Effects of Two Different Growth Media on the Postantifungal Effect Induced by Polyenes on *Candida* Species

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There are no data on the effects of different growth media on polyene-induced postantifungal effect (PAFE) in *Candida* species. Hence, the nystatin- and amphotericin B-induced PAFEs in six *Candida* species (26 isolates) grown in Sabouraud’s dextrose broth (SAB) and RPMI broth were evaluated, following limited exposure to the MICs of the two polyenes, using an automated turbidometric method. For nystatin, PAFE varied between 1.88 and 4.87 h in SAB and 0.66 and 7.01 h in RPMI, and for amphotericin B, the equivalent values were 3.13 to 10.98 h in SAB and 0.97 to 7.01 h in RPMI. These highly significant (*P* < 0.001) variations in the PAFE with both drugs, noted with most *Candida* strains grown in different media, call for standardization of intralaboratory methodology in measuring this parameter in order to obtain universally comparable data.

Opportunistic oral infections caused by both *Candida albicans* and non-*C. albicans* *Candida* species are becoming increasingly common in immunocompromised patients. For instance, the vast majority of human immunodeficiency virus-infected patients suffer from oral candidosis, the most common AIDS-associated oral infection, during the course of their disease (1).

Nystatin and amphotericin B, which belong to the polyene group of antimycotics, are common therapeutic agents for oropharyngeal candidosis (5). Nystatin is widely used as a topical agent in the management of oral candidosis, and oral application of this antimycotic can also be of considerable benefit in preventing the systemic spread of oral candidosis in immunocompromised persons (5). Though not popular, topical amphotericin B oral preparations are available and used by some clinicians for the management of oral candidosis (5). However, due to the diluent effect of saliva and the cleansing effect of the oral musculature, their availability tends to fall below the effective therapeutic concentrations and the organisms undergo only a brief exposure to antifungal agents (7). Hence, the term “postantifungal effect” (PAFE) has been used in recent years to describe the suppression of fungal growth that persists after brief exposure of organisms to the antifungal agent in question. PAFE could be used as a secondary benchmark in determining the antifungal activity of an antimycotic in addition to the conventional MIC measurement. It may also have therapeutic relevance in determining the antifungal dosing regimens in a clinical setting (2).

There are only a few reports on the PAFE of polyene antifungal agents, particularly with non-*C. albicans* *Candida* isolates. These studies to determine the PAFE have essentially investigated the interactions of a few isolates with mainly a single polyene agent (4, 10, 11). There is also no comprehensive information on amphotericin B-induced PAFE on different oral *Candida* species or the impact of growth media on the PAFE. Hence, the aim of this study was to compare the PAFE on oral isolates of *Candida* belonging to six different species following limited exposure to nystatin and amphotericin B in two commonly used growth media, namely, Sabouraud’s dextrose broth (SAB) and RPMI 1640 broth.

A total of 26 *Candida* isolates were studied: four isolates each of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, and *C. guilliermondii*, plus *C. albicans* ATCC 90028 and *C. tropicalis* ATCC 13803 used as the reference laboratory strains. All isolates were identified using API-20C AUX (BioMérieux, Basingstoke, United Kingdom). Stock cultures were maintained at −70°C. After recovery, these were maintained on Sabouraud’s dextrose agar and stored at 4 to 6°C during the experimental period.

Nystatin and amphotericin B (both from Sigma, St. Louis, Mo.) were dissolved in a mixture of dimethyl sulfoxide (DMSO) and absolute ethanol (3:2 ratio), respectively. They were prepared initially as 2,000 µg/ml solutions and stored at −70°C before use. Since the antifungal agents used were dissolved in DMSO and absolute ethanol, equivalent amounts of the latter chemicals were tested to ascertain whether they had an effect on the isolates tested. The minute volumes of the chemicals used did not have any effect on yeast growth compared with the controls.

SAB (Oxoid, Unipath Ltd, Basingstoke, Hampshire, England) was prepared with double-distilled, sterile water. RPMI 1640 medium, buffered with 0.165 M MOPS [3-(N-morpholino)propanesulfonic acid] containing L-glutamine and lacking sodium bicarbonate (Gibco BRL Products, Life Technology, Gaithersburg, Md.), was dissolved in 1 liter of sterile distilled water, adjusted to pH 7.2, and filter sterilized.

MICs of nystatin and amphotericin B for each isolate were determined in duplicate using the twofold broth microdilution technique as outlined by the National Committee for Clinical Laboratory Standards (9). Both RPMI 1640 and SAB were used to determine the MIC. In brief, 10 µl of the 1 × 10³- to 5 × 10³-CFU/ml inoculum of the cell suspension was inoculated into each well of a 96-well microplate containing 150 µl of medium with a twofold-diluted concentration of the drug. The MIC was read as the highest dilution of the drug that
inhibited growth after 24 h of incubation at 37°C in a shaking incubator (Lab-Line).

For the PAFE assay, yeast cells maintained on Sabouraud’s dextrose agar were inoculated onto fresh plates and incubated overnight prior to use. The organisms were harvested, and a cell suspension was prepared in 0.15 M phosphate-buffered saline (pH 7.2; PBS) to an optical density at 520 nm of 1.5. From this cell suspension, 0.5 ml was added to tubes containing 2 ml of medium (control) and 2 ml of medium-drug solution (test): the drug concentration was the MIC of the drug. This gave a cell suspension of 10^6 to 10^7 cells/ml in each assay tube.

The control and test tubes were then incubated at 37°C for 1 h in a shaker incubator. Following this procedure, the drug that was carried over and contaminating the yeast cells was removed 10,000-fold (2), thereby greatly minimizing any carryover effect.

As demonstrated previously (3, 4, 6), growth curves revealed a period of fungistasis after removal of the polyene antimycotics, illustrating a PAFE, in both SAB and RPMI broth (Fig. 1). A significant interspecies variation in polyene-induced PAFE was also observed (Table 1). For instance, the mean values of amphotericin B-induced PAFE in SAB ranged from 3.13 (for C. tropicalis) to 10.98 h (for C. tropicalis), whereas equivalent figures for amphotericin B were 3.13 to 10.98 h in SAB and 0.97 to 7.01 h in RPMI broth (Table 1).

### Table 1. Mean PAFE of nystatin and amphotericin B on Candida species in SAB and RPMI 1640 broth

<table>
<thead>
<tr>
<th>Species (no. of isolates)</th>
<th>Nystatin</th>
<th>Amphotericin B</th>
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<tbody>
<tr>
<td></td>
<td>SAB</td>
<td>RPMI</td>
</tr>
<tr>
<td>C. albicans (5)</td>
<td>1.88 (0.08)</td>
<td>3.09 (0.24)</td>
</tr>
<tr>
<td>C. glabrata (4)</td>
<td>2.63 (0.08)</td>
<td>3.39 (0.26)</td>
</tr>
<tr>
<td>C. guilliermondii (4)</td>
<td>3.70 (0.37)</td>
<td>1.71 (0.21)</td>
</tr>
<tr>
<td>C. krusei (4)</td>
<td>4.87 (0.18)</td>
<td>6.89 (0.33)</td>
</tr>
<tr>
<td>C. parapsilosis (4)</td>
<td>3.18 (0.20)</td>
<td>5.64 (0.94)</td>
</tr>
<tr>
<td>C. tropicalis (5)</td>
<td>3.14 (0.24)</td>
<td>0.66 (0.08)</td>
</tr>
</tbody>
</table>

*In hours. Data are means for three experiments conducted on separate occasions in duplicate for each isolate (i.e., means of 30 raw values for C. albicans and C. tropicalis and 24 values for the other species).*

Although a reasonable database on the PAFE of the major antifungals against a number of Candida species is available (3, 4, 6), the impact of different growth media on the PAFE of these antifungicals has not been studied. The media used in our study, namely, SAB and RPMI 1640 broth, differ in their composition and their pH. SAB contains pancreatic digest of casein, peptic digest of fresh meat, and dextrose, and its pH is 5.7 (Oxoid manual). RPMI 1640 broth is a chemically defined medium.
medium which contains a range of amino acids, vitamins, salt, and glucose, and its pH is 7.2 (Gibco BRL product guide). SAB is the recommended supporting medium for laboratory culture of fungi and has been so used for decades (2). RPMI 1640, on the other hand, was originally formulated for suspension cultures or monolayer cultures of human leukemic cells. It was subsequently recommended as the medium of choice for antifungal susceptibility testing by the National Committee for Clinical Laboratory Standards (9). Both these media are used extensively in mycological research, especially in studies of Candida.

Our results indicate clearly that the growth patterns of the isolates in SAB and RPMI 1640 broth were variable and elicited different PAFE values depending on the medium, even with the same Candida isolate. In general, Candida growth was extensive in SAB, whereas it was comparatively low in RPMI 1640. One reason for this may be the limited nutritional sources in RPMI 1640 broth compared with SAB. Despite the differences in the media, a PAFE was observed for all isolates following brief exposure to the polyenes, and this is likely to be due to the time taken by Candida to recover before active multiplication after such exposure. Similar polyene-induced PAFE values have been observed, particularly with C. albicans, in previous studies (3, 4, 6). However, this study is the first to report the significant differences in the polyene-induced PAFE on a battery of six different Candida species in different growth media.

The present results therefore clearly illustrate that standardization of laboratory regimens is essential in order to obtain globally comparable data on PAFE of antifungal agents. As only a few reports from a handful of laboratories on the subject of PAFE are yet available, authorities such as the NCCLS should take early steps to design and issue guidelines (akin to that for MIC measurement of antifungals) that could be universally used in evaluation of the PAFE and to generate data that are globally acceptable.

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REFERENCES


