In vitro antifungal effects of some chemotherapeutic agents against fungi commonly isolated from repeat breeder animals

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Abstract: The sensitivity of fungi isolated from the female genital organs with or without clinical signs of farm animals that failed to conceive after being bred with fertile males more than two times, to some antifungal agents was determined. Seven antifungal therapeutic agents were tested against seventeen fungal isolates from repeat breeder cows (6 isolates), buffaloes (6) and mares (5) using the disc diffusion method. The most effective antimitotic agents were nystatin followed by terbinafine, ketoconazole, miconazole, flucanazole and povidine iodine. Griseofulvin, on the other hand, was not effective against any of the fungi tested. The minimum inhibitory concentration of nystatin ranged from 156.25 to 1250 IU/ml (31.0 - 250 µg/ml), terbinafine from 78.125 µg/ml to 5 mg/ml, ketoconazole from 156.25 µg/ml to 5 mg/ml, miconazole from 1.25 to 10 mg/ml and flucanazole from 2.5 to 10 mg/ml. The MICs of povidine iodine (betadine antiseptic) ranged from 50 to 100 mg/ml. Fungal isolates belonging to Acremonium strictum, Aspergillus flavus, Emericella nidulans and Penicillium chrysogenum showed high sensitivity to terbinafine at concentration ranging from 78.125 to 156.25 µg/ml. Candida albicans was more sensitive to nystatin and ketoconazole than other antifungal agents.

Key words: antifungal therapeutic agents, fungi, cows, buffaloes, mares, repeat breeders.

Introduction

The most prevalently utilized antifungal agents include the polyenes and the azoles. The polyenes are effective by binding to ergosterol, the fungal membrane sterol, resulting in an increased permeability to the cell wall and eventual cell death (Horowitz et al. 1987). The polyenes include amphotericin B, nystatin and natamycin (Carter and Chengappa 1995). The azoles function by inhibition of the cytochrome P-450-mediated removal of the C-14 methyl group from the ergosterol precursor, lanosterol (Vanden Bossche et al. 1987). The azole derivatives include clotrimazole, econazole, ketoconazole, fluconazole and itraconazole of which fluconazole and itraconazole are members of the triazoles with 3 nitrogen molecules, whereas clotrimazole, miconazole and ketoconazole are imidazoles with 2 nitrogen molecules (Carter and Chengappa 1995).

Griseofulvin was found earlier to have no inhibitory action on yeasts (Aller- Gancedo 1978) or species of Candida (Jand et al. 1978), however, it was reported to have effect only on dermatophytes (Sandhu and Randhawa 1964, Karaca and Koc 2004). On the other hand, intraterine infusions or irrigation of animal endometritis and/or cervicitis with povidine-iodine solution showed good results for the recovery and gave negative isolations (Osman and Abou-Gabal 1975, Koujan et al. 1996). Betadine solution was reported to have antimitotic activity against Candida albicans, Proactinomyces ligniteresi, Aspergillus fumigatus, Absidia corymbifera and Mucor pusillus (Kremlev and Banakova 1979) and Candida albicans, Cryptococcus neoformans, Cryptococcus uniguttulatus and Rhodotorula rubra (Theraud et al. 2004).


Nystatin was found to be active against species of Candida and Rhodotorula (Kucharski and Rozewicka 1974, Saxena and Ishaque 1977, Jand et al. 1978, Carrillo-Munoz et al. 2002, Pal 2002) and on Penicillium sp., A. flavus, A. niger, A. fumigatus, Aureobasidium sp., Rhizomucor pusillus, Cladosporium cladosporioides, C. albicans, C. tropicalis, Cryptococcus laurentii, and R. rubra (Sirohi and Khar 2000). Terbinafine (Lamisil) showed also a potent
activity in vitro against A. flavus and A. niger, Penicillium spp., Acremonium spp., Cladosporium spp. and Alternaria alternata (Moore et al. 2001, Garcia-Effron et al. 2004) and was active against fungi from clinical specimens e.g. Rhizopus microsporus, R. oryzae, A. corymbifera, Cunninghamamella bertholletiae, R. pusillus, Mucor ramesissimus and M. circinelloides (Gomez-Lopez et al. 2003), Trichophyton rubrum, T. mentagrophytes, T. tonsurans and T. verrucosum (Karaca and Koc 2004) and C. albicans (Garg et al. 2006). However, Scopulariopsis brevicaulis showed resistance in vitro to terbinafine (Garcia-Effron et al. 2004).

The present work was designed to study the sensitivity of fungal species isolated from the genital organs of farm animals that failed to conceive after being bred with fertile males more than twice to some antifungal agents. Also, the minimum inhibitory concentration for each antifungal agent was determined.

Materials and Methods

Table 1: Antifungal agents used in the sensitivity test

<table>
<thead>
<tr>
<th>No</th>
<th>Trade name</th>
<th>Scientific name (active ingredient)</th>
<th>Formulation</th>
<th>Concentration</th>
<th>Producing company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Betadine</td>
<td>Povidine iodine</td>
<td>Antiseptic solution</td>
<td>10%</td>
<td>Nile Company, Egypt</td>
</tr>
<tr>
<td>2</td>
<td>Fungican</td>
<td>Fluconazole</td>
<td>Capsules</td>
<td>150 mg/cap</td>
<td>Amoun Pharmaceutical Company, Egypt</td>
</tr>
<tr>
<td>3</td>
<td>Lamisil</td>
<td>Terbinafine</td>
<td>Tablets</td>
<td>125 mg/tab</td>
<td>Novartis Pharma S.A.E.</td>
</tr>
<tr>
<td>4</td>
<td>Miconaz</td>
<td>Miconazole nitrate</td>
<td>Powder</td>
<td>2 g/100 g powder</td>
<td>Medical Union Pharmaceutical Company</td>
</tr>
<tr>
<td>5</td>
<td>Nizoral</td>
<td>Ketoconazole</td>
<td>Tablets</td>
<td>200 mg/tab</td>
<td>Janssen Cilag</td>
</tr>
<tr>
<td>6</td>
<td>Nystatin</td>
<td>Nystatin</td>
<td>Oral drops</td>
<td>100.000 I.U/ml</td>
<td>EIPICO (Egyptian International Pharmaceutical Industries Co.)</td>
</tr>
<tr>
<td>7</td>
<td>Ultragriseofulvin</td>
<td>Griseofulvin</td>
<td>Tablets</td>
<td>125 mg/tab</td>
<td>Kahira Pharmaceutical &amp; Chemical Industries Company, Egypt</td>
</tr>
</tbody>
</table>
The sensitivity test

The method described by Cruickshank (1965) was carried out to assess the activity and the minimal inhibitory concentrations of the antifungal agents used. Double-fold serial dilution from each antifungal agent was prepared in its suitable solvents. The filter paper discs were immersed in each concentration of the antifungal solution and left until full saturation. Discs were placed on the surface of Sabouraud dextrose agar (Moss & McQuown, 1969) seeded with the test isolate (5 µl of the fungal suspension prepared by adding 3 ml of sterile distilled water to slant culture). Discs saturated with solvents served as controls. Cultures were incubated at 25°C for 48 hours after which the zones of inhibition of fungal growth around discs were measured in mm (Venugopal and Venugopal 1994). For each fungal isolate the minimum inhibitory concentration (MIC) was determined as the lowest concentration of the antifungal agent that prevents visible fungal growth after its optimal incubation period.

Results and Discussion

Sensitivity of fungal isolates to antifungal agents

Results in Table (2) reveal that nystatin followed by terbinafine and ketoconazole were highly effective on the tested fungal isolates. Nystatin was active against all fungi tested at 1250 IU/ml (250 µg/ml) concentration. The MIC of nystatin on Alternaria alternata (isolated from cow) was 156.25 IU/ml (=31.25 µg/ml) but, on A. flavus, A. niger, P. chrysogenum and Rhodotorula mucilaginosa (from cows), Aphanoascus fulvescenes, A. flavus, A. niger, Chrysosporium tropicum (from buffaloes) and C. albicans, C. cladosporioides and Rhodotorula mucilaginosa (from mares) was 625 IU/ml (=125 µg/ml). However, the MIC of nystatin was much higher (1250 IU/ml) (=250 µg/ml) on A. strictum (from cow), C. albicans and E. nidulans (from buffaloes) and P. chrysogenum and Scopulariopsis brevicaulis (from mares). In this respect, Sirohi and Khar (2000) found the MIC of mycostatin on Penicillium sp. and Cryptococcus laurentii was 25 IU/ml, Aspergillus niger, A. fumigatus, Aureobasidium sp., C. albicans and C. tropicalis was 50 IU/ml and A. flavus, Rhizomucor pusillus, C. cladosporioides and R. rubra was 100 IU/ml. Besbes et al. (2002) found also that nystatin was effective against S. brevicaulis from otomycosis. In vitro studies of Kucharski and Rozewicka (1974), Jand et al. (1978), Carrillo-Munoz et al. (2002), Pal (2002) and Munguia and Daniel (2008) revealed also the sensitivity of C. albicans to mycostatin. Also, nystatin at 1.42 µg/ml was active on all strains of Candida tested by Aller Gancedo (1978). However, it was slightly inhibitory to C. albicans from repeat breeding bovines (Saxena and Ishaque 1977).

Terbinafine (lamisil) at 5 mg/ml was active against all fungi tested in the present study. The MIC of terbinafine on A. strictum and A. flavus (from cows), A. flavus and E. nidulans (from buffaloes) and P. chrysogenum (from mare) was 78.125 µg/ml, but on P. chrysogenum (from cow), A. fulvescenes, A. niger and C. tropicalis (from buffaloes) and C. cladosporioides (from mare) was 156.25 µg/ml. However, much higher concentrations of terbinafine were needed to inhibit A. niger from cow (312.5 µg/ml), C. albicans from buffalo and mare (1.25 µg/ml) and on A. alternata, R. mucilaginosa from cows, R. mucilaginosa and Scopulariopsis brevicaulis from mares (5 mg/ml). In this respect, Garg et al. (2006) found terbinafine to be active against C. albicans with MIC determined by a macrodilution method ranging from 2-4 µg/ml. On the other hand, Garcia-Effron et al. (2004) stated that terbinafine showed a potent activity in vitro against A. flavus and A. niger with MIC ranging from 0.03-4 and 0.06-2 µg/ml respectively using broth microdilution method with modification. Also, the in vitro activity of terbinafine was superior against A. flavus and A. niger with MICs being determined using a microtiter method on two different media ranging from ≤0.03->16, ≤0.03->16 and 0.125-1, 0.06-1 µg/l respectively (Moore et al. 2001). Terbinafine showed also strong activity in vitro against species of Penicillium, Acremonium, Cladosporium, Scopulariopsis and Alternaria with MIC much lower in all cases than that reported in the current study, where their MICs, respectively, were 0.03-8, 0.06-8, 2, 1-16, and 0.25 mg/L (Garcia-Effron et al. 2004). Terbinafine was also found active against other fungi from clinical specimens e.g. Rhizopus microsporus, R. oryzae, Absidia corymbifera, Cunninghamella bertholletiae, Rhizomucor pusillus, Mucor ramosissimus and M. circinelloides (Gomez-Lopez et al. 2003), Trichophyton rubrum, T. mentagrophytes, T. tonsurans and T. verrucosum (Karaca and Koc 2004).
Table 2: Minimum inhibitory concentrations (MICs) of antifungal agents tested on fungi commonly isolated from cows, buffalo-cows and mares that failed to conceive.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Source</th>
<th>AUMC no.</th>
<th>PI (mg/ml)</th>
<th>F (µg/ml)</th>
<th>M (µg/ml)</th>
<th>K (µg/ml)</th>
<th>T (µg/ml)</th>
<th>N (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acremonium strictum W. Gams</td>
<td>Cow</td>
<td>3367</td>
<td>100</td>
<td>2.5</td>
<td>5</td>
<td>625</td>
<td>78.125</td>
<td>1250</td>
</tr>
<tr>
<td>Alternaria alternate (Fries) Keissler</td>
<td>Cow</td>
<td>3370</td>
<td>100</td>
<td>5</td>
<td>2.5</td>
<td>312.5</td>
<td>5</td>
<td>156.25</td>
</tr>
<tr>
<td>Aphanomyces fulvescens (Cooke) Apienis</td>
<td>Buffalo</td>
<td>3376</td>
<td>100</td>
<td>2.5</td>
<td>5</td>
<td>312.5</td>
<td>156.25</td>
<td>625</td>
</tr>
<tr>
<td>Aspergillus flavus Link</td>
<td>Buffalo</td>
<td>3375</td>
<td>50</td>
<td>2.5</td>
<td>5</td>
<td>156.25</td>
<td>78.125</td>
<td>625</td>
</tr>
<tr>
<td>Aspergillus flavus Link</td>
<td>Cow</td>
<td>3365</td>
<td>100</td>
<td>5</td>
<td>2.5</td>
<td>156.25</td>
<td>78.125</td>
<td>625</td>
</tr>
<tr>
<td>Aspergillus niger van Tieghem</td>
<td>Cow</td>
<td>3364</td>
<td>100</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>312.5</td>
<td>625</td>
</tr>
<tr>
<td>Aspergillus niger van Tieghem</td>
<td>Buffalo</td>
<td>3371</td>
<td>100</td>
<td>5</td>
<td>5</td>
<td>1.25 mg</td>
<td>156.25</td>
<td>625</td>
</tr>
<tr>
<td>Candida albicans (Robin) Berkhout</td>
<td>Buffalo</td>
<td>3374</td>
<td>50</td>
<td>2.5</td>
<td>5</td>
<td>156.25</td>
<td>1.25 mg</td>
<td>1250</td>
</tr>
<tr>
<td>Candida albicans (Robin) Berkhout</td>
<td>Mare</td>
<td>3379</td>
<td>100</td>
<td>2.5</td>
<td>1.25</td>
<td>625</td>
<td>1.25 mg</td>
<td>625</td>
</tr>
<tr>
<td>Chrysosporium tropicum Carmichael</td>
<td>Buffalo</td>
<td>3377</td>
<td>100</td>
<td>2.5</td>
<td>1.25</td>
<td>312.5</td>
<td>156.25</td>
<td>625</td>
</tr>
<tr>
<td>Cladosporium cladosporioides (Fresenius) de Vries</td>
<td>Mare</td>
<td>3381</td>
<td>50</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
<td>156.25</td>
<td>625</td>
</tr>
<tr>
<td>Emericella nidulans (Eidam) Vuillemin</td>
<td>Buffalo</td>
<td>3378</td>
<td>50</td>
<td>2.5</td>
<td>1.25</td>
<td>625</td>
<td>78.125</td>
<td>1250</td>
</tr>
<tr>
<td>Penicillium chrysogenum Thom</td>
<td>Cow</td>
<td>3368</td>
<td>100</td>
<td>10</td>
<td>2.5</td>
<td>156.25</td>
<td>156.25</td>
<td>625</td>
</tr>
<tr>
<td>Penicillium chrysogenum Thom</td>
<td>Mare</td>
<td>3382</td>
<td>50</td>
<td>5</td>
<td>1.25</td>
<td>156.25</td>
<td>78.125</td>
<td>1250</td>
</tr>
<tr>
<td>Rhodotorula mucilaginosa (Jorgensen) F.C. Harrison</td>
<td>Cow</td>
<td>3369</td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>1.25 mg</td>
<td>5</td>
<td>625</td>
</tr>
<tr>
<td>Rhodotorula mucilaginosa (Jorgensen) F.C. Harrison</td>
<td>Mare</td>
<td>3380</td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>1.25 mg</td>
<td>5</td>
<td>625</td>
</tr>
<tr>
<td>Scopulariopsis brevicaulis (Saccardo) Bainier</td>
<td>Mare</td>
<td>3383</td>
<td>100</td>
<td>10</td>
<td>2.5</td>
<td>1.25 mg</td>
<td>5</td>
<td>1250</td>
</tr>
</tbody>
</table>

- AUMC: Assiut University Mycological Centre culture collection, PI: Povidine iodine (mg/ml); F: Fluconazole (mg/ml); M: Miconazole (mg/ml); K: Ketokonazole (µg or mg/ml); T: Terbinafine (Lamisil) (µg or mg/ml); N: Nystatin (IU/ml).

**Ketoconazole** (Nizoral) was active against all fungi tested at 5 mg/ml concentration (Table 2). The MIC of ketoconazole on *A. flavus* and *P. chrysogenum* (from cows), *A. flavus* and *C. albicans* (from buffaloes) and *P. chrysogenum* (from mare) was 156.25 µg/ml, while on *A. alternata* (from cow) and *A. fumigatus* and *C. tropicum* (from buffaloes) was 312.5 µg/ml, however, on *A. strictum* (from cow), *E. nidulans* (from buffaloes) and *C. albicans* (from mare) was 625 µg/ml. It has been reported that the MIC of ketoconazole on isolates of *C. albicans* ranged between 0.125-64 µg/ml using an agar dilution assay (Shams-Ghahfaroki et al. 2006), between 0.031125-16 µg/ml using a macrodilution method (Garg et al. 2006) and between ≤0.03-16 µg/ml using a broth microdilution method (Barchiesi et al. 2000). In the study of Carrillo-Munoz et al. (2002) on 75 clinical isolates of *C. albicans*, 8 strains were resistant, 19 of intermediate susceptibility and 48 susceptible to ketoconazole (using 15µg/ml). Moreover, ketoconazole has shown an efficacy of 95-100% in vitro against Aspergillus species and *C. albicans* (Ho et al. 2006, Munguia & Daniel 2008).

On the other hand, the MIC of ketoconazole was much higher (1.25 mg/ml) on *R. mucilaginosa* (from cow), *A. niger* (from buffalo) and *R. mucilaginosa* and *S. brevicaulis* (from mares) while it was 5 mg/ml on *A. niger* (from cow) and *C. cladosporioides* (from mare). In this respect, the MIC of ketoconazole ranged between 0.5-2.0 µg/ml for isolates of clinical specimens origin of *R. rubra* (Barchiesi et al. 2000) and 1-16 µg/ml for *S. brevicaulis* (Aguilar et al. 1999) using a broth microdilution method.

**Miconazole** (Miconaz, one of the imidazole derivatives) was active on all fungi tested at 10 mg/ml concentration. Its MIC on *C. tropicum* and *E. nidulans* (from buffaloes), *C. albicans* and *P. chrysogenum* (from mares) was 1.25 mg/ml, however for *A. alternata*, *A. flavus* and *P. chrysogenum* (from cows) and *C. cladosporioides* and *S. brevicaulis* (from mares), the MIC was 2.5 mg/ml. On the other hand, the MIC of miconazole on *A. strictum* (from cow) and *A. fumigatus*, *A. flavus*, *A. niger* and *C. albicans* (from buffaloes) increased to 5 mg/ml. In this respect, Sirohi and Khar (2000) stated that the antifungal drug miconazole had no much higher value, where
only 29.27% of the 41 isolates tested (related to A. niger, 10 of 17, and C. albicans, 2 of 2 were sensitive to 600 µg/ml concentration and its MIC on C. albicans and A. niger was 300 µg/ml and 150 µg/ml respectively. Aller Gancedo (1978) reported that some species of Candida were sensitive to miconazole as the MIC on all strains was 6.54 µg/ml using serial dilutions in a liquid medium. During their study of the in vitro susceptibility of 75 clinical isolates of C. albicans to miconazole, Carrillo-Munoz et al. (2002) found that 9 strains were resistant, 15 were of intermediate susceptibility and 51 were susceptible to miconazole. On the other hand, five isolates of S. brevicaulis from clinical specimens tested by Aguilar et al. (1999) displayed moderate susceptibility to miconazole, with MIC ranging from 4->16 µg/ml using broth microdilution method. Also, MIC of imidazole derivatives (including miconazole) for C. albicans, A. flavus, A. niger and E. nidulans and Penicillium spp. were measured with liquid and solid media under a variety of experiment conditions and it was concluded that the concept that imidazoles act similarly on all types of fungi is not applicable (Odds 1980).

Fluconazole (fungican) at 10 mg/ml affected all fungi tested, while it had no effect at 1.25 mg/ml. The MIC of fluconazole on A. strictum (from cow), A. fumigatus, A. flavus, C. albicans, C. tropicalis and E. nidulans (from buffaloes) and C. albicans and C. cladosporioides (from mares) was 2.5 mg/ml. However, its MIC for A. alternata, A. flavus and A. niger (from bovine and buffaloes) and P. chrysogenum (from mare) was 5 mg/ml, while for P. chrysogenum and R. mucilaginosa (from cows) and R. mucilaginosa and S. brevicaulis (from mares) was 10 mg/ml (Table 2). In this respect, fluconazole was found to be active against C. albicans from clinical specimens with MICs ranging from 0.25 to 16 µg/ml by macrodilution method (Garg et al. 2006), from 0.12 to >128 µg/ml by broth microdilution tests (Pfaller et al. 2004) and from ≤0.125 to >64 µg/ml (Barchiesi et al. 2000) or 0.016 to 256 µg/ml by E test strips (Mallie et al. 2005). The in vitro study of Corrillo-Munoz et al. (2002) on susceptibility of 75 clinical isolates of C. albicans to fluconazole, revealed that 6 strains were resistant, 4 were intermediate susceptible and 65 were susceptible to fluconazole using the agar diffusion method. Fluconazole was also found to be active against R. rubra from clinical specimens and the MIC was ≥64 µg/ml using a broth microdilution method (Barchiesi et al. 2000), however in our study the MIC for R. mucilaginosa was 10 mg/ml where disc diffusion method was used.

Isolates of S. brevicaulis of clinical origin tested by Aguilar et al. (1999) (5 isolates) and Carrillo-Munoz et al. (2005) (19 out of 20 isolates) were highly resistant to fluconazole with MIC being >64 µg/ml by microdilution method and 25 µg by tablets. However, our isolate of S. brevicaulis was more resistant and the MIC was much higher (10 mg/ml).

Betadine (povidone-iodine) antiseptic solution was effective on all fungal isolates tested in vitro at 100 mg/ml, while it had no effect on these fungi at lower concentrations (12.5 and 25 mg/ml). For A. flavus, C. albicans and E. nidulans (all from buffaloes) and C. cladosporioides and P. chrysogenum (from mares), the MIC was 50 mg/ml. For A. strictum, A. alternata, A. flavus, A. niger, P. chrysogenum and R. mucilaginosa (from cows), A. fulvescenes, A. niger and C. tropicalis (from buffaloes) and C. albicans, R. mucilaginosa and S. brevicaulis (from mares), the MIC of betadine was 100 mg/ml. In this respect, Theraud et al. (2004) in their study on antiseptic betadine 10% (= 100 mg/ml) proved its efficiency as fungicidal for the five isolates they tested (3 clinical: C. albicans, Cryptococcus neoformans and R. rubra and 2 environmental: C. albicans and Cryptococcus uniguttulatus). Kremlev and Banakova (1979) found also that iodinol (iodine and potassium iodide in polyvinyl alcohol) at 125 µg/ml concentration inhibited the growth of C. albicans, Paecilomyces lilimum, Aspergillus fumigatus, Absidia corymbifera and Mucor pusillus. However, the efficacy of betadine intrauterine infusions was studied on 112 repeat breeder Holstein cows (aged 3-7 years) with endometritis revealed good results for the recovery and the conception rates (Koujan et al. 1996). Also, intrauterine irrigation of infected genitalia with 5% v/v of Lugol’s solution (5 gm iodine and 8 gm potassium iodide in 100 ml distilled water) revealed negative mycotic isolations (Osman and Abou Gabal, 1975). Also, Povidine iodine was active against species of Candida, Penicillium, Aspergillus, Epidermophyton and Microsporum at different concentrations (Jayaraja Kumar et al. 2009). It was also concluded that diluted Lugol’s iodine was a useful treatment of repeat breeder cows (Mutiga 1978).

Griseofulvin at 12.5 up to 200 mg/ml has no inhibitory effect on all fungi tested. In accordance with the current results, Jand et al. (1978) found that griseofulvin at 25-250 µg/ml has no inhibitory effect on C. albicans, C. stellatooides, C. parapsilosis, C. guillermondii and Saccharomyces sp. Griseofulvin had also no inhibitory action on yeasts (Aller Gancedo 1978). From the available literature, griseofulvin
is only inhibitory against dermatophytes (Sandhu and Randhawa 1964, Karaca and Koc 2004).

It could be concluded that nystatin followed by terbinafine and ketoconazole had the highest effect on most isolated fungi from repeat breeder animals.

References


