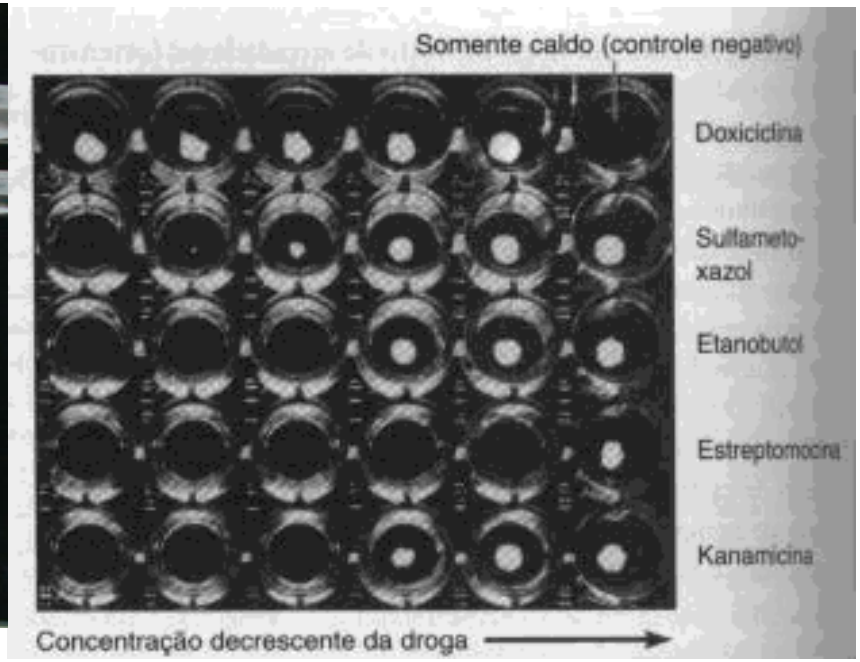
Figure 1
Fluconazole (FL): change of morphology at endpoint. MIC, 48 mg/litre; itraconazole (IT): sharp end point. MIC, 0.5 mg/litre.

Diluição em caldo

- Técnica de diluição em caldo
  - Leveduras - documento M60 (CLSI, 2017) e documento E.DEF. 7.3.1 (Eucast, 2017)
  - Filamentoso – documento M38-A2 – CLSI, 2008
  - CIM – inibição de 50% e 90% do crescimento fúngico



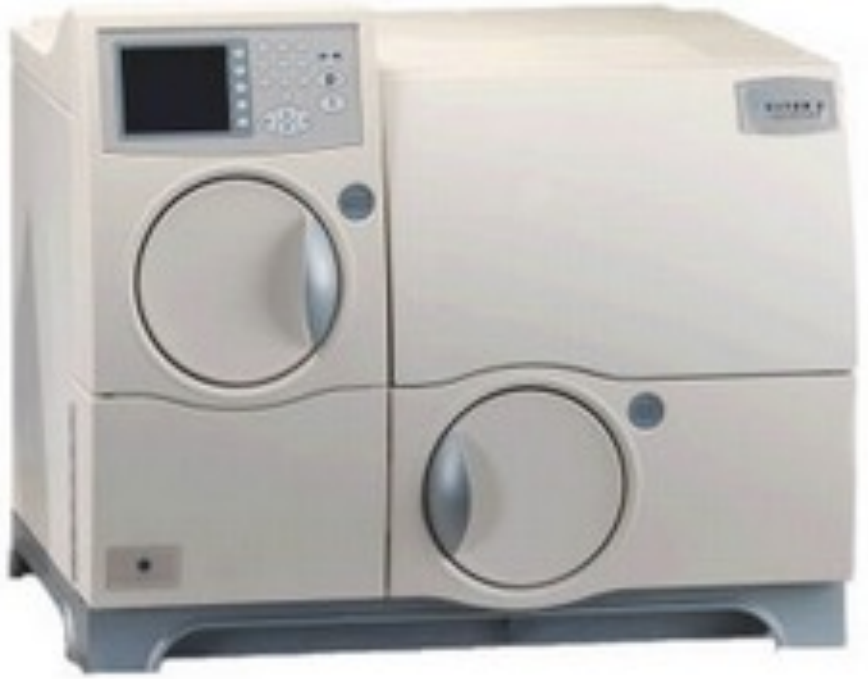
Macrodiluição

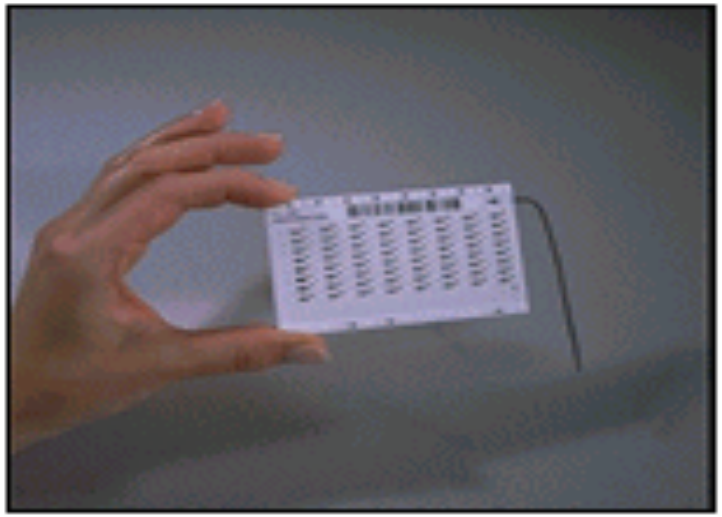


Microdiluição

Automação

Identificação e testes de susceptibilidade e resistência





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Navigate to "virtual cassette" workspace.

Inoculate tubes and place in cassette.

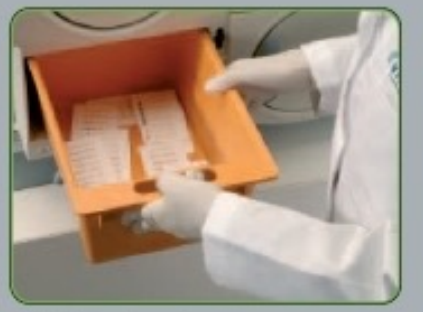
Scan the appropriate card and place it in the cassette.

Scan in the isolate identification number, or use the keyboard to type it in.

Place cassette in filler.

Place cassette in reader.

Results print automatically when card is complete.



Finished cards are automatically sent to waste bin for safe disposal.

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