IDENTIFYING Fungal Appropriate Fungal Treatments: A Look at Antifungal Susceptibility Testing

Clinical susceptibility testing of fungal isolates is a useful tool for proper treatment of fungal infections. Clinicians should be aware of qualitative and quantitative susceptibility testing methods as well as automated and commercial testing. This issue of CLIPs briefly summarizes an article that addresses the most common antifungal susceptibility tests, in addition to other pertinent antifungal susceptibility testing factors that can affect therapeutic patient care decisions. If you need further information, please contact the Samford University Drug Information Service at (205) 726-2659.


Review of Antifungal Agents:

<table>
<thead>
<tr>
<th>Site of Action or MOA</th>
<th>Drug Names</th>
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<tbody>
<tr>
<td>Intracellular; Distinct MOA</td>
<td>griseofulvin; flucytosine</td>
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<tr>
<td>Cell Wall; Synthesis Inhibition of β-(1,3)-D-glucan</td>
<td>micafungin, caspofungin, anidulafungin</td>
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<tr>
<td>Cell Wall; Synthesis Inhibition of 14-α-demethylase</td>
<td>ketoconazole, fluconazole, itraconazole, voriconazole, posaconazole.</td>
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<tr>
<td>Cell Membrane; binds ergosterol &amp; ↑ membrane permeability</td>
<td>amphotericin</td>
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<tr>
<td>Enzymatic Inhibition of squalene monoxygenase</td>
<td>allylamine: terbinafine</td>
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MOA-mechanism of action

Provoking Factors for Use of Antifungal Susceptibility Testing:

- **Candida** Resistance: varies depending on the site of action of the agent
  - Azoles: induction of efflux pumps, alteration of target site (via gene mutation), increased enzyme expression, pathway alterations, biofilm production and alteration of mitochondrial function.
  - Polyenes: decrease ergosterol
  - Echinocandins: gene mutation
- Epidemiological data indicating rise in prevalence of fungal infections:
  - **Candida** species (i.e., are most prevalent fungi in the inpatient setting (70-90% of infections of fungal type) and prevalence is on the rise.
  - In an analysis of 24,179 cases of nosocomial infections of the blood **Candida** species had a crude mortality rate of 39.2% when compared to bacterial infections. In the same study **Candida** species were listed as the 4th major contributor of bloodstream infections among all organisms.
  - It is postulated that the collective increase in fungal resistance has been precipitated by antifungal overuse as well as an increase in broad spectrum antibiotic use, age of the general population, organ transplantation processes, and geographical factors.

Challenges of Antifungal Susceptibility Testing

- Cultivating fungal isolates can be difficult due to slow growth habits and the need for ideal conditions.
- Lack of standardization of testing methods.
- Availability of testing supplies in health systems

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Susceptibility Testing Methods:

- Two Types: results reported as susceptible, susceptible-dose dependent/intermediate, and resistant.
  1. Qualitative: Kirby-Bauer method – an agar plate that is inoculated with an organism has antimicrobial filled disks placed upon it. Drug then travels down a concentration gradient, leading to a growth inhibition circle (also known as zone of inhibition). The size of the circle of inhibition is dependent upon organism drug susceptibility. The circle is measured and the results are compared to ranges that indicate whether the organism is resistant or susceptible to the drug.
    - Considerations: If the diameter of the inhibition zone is measured improperly, susceptibility may be skewed, therefore it is important to consider:
      - Status of the fungus: fungistatic or fungicidal
      - Colony growth in the zone of inhibition
  2. Quantitative: valued as standard methods, broth microdilution or macrodilution producing a minimum inhibitory concentration (MIC with units in mcg/ml). Each method has an incubation period.
    - Macrodilution: consists of 1 ml test tubes with varying concentrations of an antifungal.
    - Microdilution: now used more than macrodilution, includes the use of a plate with 96 reservoirs each holding 200 microliters per container and a different concentration of antifungal.
    - Considerations for both quantitative measures:
      - Breakpoint: FDA approved data-driven MIC’s that distinguish between wild-type microbes and microbes that are resistant. Laboratory automated testing systems must use the FDA approved MIC (breakpoint).

Available Susceptibility Guidelines

- Guidelines of susceptibility testing are available from a global, not for profit, organization called Clinical Laboratory Standards Institute (CLSI). Guidelines are available for the following:
  - Candida species: susceptibility guidelines from CLSI are provided for fluconazole, vorconazole, itraconazole, flucytosine and echinocandins. No CLSI guidelines exist regarding amphotericin.
  - Aspergillus sp., Fusarium sp, Rhizopus oryzae, Trichophyton and dermatophytes (molds): amphotericin B, caspofungin, itraconazole, posaconazole and voriconazole all have susceptibility guidelines available.
- Methods of commercial susceptibility tests are not necessarily in the guidelines.

Commercial Susceptibility Tests with FDA Approval:

1. Vitek 2 by bioMeriux
2. Yeastone by Trek Diagnostics
   - Each of the Vitek and Yeastone commercial tests are capable of susceptibility testing for fluconazole, caspofungin, and voriconazole regarding Candida.
   - In addition, Yeastone can be used to test other antifungal susceptibility.
3. Etest by bioMeriux: capable of testing for fluconazole, flucytosine, itraconazole, and voriconazole.

Summary:

- Antifungal susceptibility testing has great use in the clinical setting for treatment and reduction of resistance.
- Although there are factors such as costs and supplies that hinder the possibility of susceptibility testing in health systems, there is incentive to implementing such a system.
- Testing onsite rather than offline, decreases turn-around time from >7 days to 3 days, which in turn can optimize patient therapy and decrease the use of unnecessary treatments, which potentially lowers the chance for resistance.
- An effort is being made to standardize susceptibility testing and update standards according to epidemiological shifting.
- In the face of healthcare reform that puts a stronger emphasis on quality of care and decreased times of hospitalizations, it is important for clinicians to stay current regarding antifungal susceptibility testing to ensure proper use of antifungal susceptibility results and provide optimum patient care.