

Antifúngicos

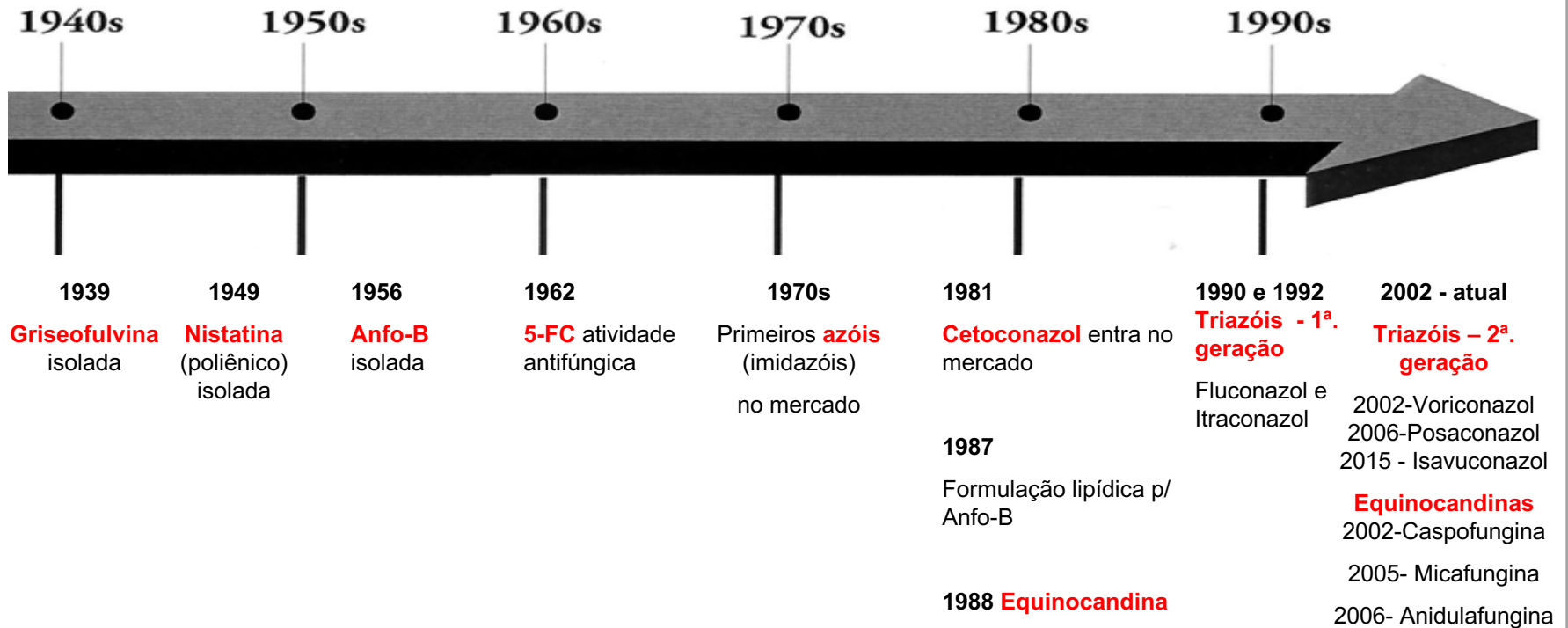
Kelly Ishida

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Instituto de Ciências Biomédicas
Departamento de Microbiologia

Classificação das micoses

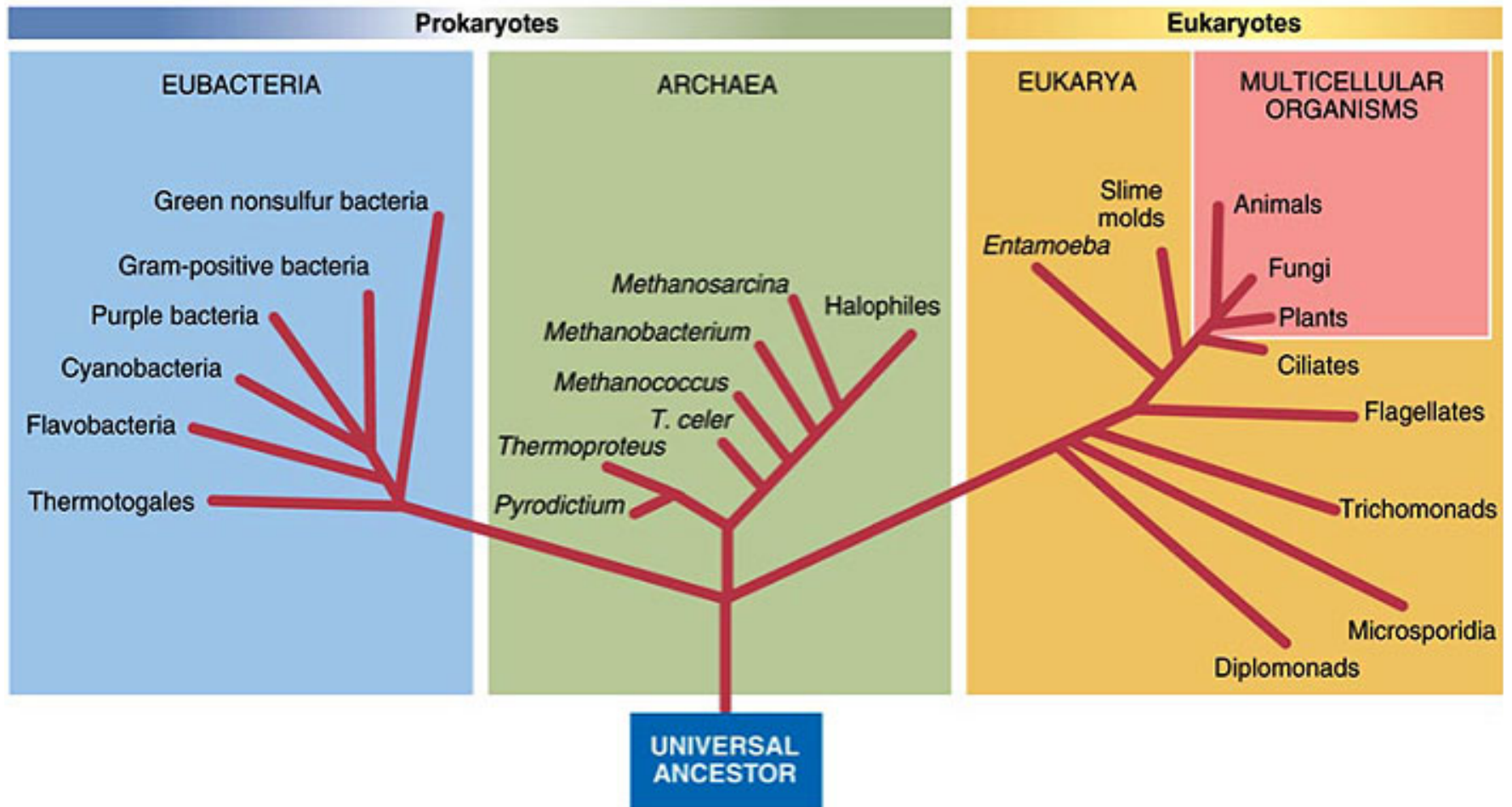
Micose	Tecido	Espécies
Superficial	Extrato córneo do tecido epitelial, pelo e cabelo	<i>Malassezia</i> spp. <i>Hortaea werneckii</i> <i>Piedraia hortae</i> <i>Trichosporon</i> spp.
Cutâneo	Porções queratinizadas da pele, pelo e cabelo	<i>Trichophyton</i> spp. <i>Microsporum</i> spp. <i>Epidermophyton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

Histórico – antifúngicos

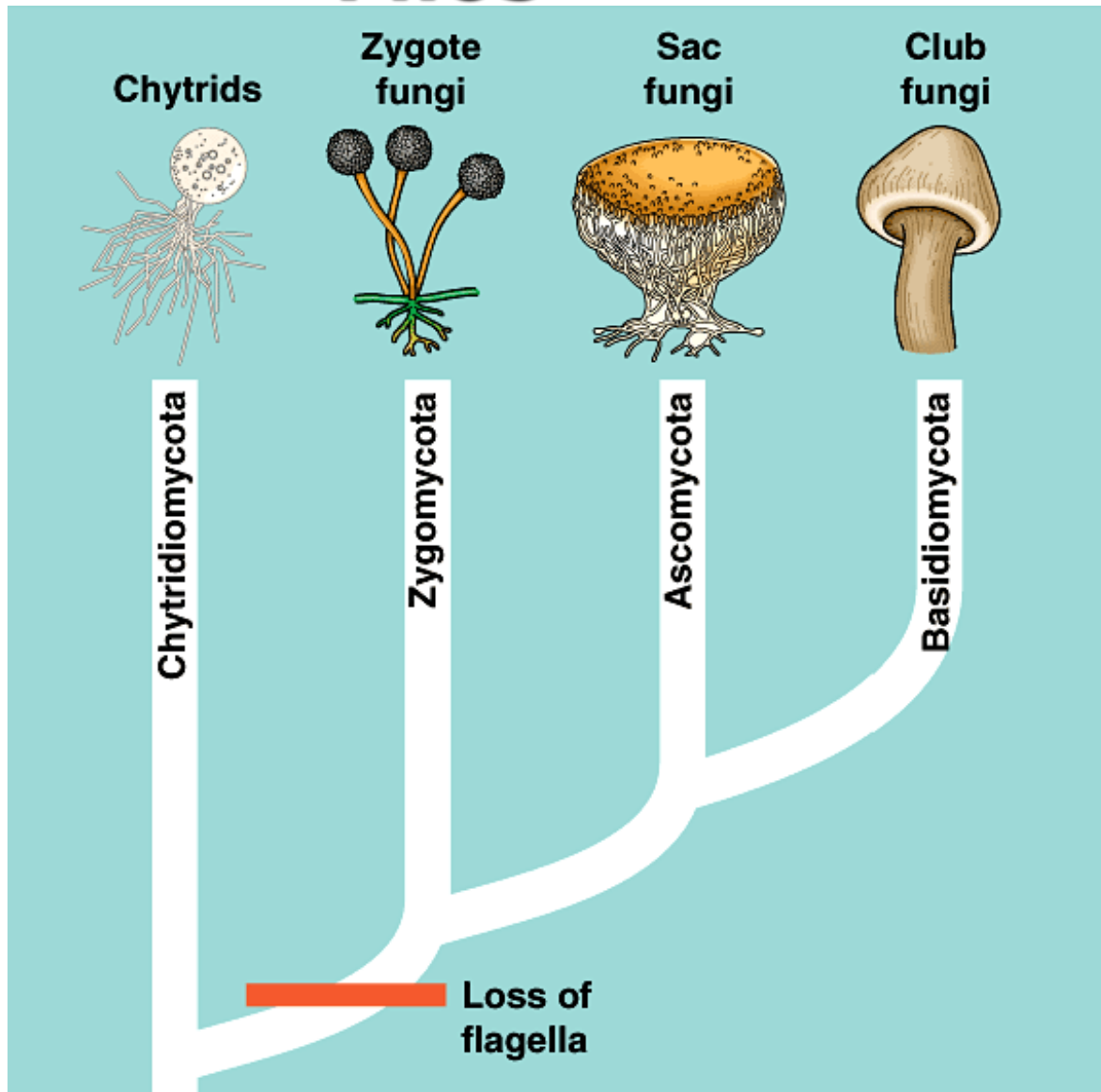


1903 – KI (iodeto de potássio)

Domínio dos seres vivos



Filos

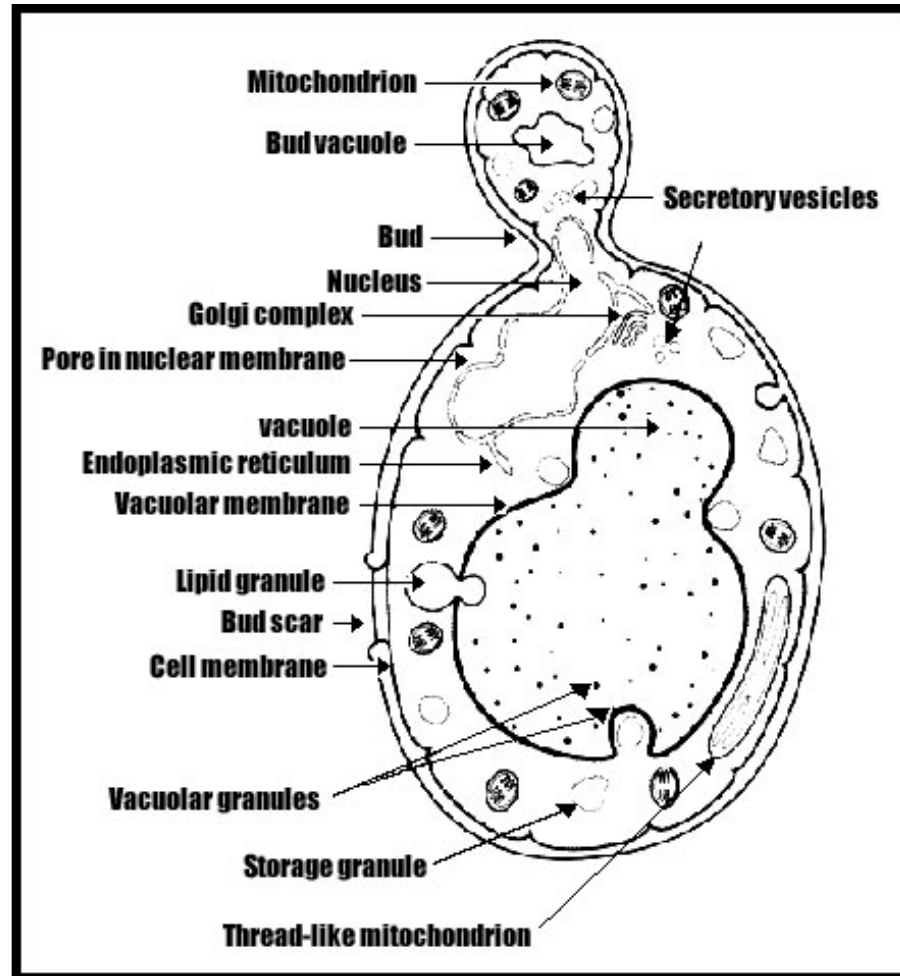


Originados de um único ancestral

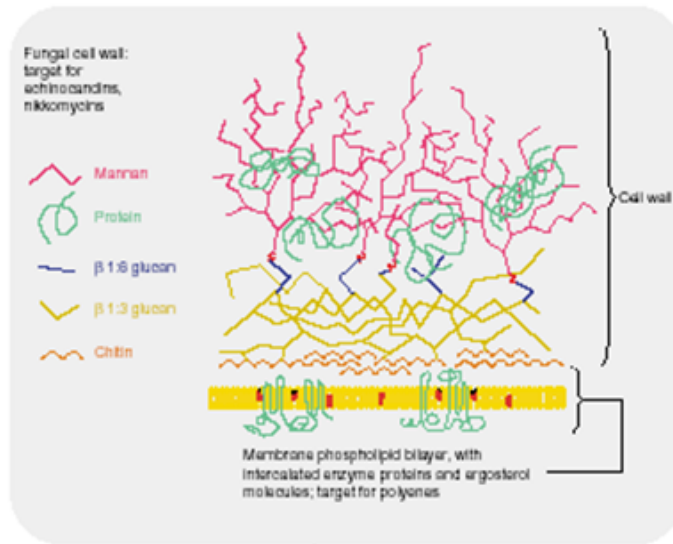


Grupo monofilogenético

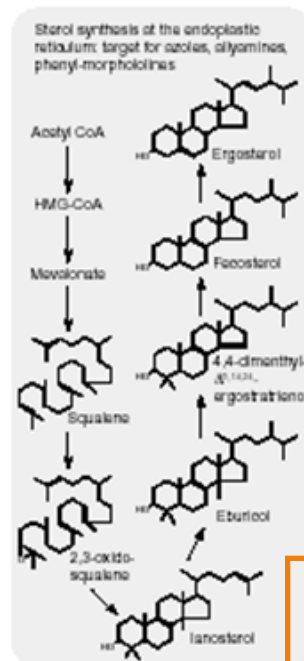
A célula fúngica



Alvos para a quimioterapia antifúngica



Equinocandinas



Poliênicos
Azóis



DNA and RNA synthesis: targets for flucytosine

5-Fluorocitosina

Microtubule assembly: target for griseofulvin

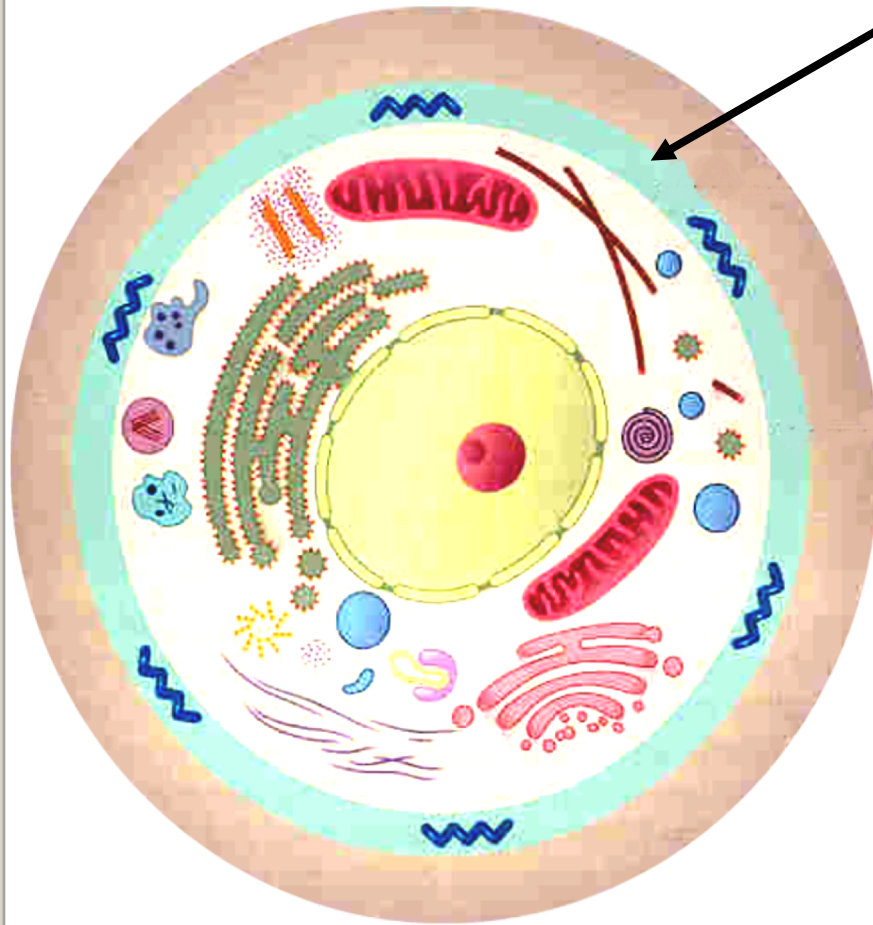
Griseofulvina

O antifúngico ideal...

- Toxicidade seletiva $\Rightarrow$ ↓ 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

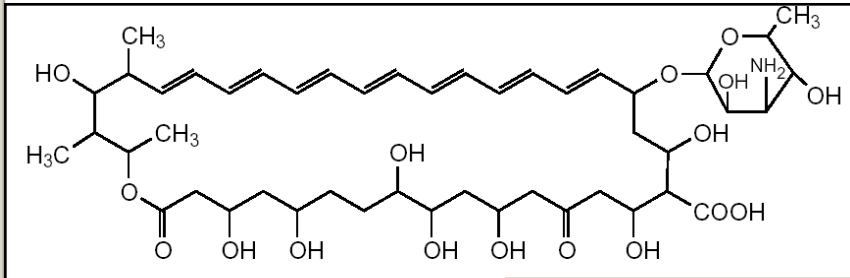


Membrana celular

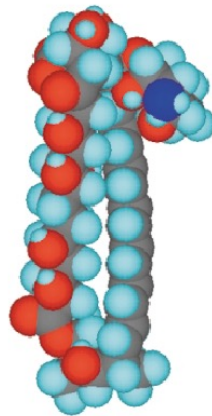
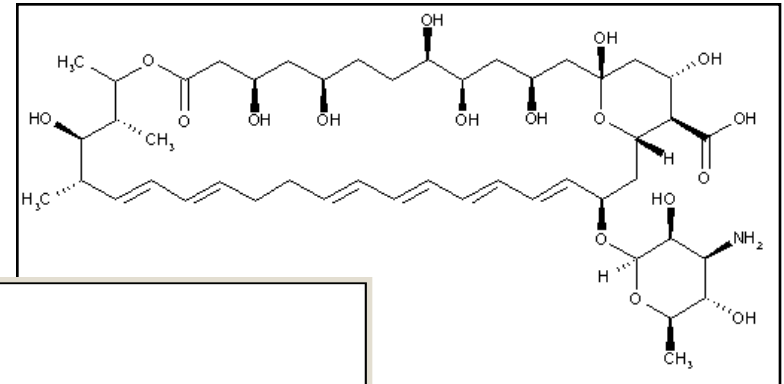
- **Poliênicos**
 - Anfotericina B e formulações lipídicas
 - Nistatina
- **Azóis**
 - Cetoconazol, Miconazol,
 - Fluconazol, Itraconazol
 - Voriconazol, Posaconazol, Isavuconazol
 - Albacozazol, Ravuconazol,
- **Alilamina** (Terbinafina, butenafina)
- **Amorolfina**

Ação sobre o ergosterol- agentes poliênicos

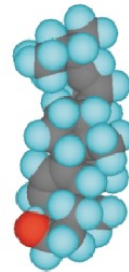
Anfotericina B



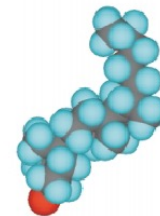
Nistatina



Anfotericina B



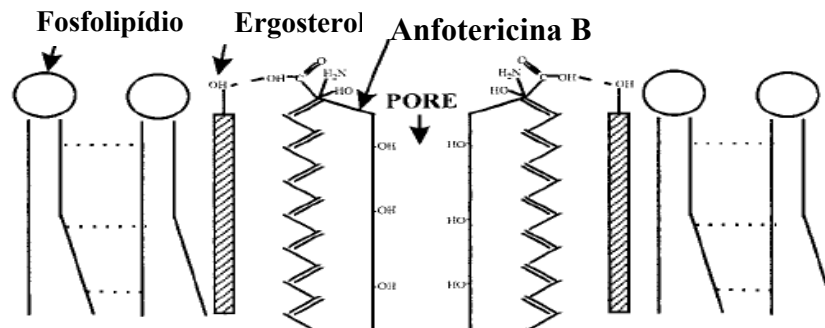
Ergosterol



Colesterol

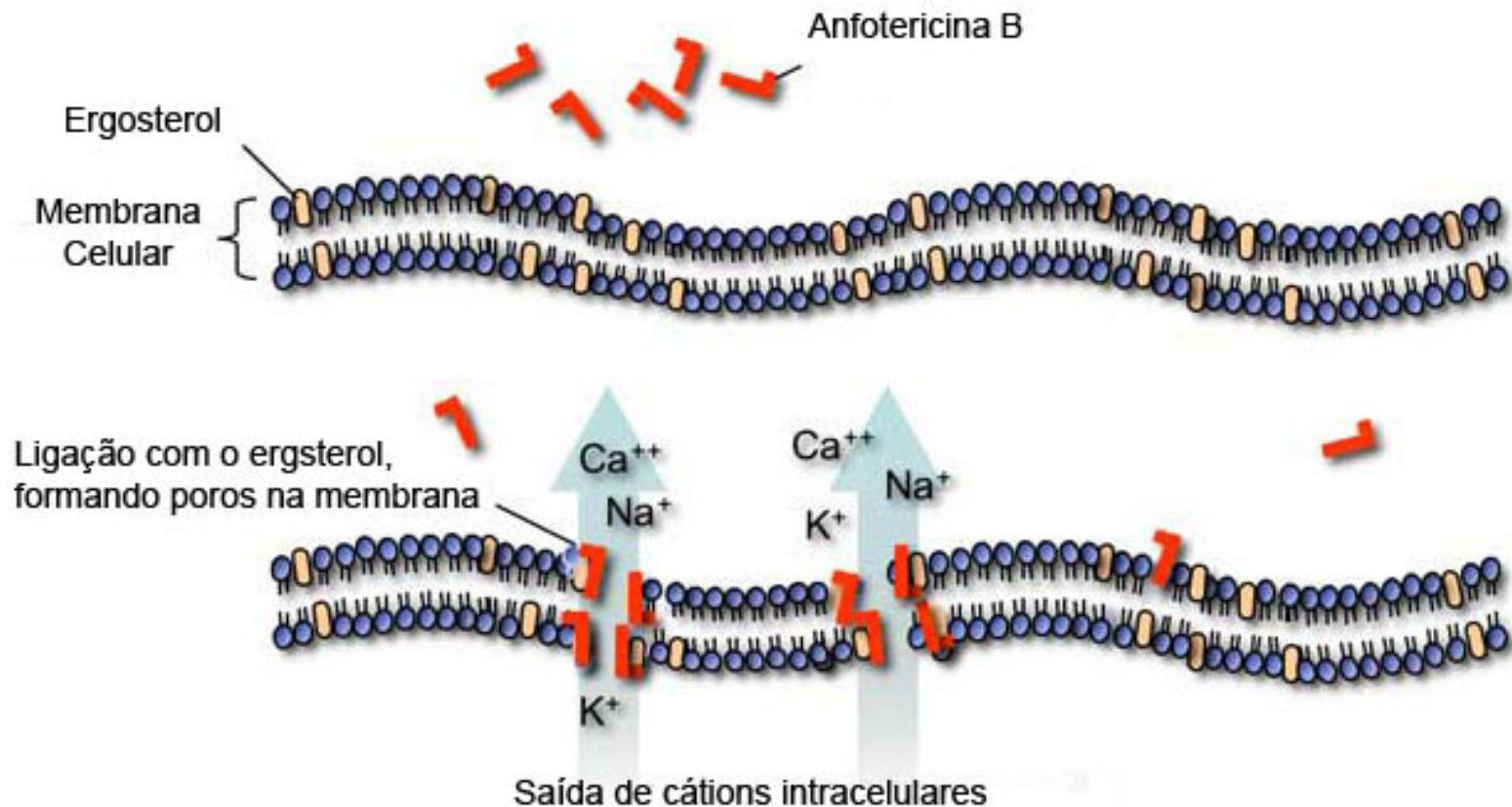
TRENDS in Microbiology

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

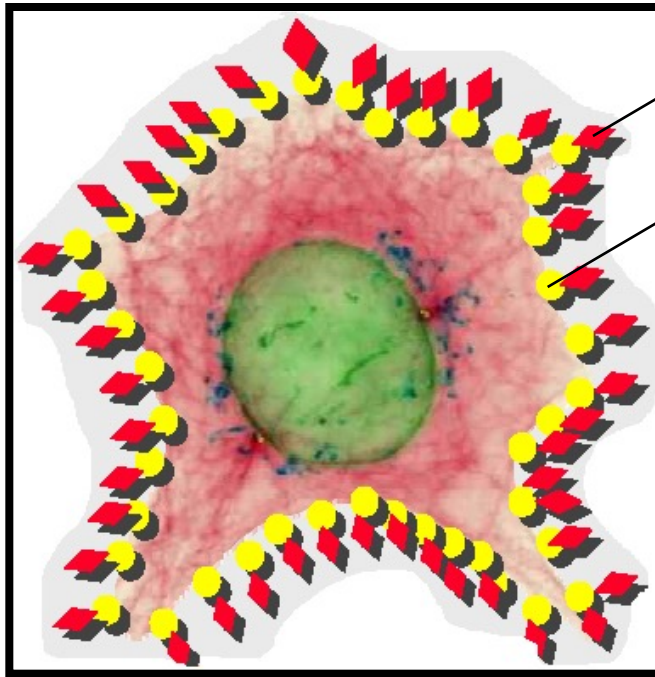


Meio aquoso
Bicamada lipídica

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*
- Altamente tóxico
- Má absorção mucocutânea
- Usado no tratamento de infecções mucocutâneas (Ex. lavagem bucal, SGI)
- Efeitos colaterais: Náuseas, vômito, diarreia com dose elevada

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)



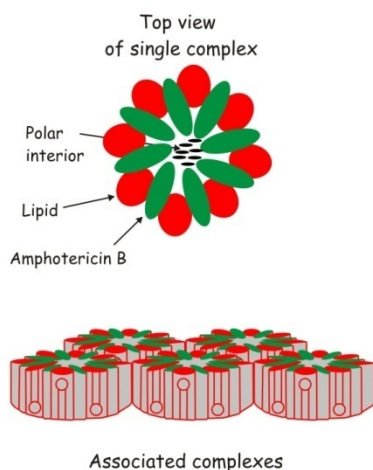
**Eficácia semelhante a
AMB desoxicolato**



Efeitos colaterais

Formulações lipídicas da AMB desoxicolato

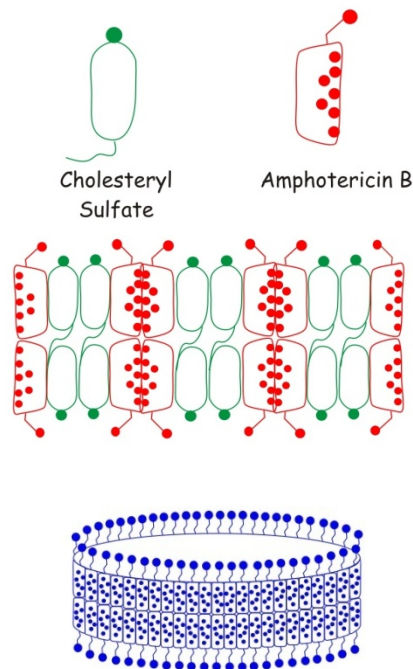
Abelcet[®] ABLC Complexo lipídico



Ribbon-like particles
Carrier lipids: DMPC, DMPG
Particle size (µm): 1.6-11

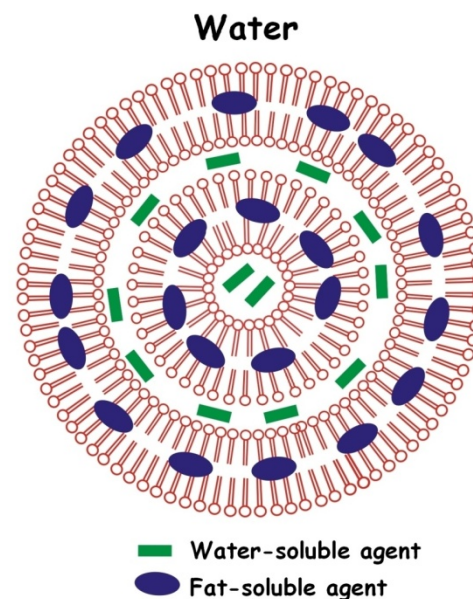
DMPC-Dimyristoyl phosphatidylcholine
DMPG- Dimyristoyl phosphatidylglycerol

Amphotec[®] ABCD Dispersão coloidal



Disk-like particles
Carrier lipids: Cholesteryl sulfate
Particle size (µm): 0.12-0.14

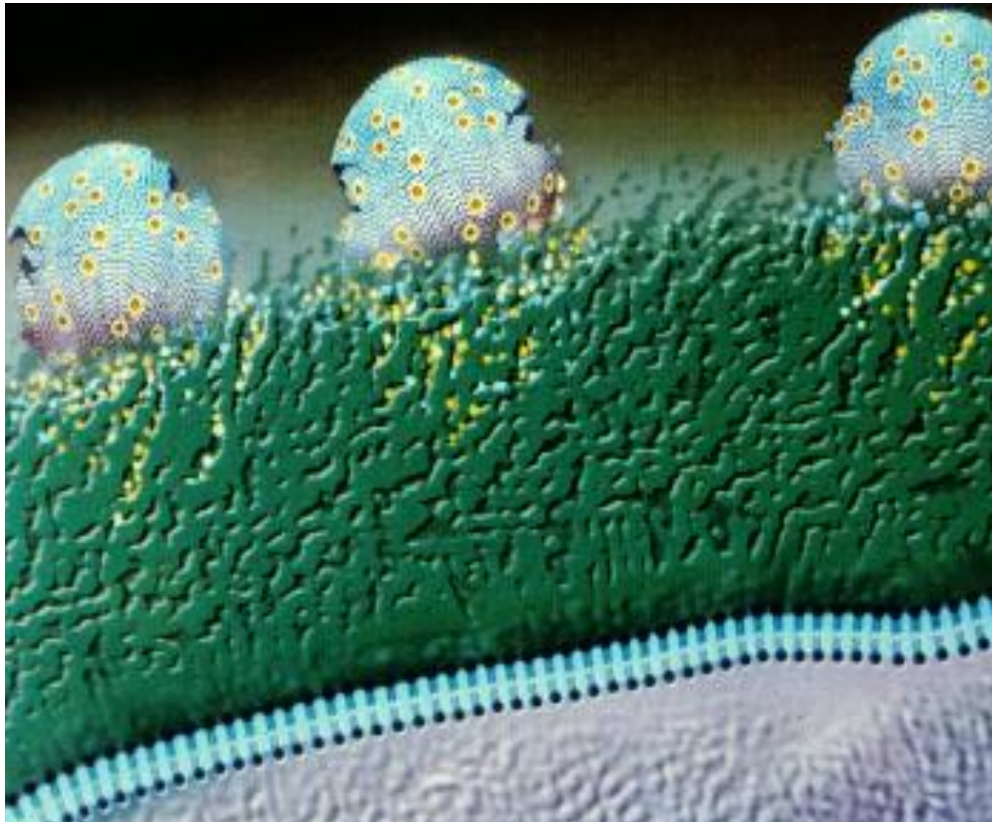
Ambisome[®] L-AMB AMB lipossomal



Unilamellar liposome
Carrier lipids: HSPC, DSPG, cholesterol
Particle size (µm) : 0.08

HSPC-Hydrogenated soy phosphatidylcholine
DSPG-Distearoyl phosphatidylcholine

AMB lipossomal - Ambissome®



- Maior afinidade pela célula fúngica
- Menor toxicidade, por impedir liberação nas células do hospedeiro
 - Permite maiores dosagens
- Custo muito elevado (~100 x a mais que a AMB desoxicolato)

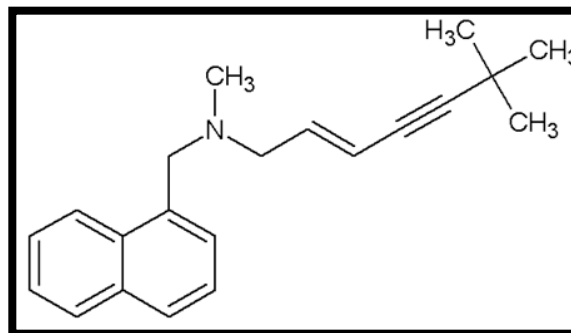
Inibição da síntese do ergosterol



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

Alilaminas: terbinafina e butenafina

Terbinafina (Lamisil, Novartis Pharmaceuticals)



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

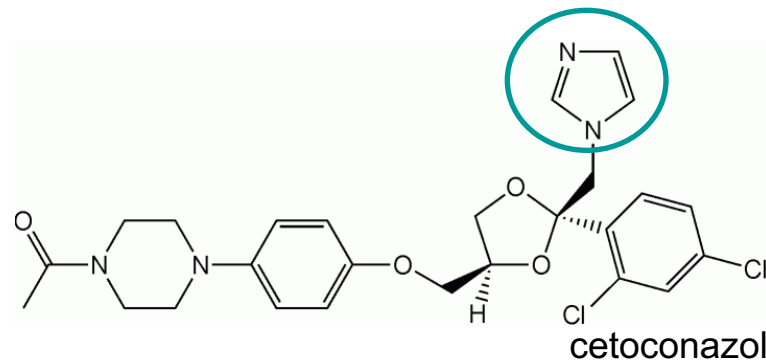
Acumula na pele, unha e tecido adiposo

Reações adversas: diarreia, náuseas, erupções cutâneas, urticária e fotossensibilidade.

Azóis: imidazóis e triazóis

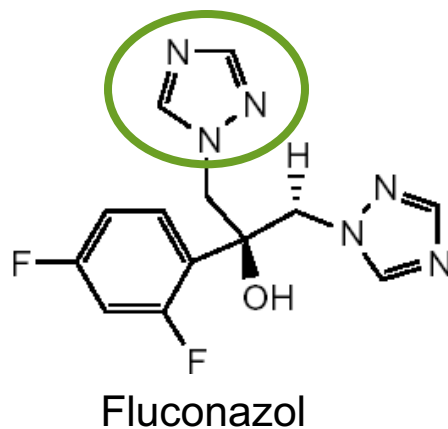
- Imidazóis

- 1ª. Geração
- 2ª. Geração

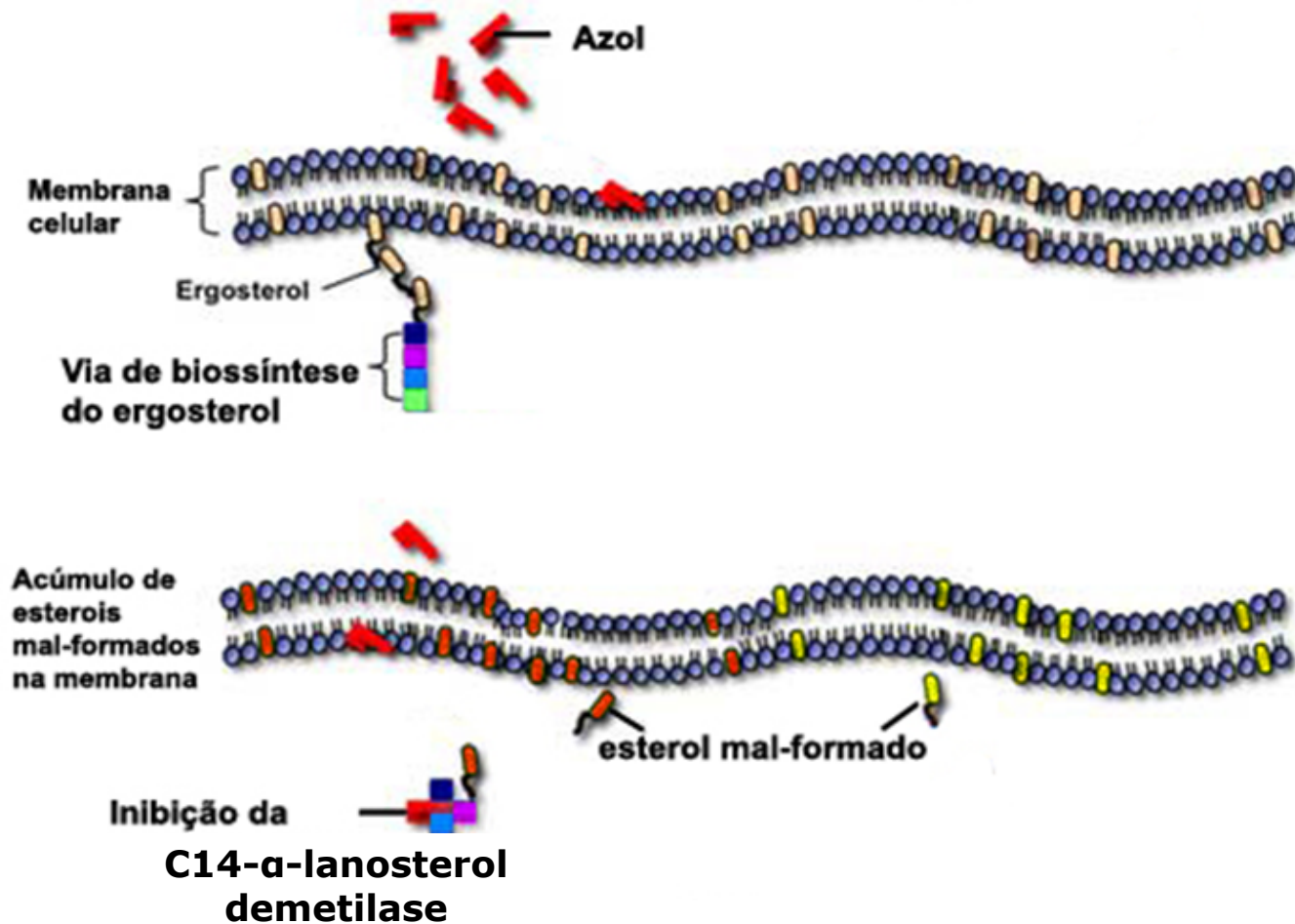


- Triazóis

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

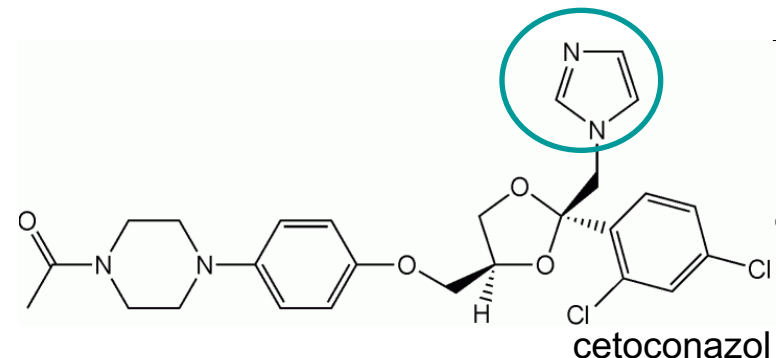


Azóis: imidazóis e trizóis



Agentes azólicos: imidazóis

- Econazol
 - Miconazol
 - Clotrimazol
- } Tópico



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

São Fungistáticos

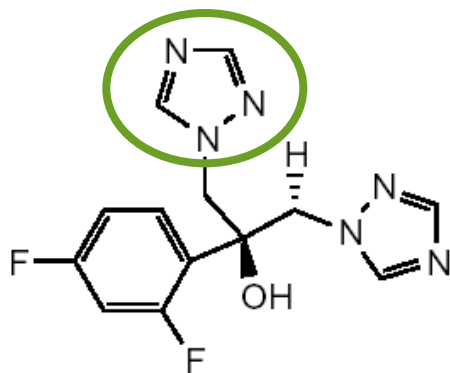
Dermatofitoses, candidíasis cutânea/mucocutânea

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

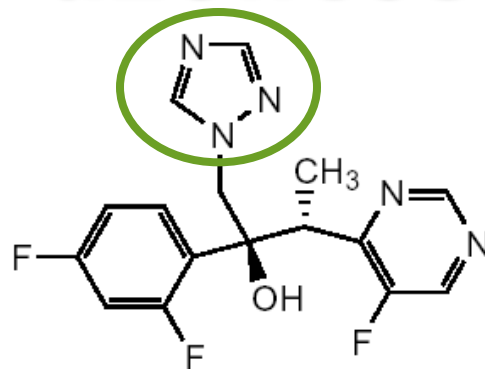
Absorção do cetoconazol – pH ácido

São hepatotóxicos, altera níveis de testosterona e cortisol

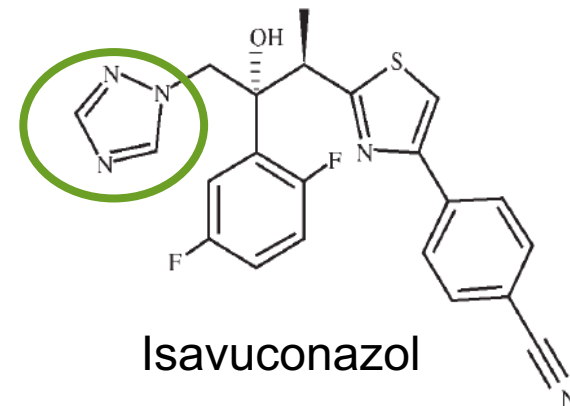
Agentes azólicos: triazóis



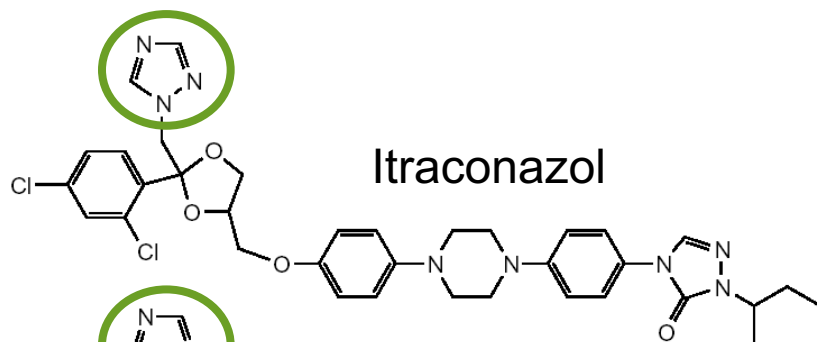
Fluconazol



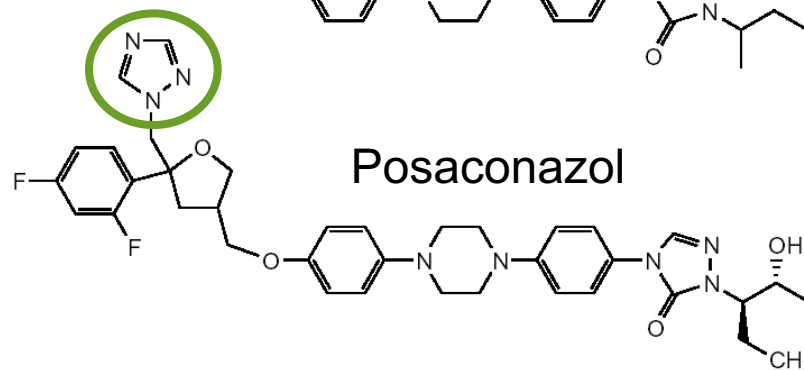
Voriconazol



Isavuconazol



Itraconazol



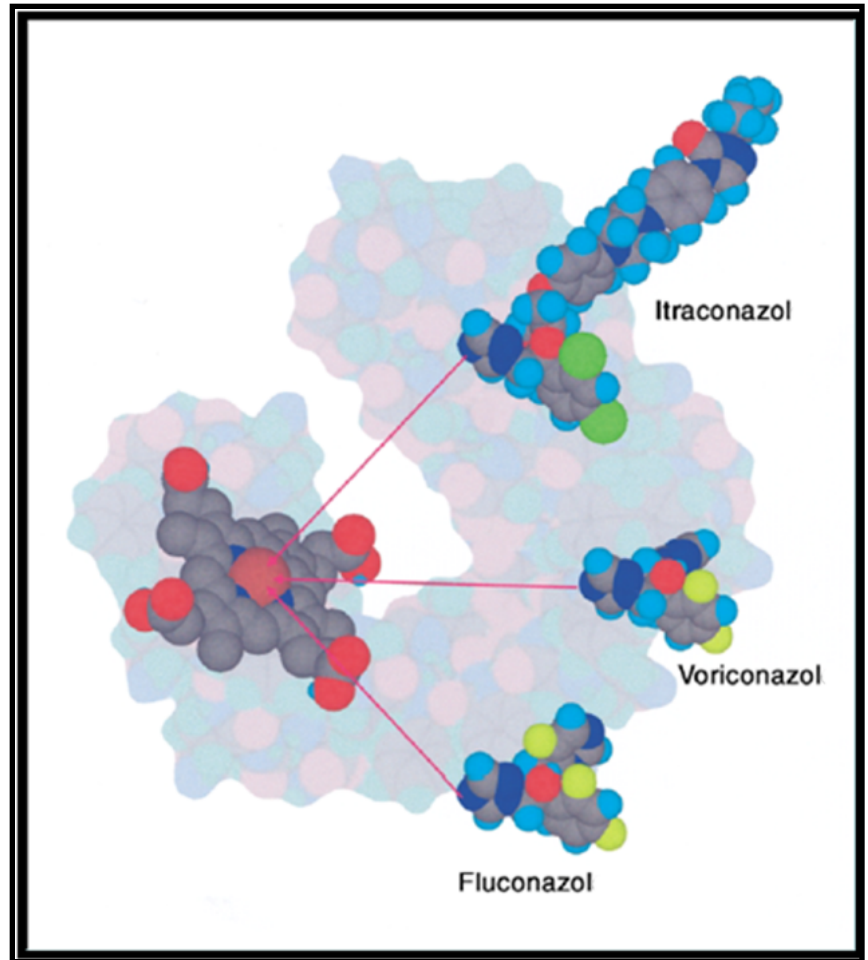
Posaconazol

No tratamento de Infecções sistêmicas.

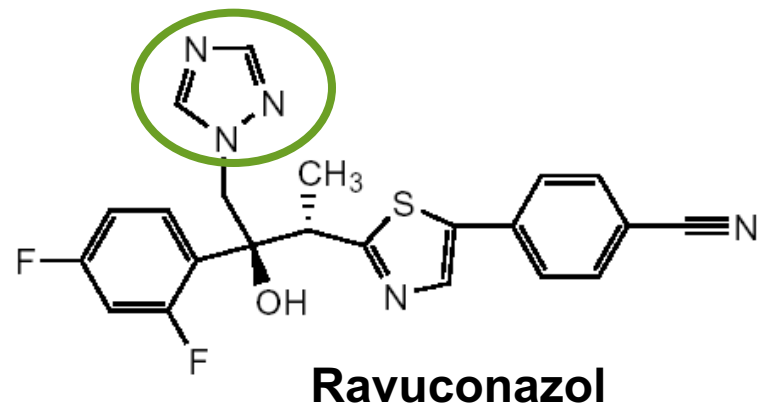
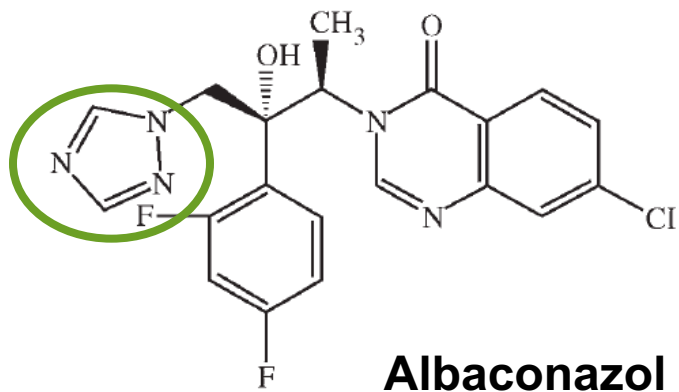
Alta afinidade pela C14- α demetilase – ↓efeitos colaterais

São mais específicos para a
enzima do fungo

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



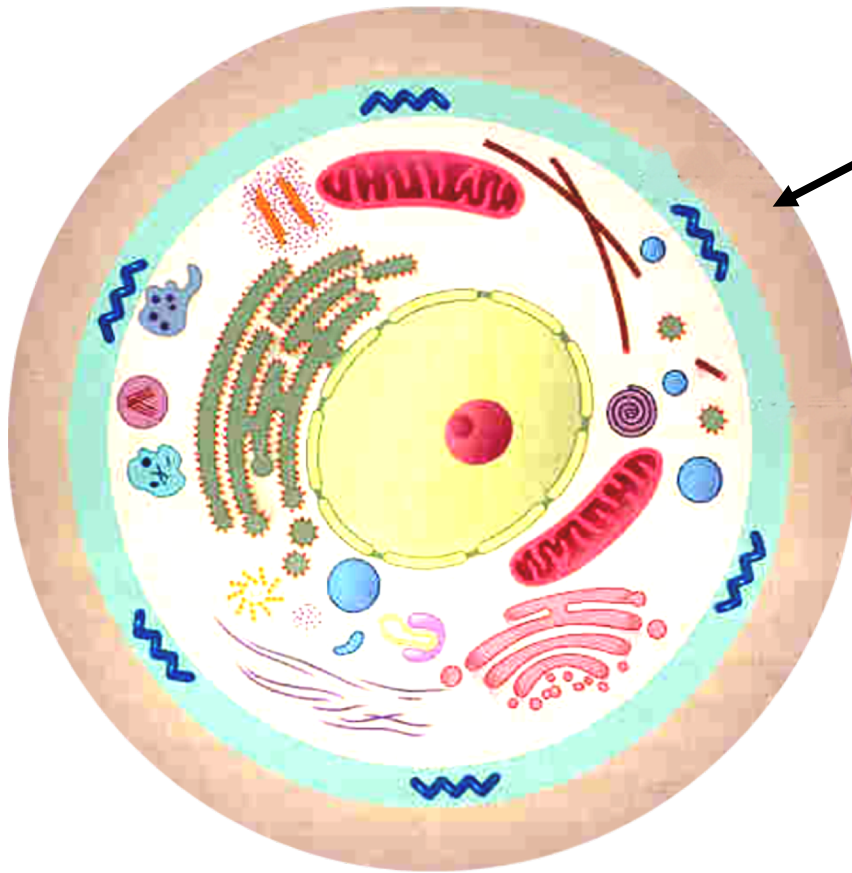
Novos triazóis



Espectro de ação dos triazóis

	<i>Candida</i>	<i>Cryptococcus</i>	<i>Aspergillus</i>	<i>Fusarium</i>	<i>Zigomicetos</i>
Flu	++	+++	-	-	-
Itra	++	++	+++	-	-
Vori	+++	+++	+++	++	-
Posa	+++	+++	+++	++	++
Isav	+++	+++	+++	+	+

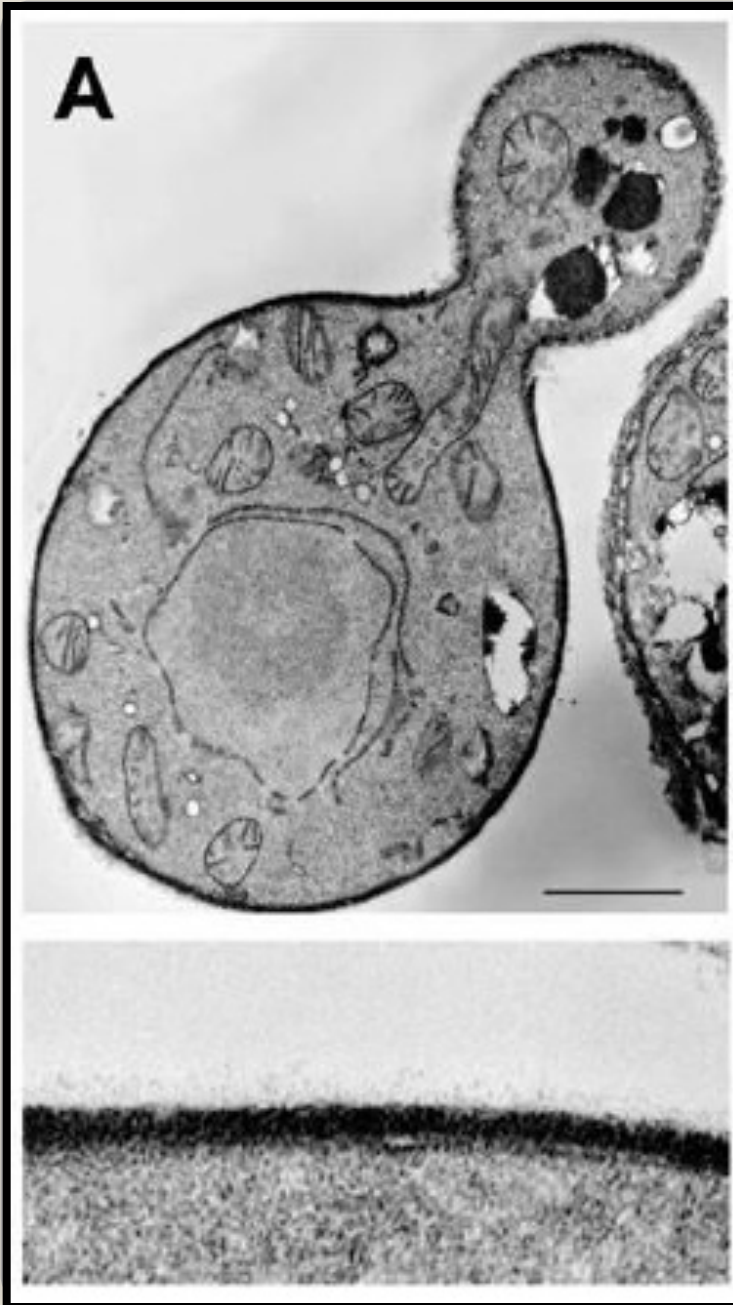
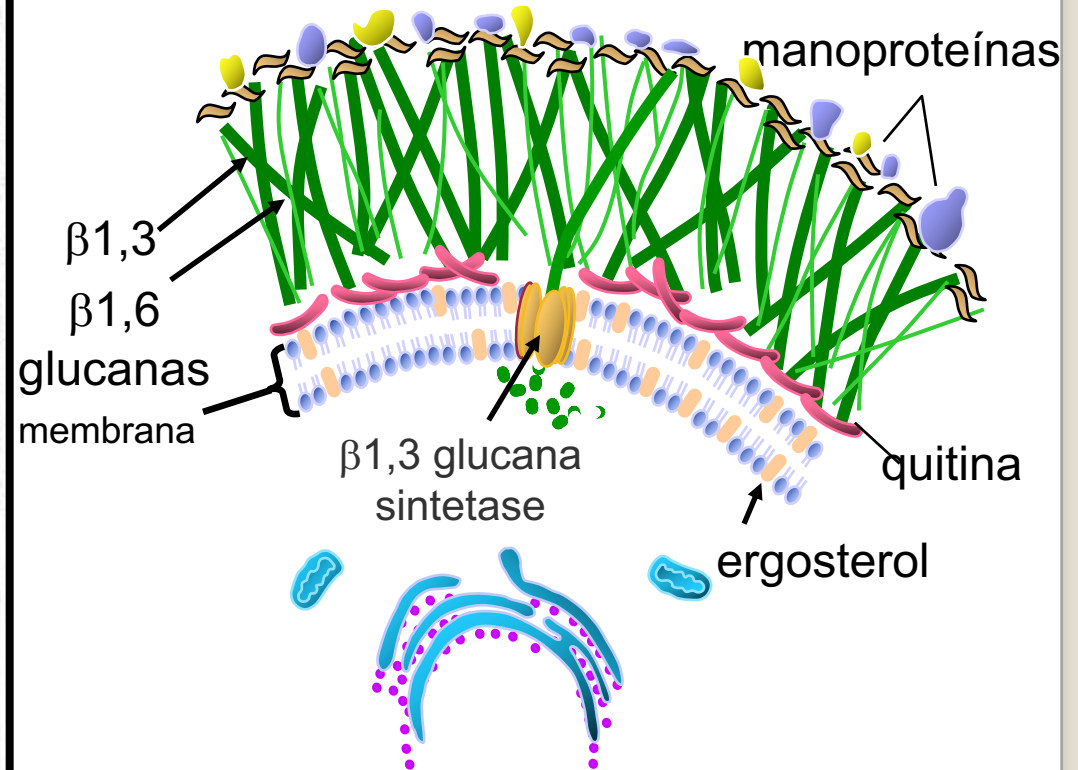
Antifúngicos que agem na síntese da parede celular



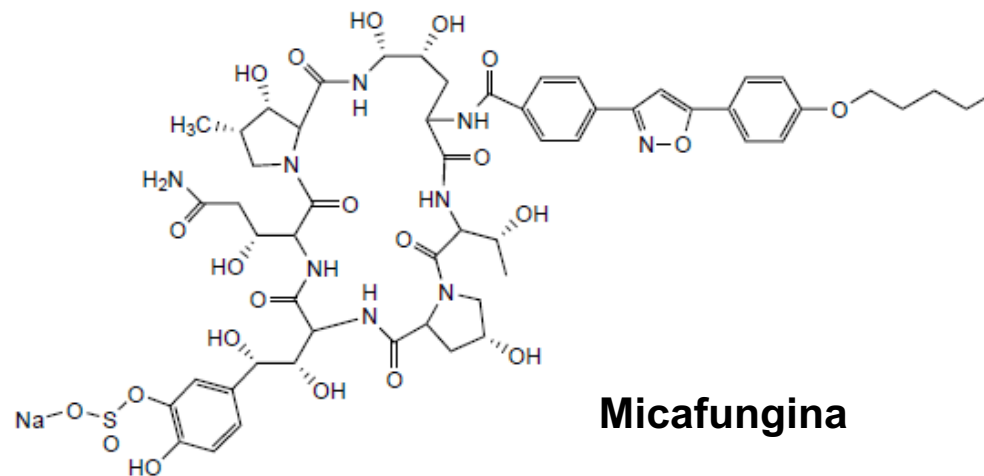
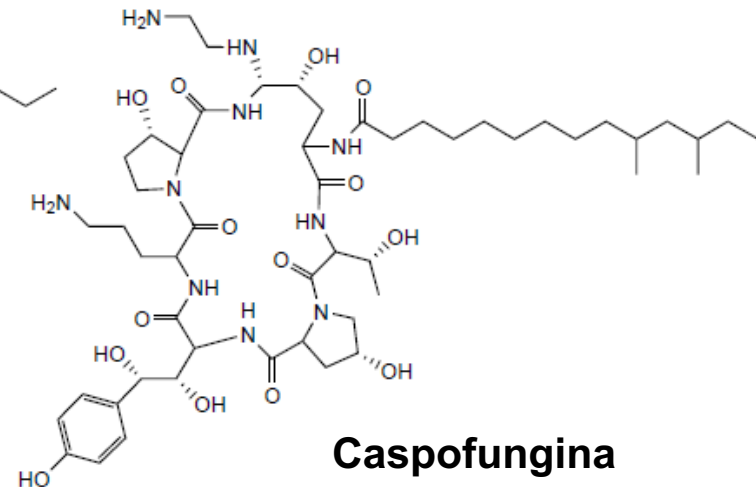
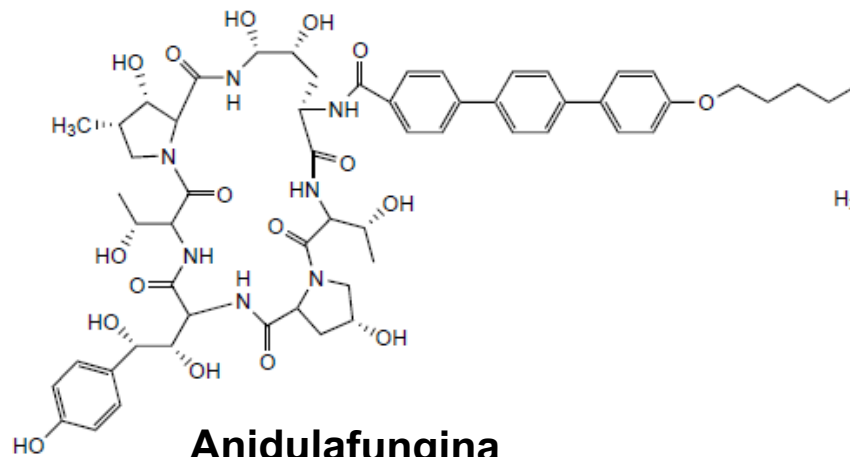
Parede celular

- Equinocandinas
 - Caspofungina
 - Micafungina
 - Anidulafungina
 - Aminocandina*

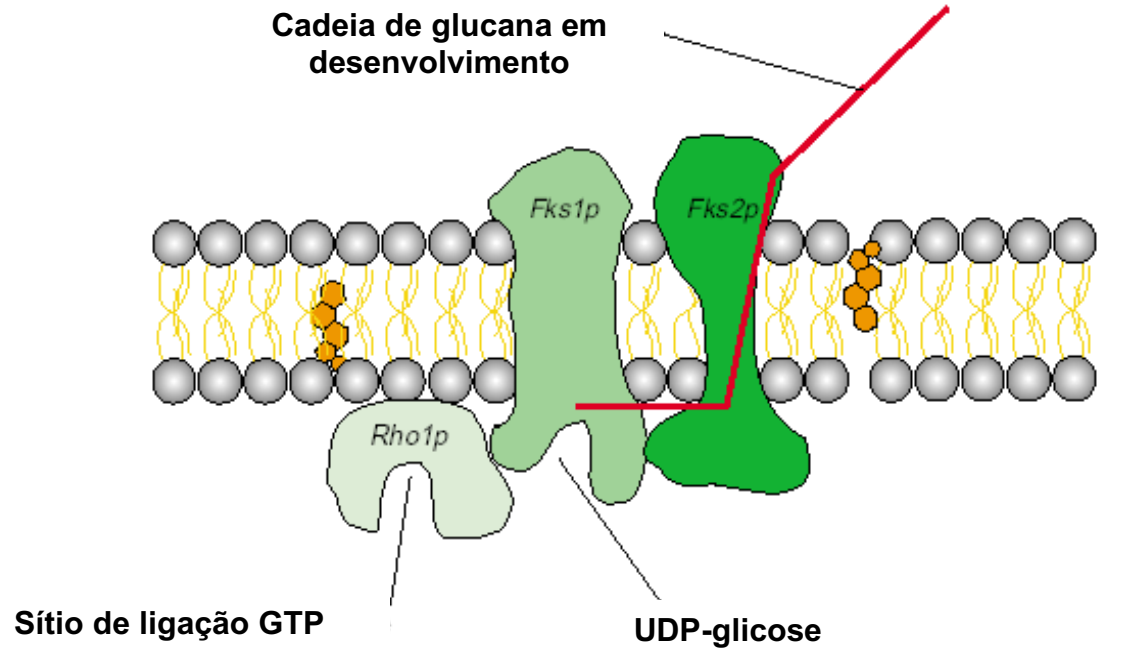
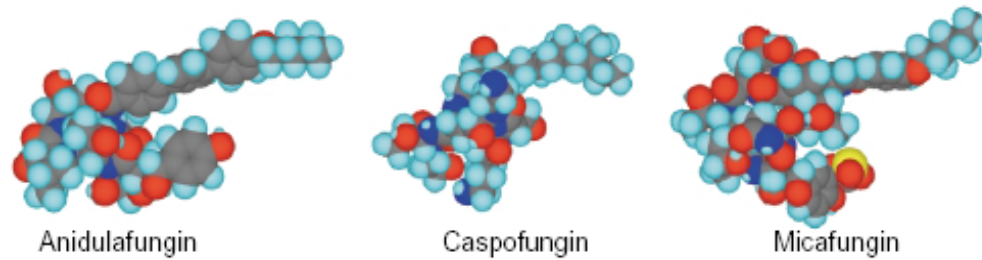
Pared celular fúngica



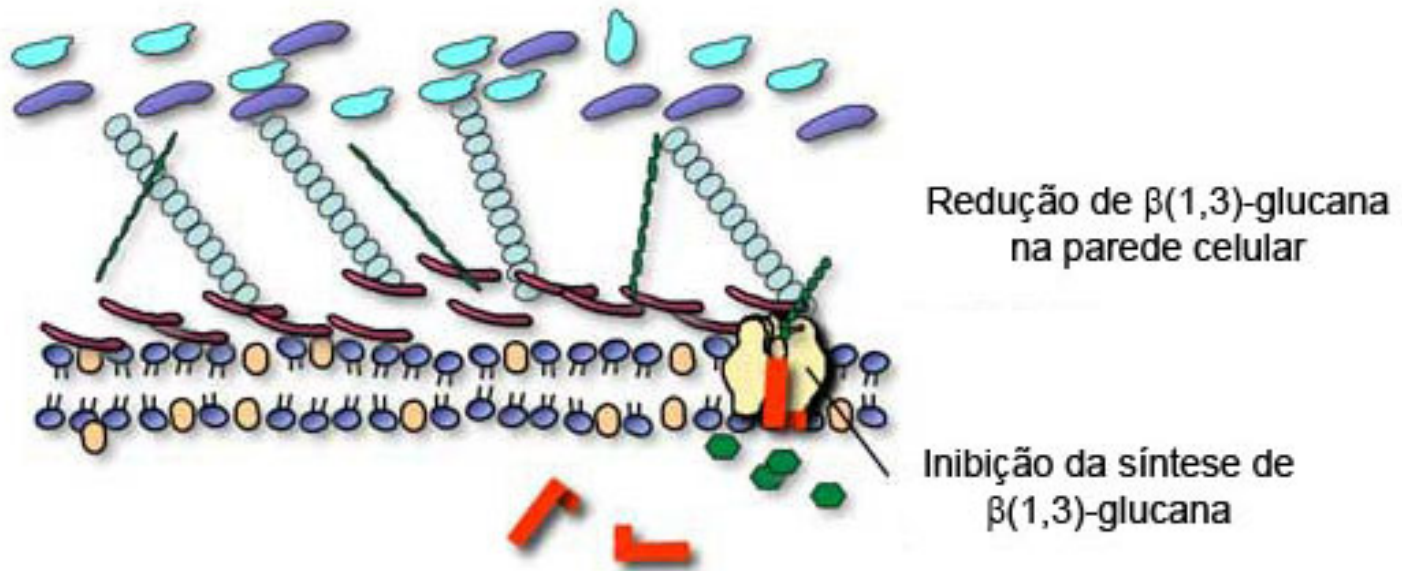
Agentes lipopeptídico: peptídio cíclico + cadeia de ácido graxo



Inibidores não-competitivos



TRENDS in Microbiology



Perda da integridade da parede celular

Fragilidade Osmótica

Efeito fungicida

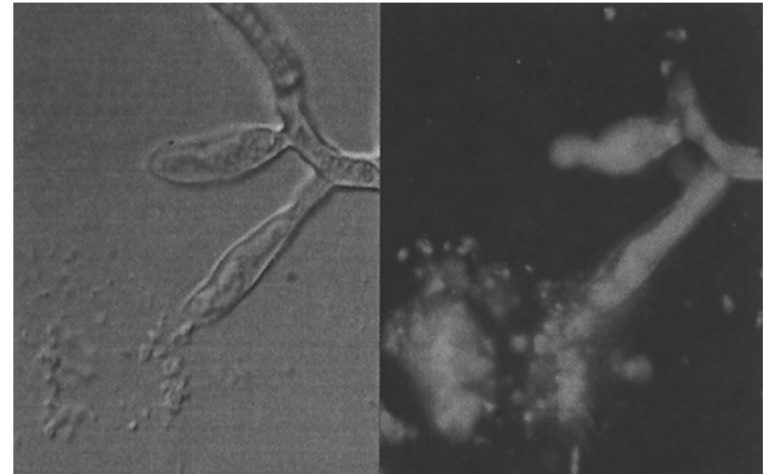
Ativos contra:

***Candida* spp. - fungicida**

e *Aspergillus* spp. - fungistático

Indicação: candidíase esofágica,
candidemia, candidíase invasiva,
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.

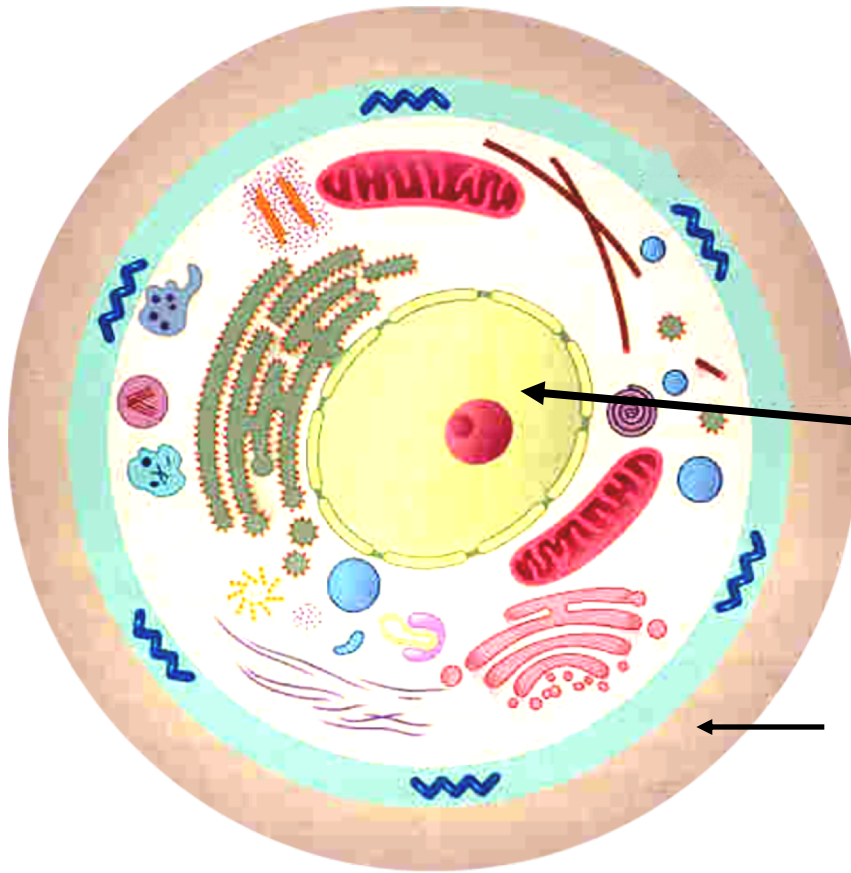
Pouca interação com outras drogas

Efeitos adversos: gastrointestinais

Não apresenta resistência cruzada os agentes azólicos

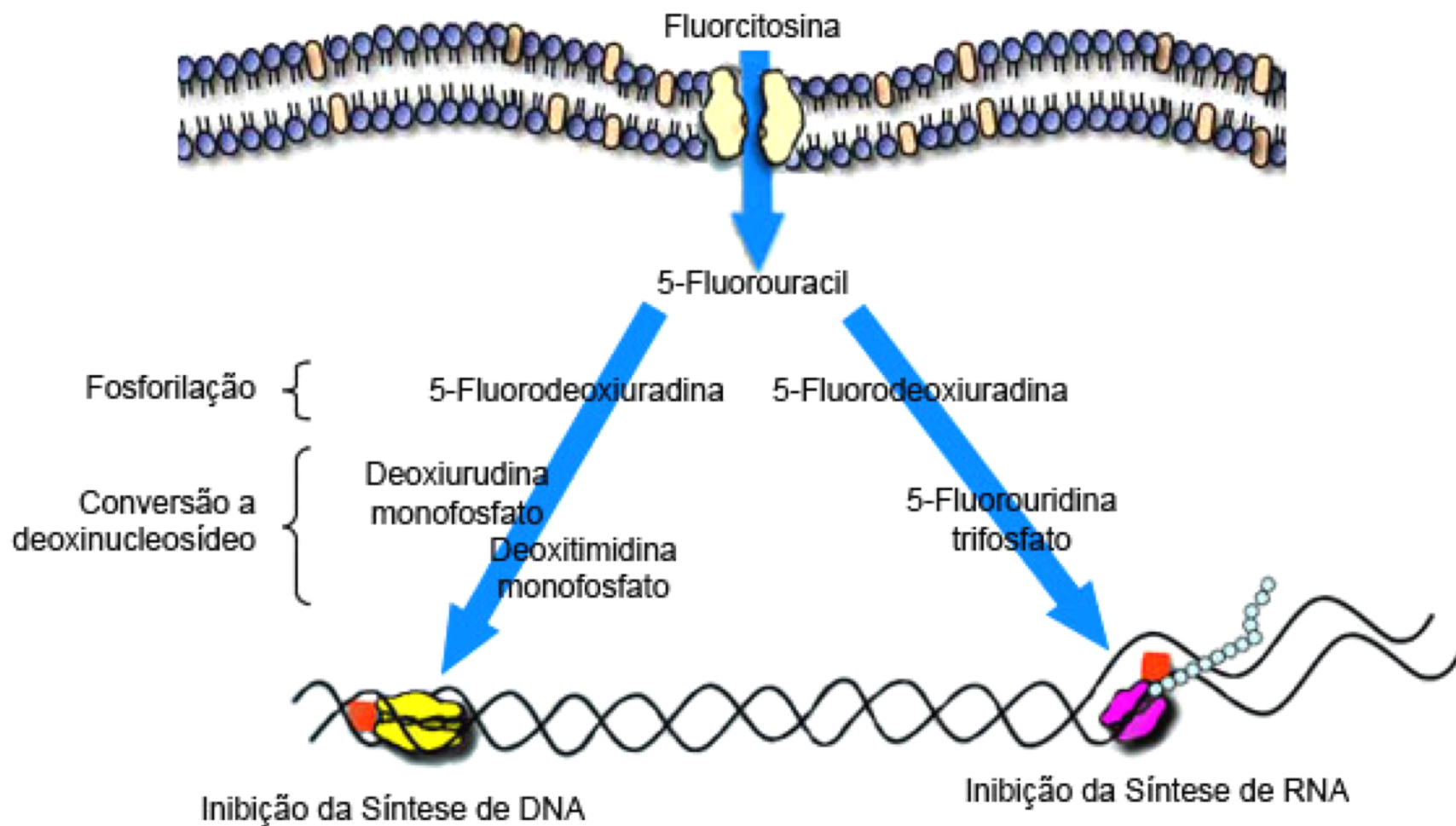
Caspofungina, Anidulafungina e Micafungina - São
similares - eficácia e segurança

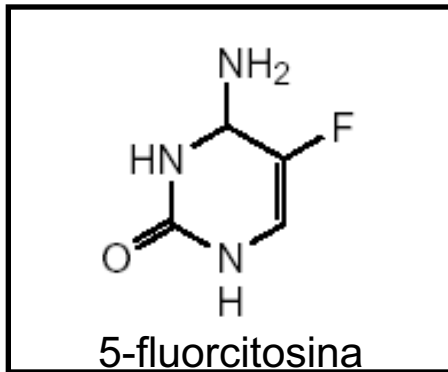
Antifúngico que inibe a síntese de ácidos nucleicos



Síntese DNA/RNA

- Análogos de Pirimidina
 - 5- Flucitosina (5-FC)



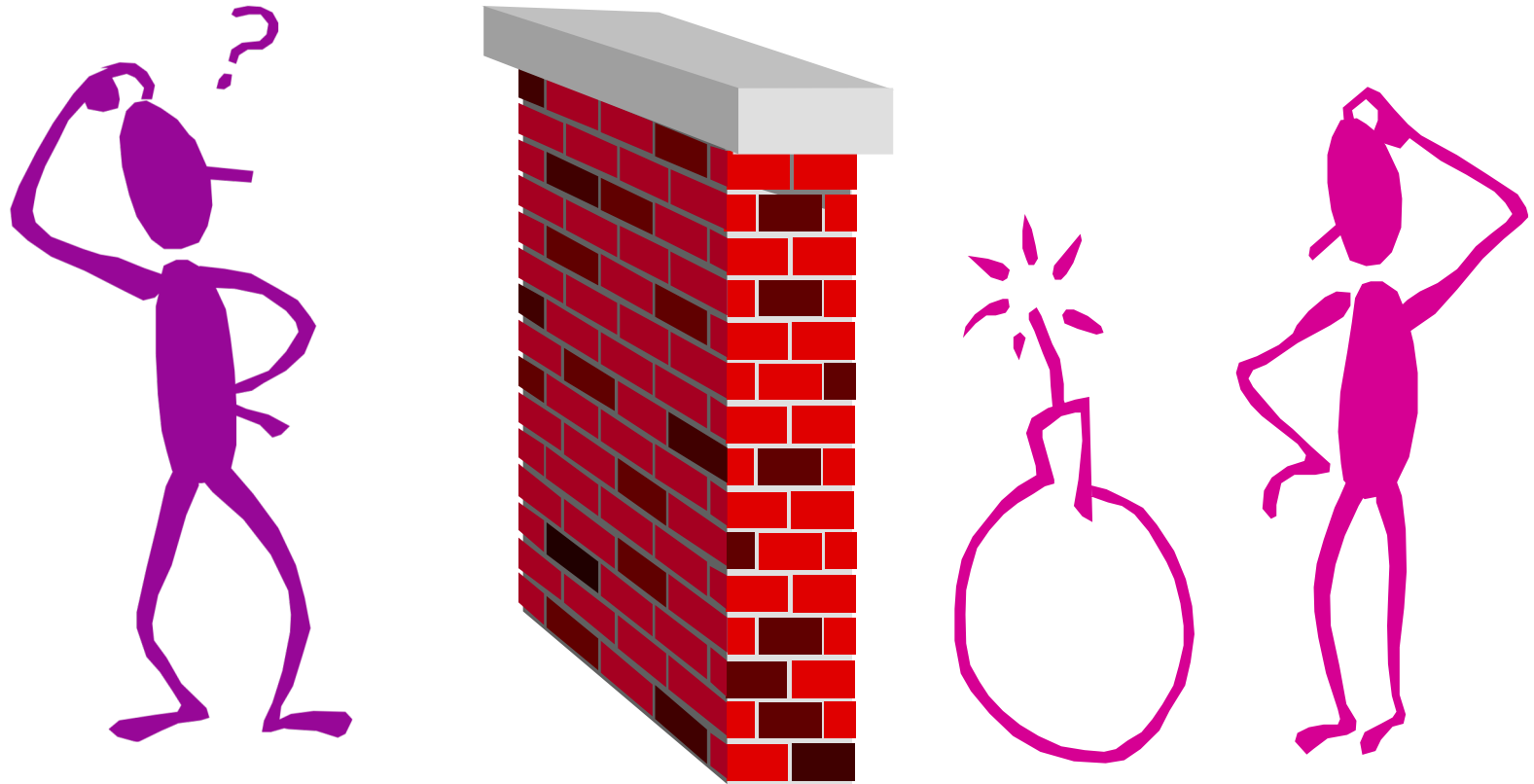


Sintetizada em 1957 como agente anti-tumoral

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

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 ($\mu\text{g/mL}$)
- **CFM:** menor concentração do antifúngico necessária para matar o fungo ($\mu\text{g/mL}$)
 - Fungicida x Fungistático

O que pode levar à resistência ?

“ O antimicrobiano não induz à resistência”

**O que ocorre é a seleção natural de
microorganismos 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

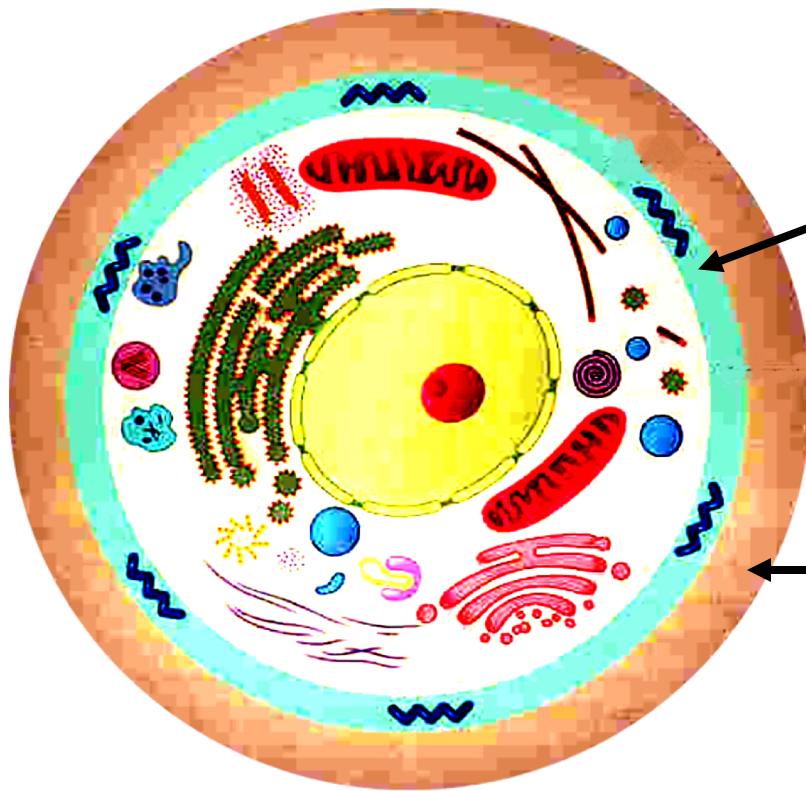
- **Resistência microbiológica** – relacionada com características do fungo
 - Primária = Intrínstica (*C. krusei* ao fluconazol)
 - Secundária = Extrínstica (*C. albicans* ao fluconazol)

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 susceptibilidade à droga 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 intrínseca: *C. lusitaniae*

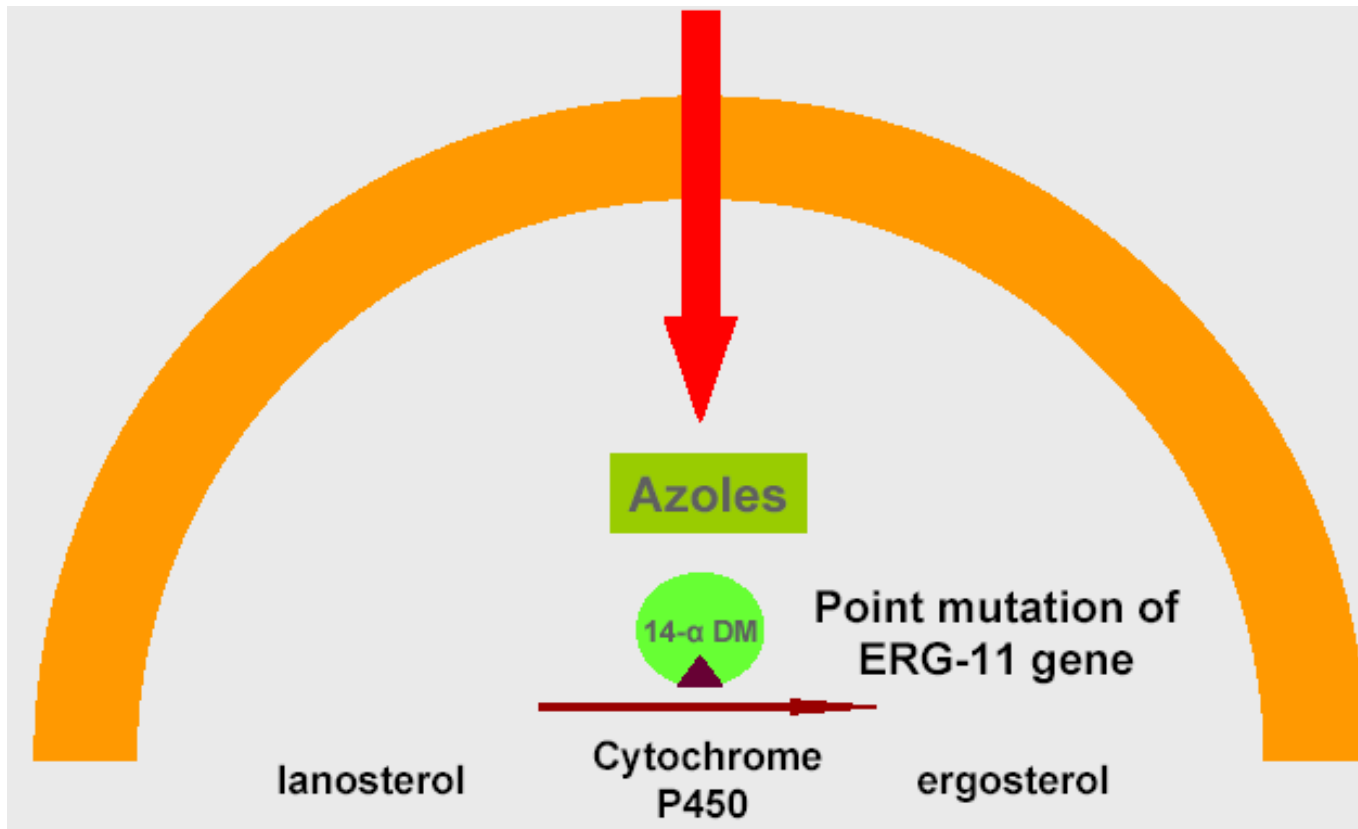
Resistência secundária: *C. albicans*, *C. tropicalis*, *C. glabrata* e *C. krusei* e outros

Resistência é rara.

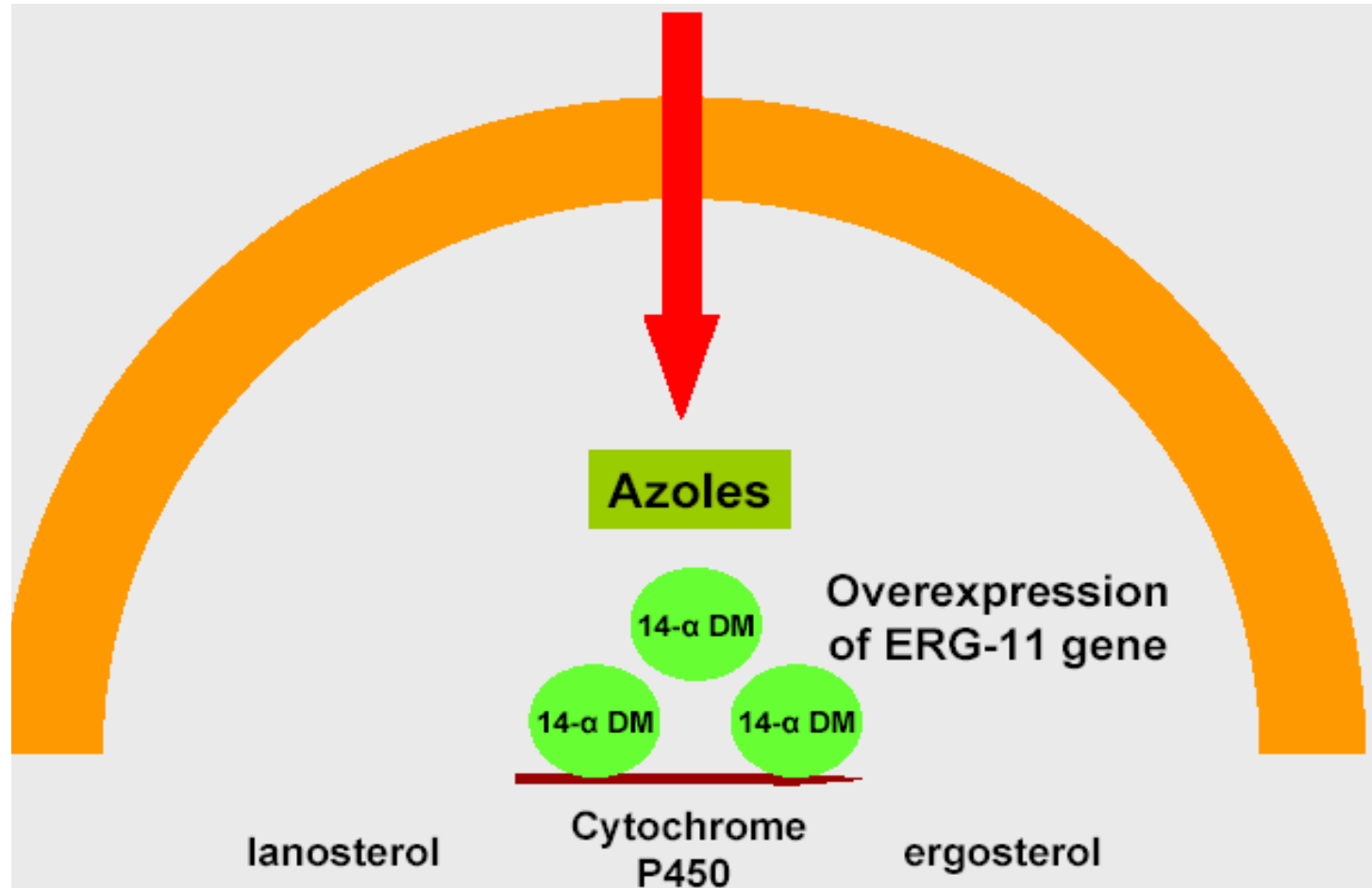
Resistência *Candida* spp. CIM>1µg/mL

Mecanismos de resistência aos azóis

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

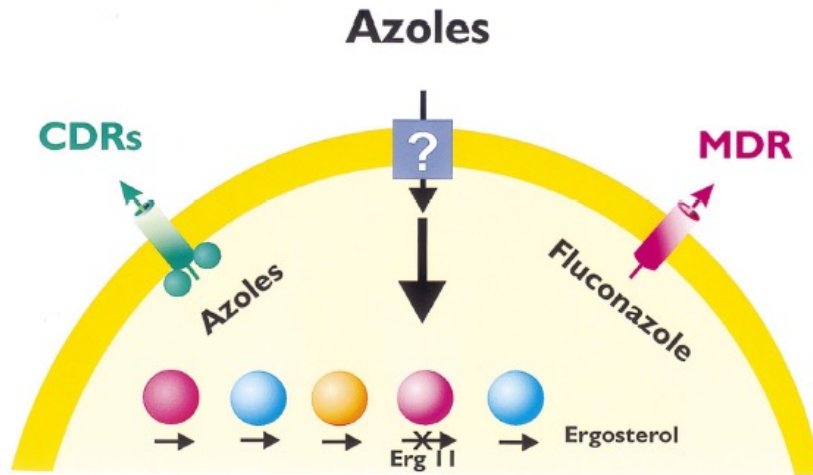


Aumento da expressão da C14- α - lanosterol demetilase

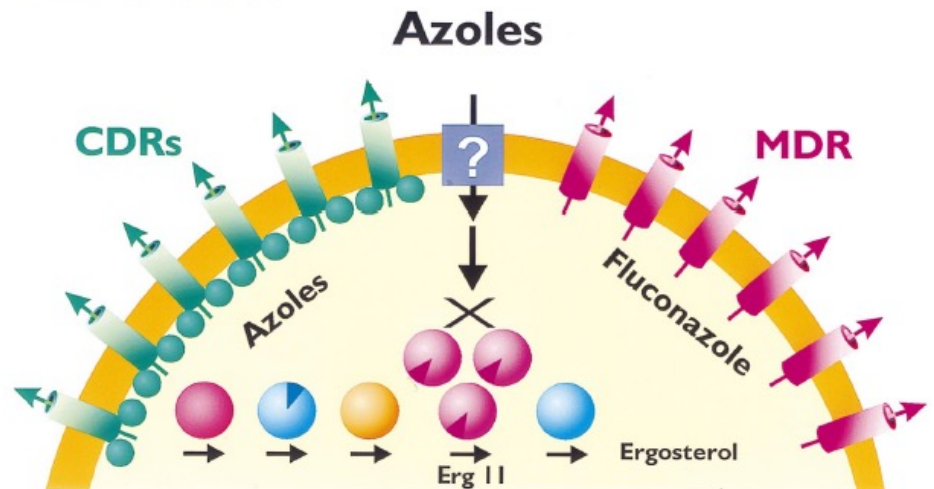


Aumento de bombas de eflujo

SUSCEPTIBLE

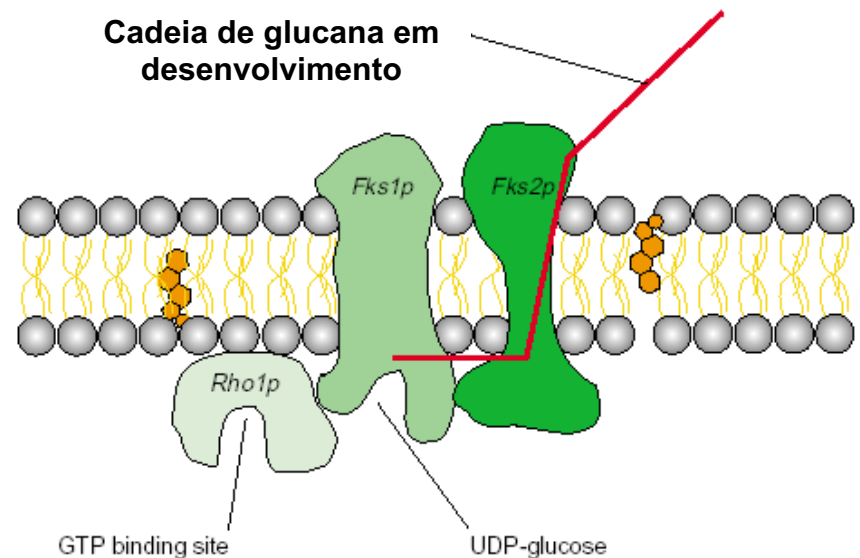


RESISTANT



Resistência às equinocandinas

- *C. parapsilosis* – menor suscetibilidade
- Aumento dos casos de resistência (~14% para *C. glabrata*)-dados de 2014
- Mecanismos:
 - Mutação no gene FKS1p (β 1,3 glucana sintetase)
 - Bomba de efluxo CDR1 e CDR2 (*C. glabrata*)



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*)

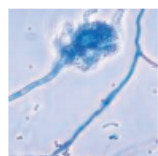
Existe relação entre a utilização de fungicidas na agricultura na seleção de fungos patógenos humanos resistentes aos agentes azólicos?



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



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

human population, this would be the first time a similar link is found between farm use of fungicides and human health. 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.

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



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

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



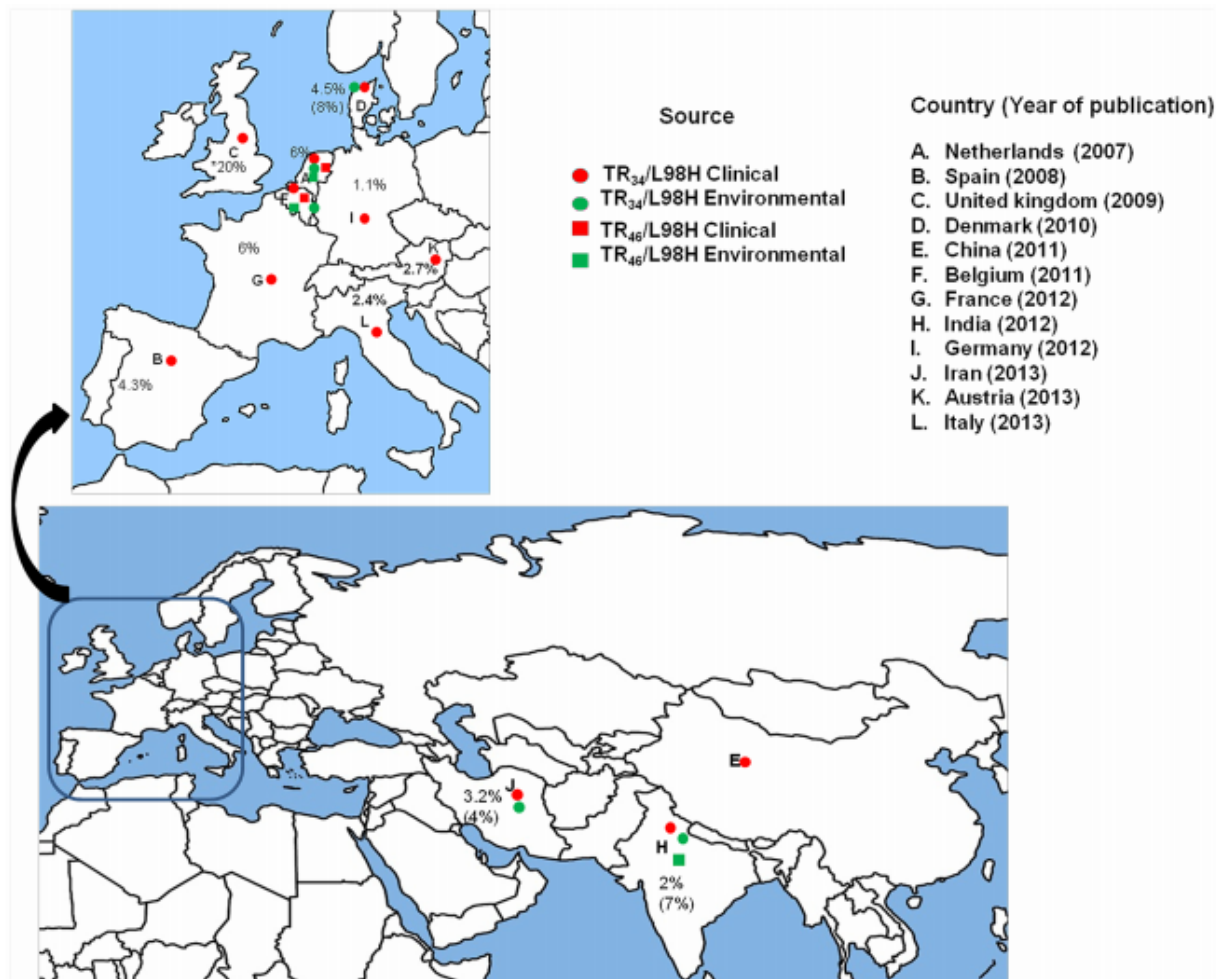


Figure 2. A global map depicting geographic distribution of multi-triazole-resistant clinical (red) and environmental (green) *Aspergillus fumigatus* strains carrying the TR₃₄/L98H (circle) and the TR₄₆/Y121F/T289A mutations (square). Countrywide prevalence rates (%) of *A. fumigatus* carrying TR₃₄/L98H 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?

M27-A3
Vol. 28 No. 14
Replaces M27-A2
Vol. 22 No. 15

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition

This document addresses the selection and preparation of antifungal agents; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.



Comitês

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

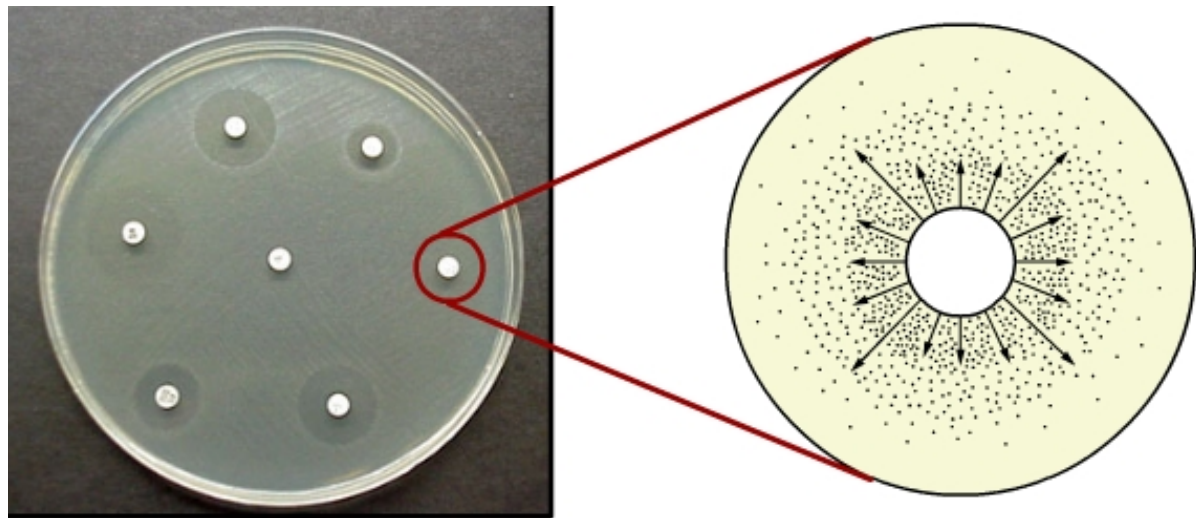
1. Padronização das técnicas
2. Critérios para interpretação dos resultados
3. Parâmetros para o controle qualidade

- Testes de susceptibilidade “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: $1-5 \times 10^6$ UFC/mL
2. Ágar Muller-Hinton
2. Discos de papel impregnados com antifúngicos:
Fluconazol – 40 μ g



E teste® - bioMérieux

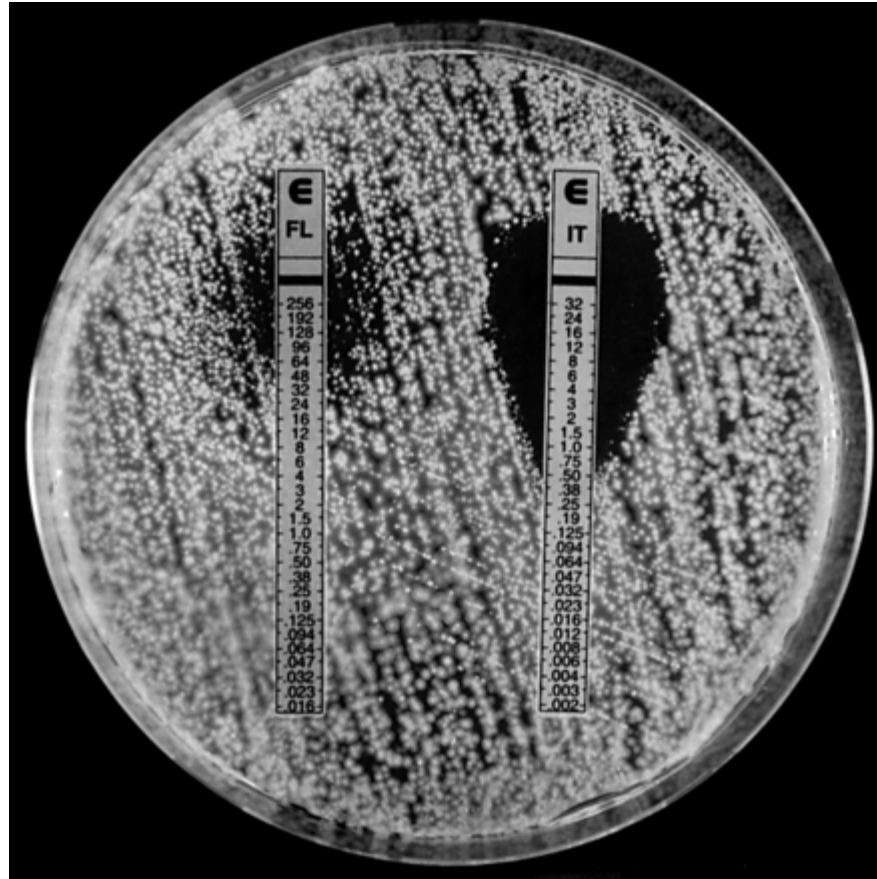


Figure 1

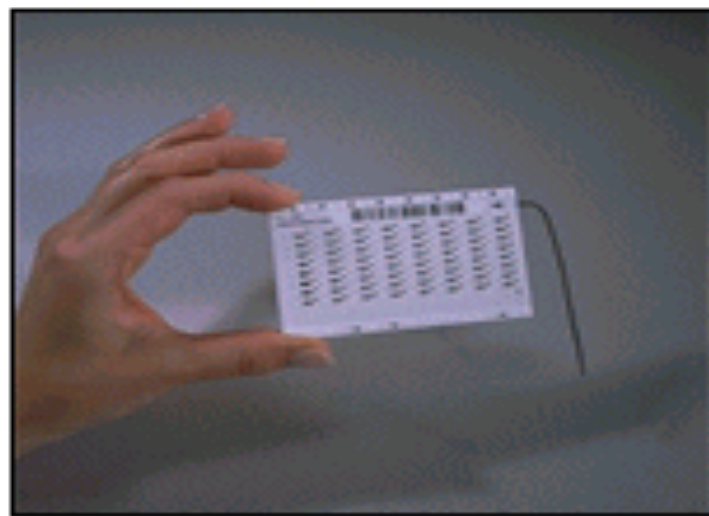
*Fluconazole (FL): change of morphology at endpoint. MIC, 48 mg/litre;
itraconazole (IT): sharp end point. MIC, 0.5 mg/litre.*

- 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



Automação

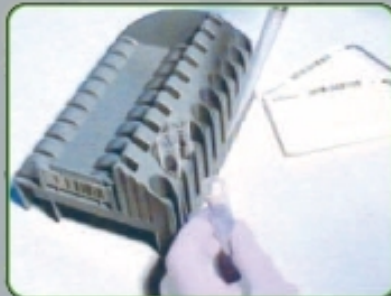
Identificação e testes de susceptibilidade e resistência



Vitek 2 bioMérieux



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.

Sensititre YeastOne (TREK Diagnostic Systems)



Referências

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