Identification of rare but clinically relevant yeast species and studying of their susceptibility to the newest antifungal agent

Doctoral (Ph.D.) theses

Szabó Zsuzsanna, M.Sc.

Semmelweis University
Doctoral School of Clinical Medicine
Program 2/16: Dermatology and venerology

Program leader: Prof. Sarolta Kárpáti M.D., Ph.D.
Supervisors: Prof. Ferenc Rozgonyi, M.D., Ph.D., D.Sc.
László Majoros, M.D., Ph.D.
Opponents: Edit Hajdú, M.D., Ph.D.
Zsuzsanna Csukás, M.D., Ph.D.
Chairman of the Final Examination Committee:
Attila Pajor, M.D., Ph.D., D.Sc.
Members of the Final Examination Committee:
Béla Szabó, M.D, Ph.D.
Edit Urbán, M.D., Ph.D.
Dóra Szabó, M.D., Ph.D.

Budapest
2010

I. INTRODUCTION

From the 1980s yeasts have been increasingly identified as a major cause of human disease, giving rise to one of the most important problems in the field of public health. According to source data merely between 1979 and 2000 incidences of annual sepsis caused by various fungi have been doubled in the USA.

According to the newest source data *C. ablicans, C. glabrata, C. parapilosis C. tropicalis,* and *C. krusei* are directly responsible for the development of candidemia. The number of those yeast species which are difficult to identify, namely the *C. guilliermondii,* the *C. lusitaniae,* the *C. kefyr,* the *C. rugosa,* the *C. famata,* the *C. inconspicua,* the *C. norvegensis* and the *C. dubliniensis,* has been increasing. The resistance of these species against certain antifungal agents can significantly differ from the well known *C. ablicans*. The change in the epidemiology of *Candida* species requires an accurate and fast identification of clinical isolates to species level, which is the first step in establishing an adequate therapy.

Isolates of *C. dubliniensis* and *C. ablicans* having a decreased FLU susceptibility but occurring phenotypically similar to yeasts are good examples for yeast infections, which occurred rarely in the past but become more and more frequent nowadays. Test used in routine laboratories are still not suitable for accurate identification of these two species.

Differentiation of *C. dubliniensis and C. albicans* in routine laboratories meant a similar problem for a long time.

Lately the *C. orthopsilosis* and *C. metapsilosis* species (previously belonged to *C. parapsilosis* II, III group) were isolated from the unmitigated *C. parapsilosis* using the methods of molecular biology. Current epidemiological studies have shown that the two new species can be isolated from patients with candidemia in 1-2%, which means that the identification of them to species level is not negligible in the future.

The knowledge of *in vitro* pharmacodynamical effects of rarely isolated *Candida* species to the newest antifungal agents is very important. Although even the most common

fluconazole (FLU) is very effective *in vitro* and *in vivo* against the majority of clinically important *Candida* species, the primary FLU resistance occurs at *C. inconspicua* isolates shows a decreased susceptibility against FLU, which can be seen from MIC values as well. During extended FLU-therapy even isolates of *C. dubliniensis* become resistant to FLU, which causes partial or total cross resistance to triazole antifungal agents as well.

In recent years, echinocandins introduced to therapy practice are at least as effective *in vivo* as the amphotericin B (AMB) and have notably less side effects. Currently echinocandins are the first line of therapy in the case of neutropenic patients with invasive candidiasis. Although it is important to note that the newly separated isolates of *C. orthopsilosis* and *C. metapsilosis* shows significantly lower MIC values against echinocandins compared to *C. parapsilosis*.

Changings in epidemiology of yeasts requires adopting new diagnostic methods to routine ones as well as getting more acquainted with relations of susceptibility-resistance of rare species.

II. OBJECTIVES

1. The objective was to identify 264 yeast clinical isolates (isolated at the Department of Microbiology at the University of Debrecen) with MICRONAUT-Candida and API ID 32C identification systems. Aiming to evaluate new test systems for these strains and to incorporate their use into routine laboratories.

2. The objective was to study the *in vitro* killing activity of fluconazole and amphotericin B against *C. inconspicua* isolates with decreased fluconazole susceptibility using time kill methodology.

3. The objective was to determine the killing activity of caspofungin both in RPMI 1640 and antibiotic medium 3 (AM3) against *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* using time kill methodology.

4. The objective was to determine *in vitro* activities of the oldest antifungal drugs, amphotericin B, 5-fluorocytosine and new triazoles, fluconazole, voriconazole and posaconazole against *C. dubliniensis* isolates using time kill methodology.

III. MATERIALS AND METHODS

III. 1. The source of the isolates

The majority of yeasts (255) were isolated mostly from throat, sputum, blood, wound, urine and vagina from both in and outpatients between the months of November 2005 and April 2006 at the Microbiology Department at the University of Debrecen.

Nine *C. dubliniensis* strains (6 were used for time-kill studies) were obtained from the University of Szeged being previously identified with rapid real time PCR method on the basis of melting point analysis.

C. inconspicua isolates (8) used for time-kill studies also were derived from the Microbiology Department at the University of Debrecen.

All *C. parapsilosis* isolates used in identification studies belonged to *C. parapsilosis sensu stricto* isolates.

For time-kill studies the use of six, three and four *C. paraspilosis, C. otrhopsilosis* and *C. metapsilosis* isolates identified with DNA-based methods. Two *C. parapsilosis*, three *C. orthopsilosis* and three *C. metapsilosis* were from Tavanti Italian collection of clinical isolates. All others were isolated at the Microbiology Department at the University of Debrecen.

Also were used the less common species (*Geotrichum*, *Rhodotorula* and *Trichosporon*) from the University of Debrecen, as well as 13 ATCC strains (*C. albicans 14053, C. albicans 10231, C. parapsilosis 22019, C. tropicalis 750, C. krusei 6258, C. inconspicua 16783, C. norvegensis 22977, C. lusitaniae 38533, C. guilliermondii 6260, C. dubliniensis CD36, C. pulcherrima 18406, C. famata 36239*, and *C. rugosa 2142*).

III. 2. Isolation and identification of the yeasts species

Identification by using commercial kits

Yeasts grown on Sabouraud dextrose agar were simultaneously tested with both identification systems, MICRONAUT-Candida System (Merlin Diagnostika, Gmbh, Borhneim, Germany) and API ID 32C (BioMérieux, Marcy l'Etoile, France) systems. The tests were performed according to the manufacturer's instruction. API ID 32C and MICRONAUT-Candida were read after 48 and 24 h, respectively. If the results were in accordance, they were accepted them as final. In case of discrepancy, both methods were repeated and additional tests were set up (microscopic morphology, esculin hydrolysis, growth at 45 ^{0}C, nitrate assimilation). Otherwise or when additional tests yielded equivocal results, PCR ribotyping results were accepted as valid identification.

Statistical analysis

The proportion of misidentification was determined for each test and relative accuracies of results were compared using Fisher's exact test. For statistical analysis we used GraphPad Prism 4.03 Windows software.

III. 3. Methods used for determination of the strain susceptibility

Broth microdilution method

MIC determination was performed according to Clinical and Laboratory Standards Institute methodology (NCCLS 2002). MIC were determined for amphotericin B (AMB), for 5-fluorocytosine (5-FC) (Sigma, Budapest, Hungary), for fluconazole (FLU), for

voriconazole (VOR) (Pfizer, Groton, CT, USA), for posaconazole (POS) (Schering-Plough, Kenilworth, NJ, USA) and for caspofungin (CAS) (Merck-Research Laboratories, Rahway, NJ, USA). *C. dubliniensis CD36, C. inconspicua 16783* and *C. parapsilosis ATCC 22019* strains were included for references.

For preparation of the inoculum we used 24 hours strains grown on Sabouraud dextrose agar.

0.9 % NaCl was used for preparation of 0.5 McFarland yeast suspension. Final inoculum concentration of 10^3 CFU/mL were standardized both in RPMI-1640 (Sigma, Budapest, Hungary) and antibiotic medium 3 (AM3, Fluka, Budapest, Hungary), too. Trays used for MIC determination were prepared in house and were stored at -20 ^{0}C until used in the study (max. 1 month).

Microdilution trays (96-well plates) also contained wells of the antifungal agents (as positive yeast control) and without antifungal agents (as negative medium control). They were incubated at 35 ^{0}C.

In case of triazoles, amphotericin B and 5-fluorocytosine MIC were read visually after 48 hours incubation at the lowest concentration of prominent or total decrease in turbidity (NCCLS 2002). In case of caspofungin the MIC was read at 24 hours growth as the lowest concentration at which partial growth inhibition was observed.

Time-kill studies

For performing time-kill experiments we used the published method by Klepser (1998). Time-kill assays were performed with all tested clinical isolates at least twice. A 10^5 CFU/mL starting inoculum was applied and drug concentration depended on the species and MIC values, its ranged from 0,5-16 x MIC. According to the protocol test tubes were continuously agitated during the course of the time-kill studies and were incubated at 35 ^{0}C for 48 hours. Samples (100 µl) were removed at determined hours (0, 2, 4, 6, 8, 12, 24, 48).

Sampling time depended as well as on tested isolates and tested antifungal agent. A tenfold dilution series of each sample in sterile saline was prepared and four times 30 µl aliquots were plated onto Sabouraud dextrose agar.

Colony counts were determined after incubation of the plates at 35 ^{0}C for 48 hours.

III. 4. Interpretation of the results

Broth microdilution method (MIC values)

The MIC of amphotericin B were read at the lowest concentration at which complete growth inhibition was observed. For the interpretive criteria of triazoles and 5-fluorocytosine partial inhibition criteria was used (the lowest concentration that produced a prominent decrease in turbidity compared to that of the drug-free control) (NCCLS 2002).

Interpretive criteria for VOR defined according to the description in CLSI (2008), susceptible (S) ≤ 1 mg/L, susceptible-dose dependent (S-DD) 1-2 mg/L and resistant (R) ≥ 4 mg/L.

In accordance with current publications the interpretive criteria for CAS and the threshold concentration ≤ 2 mg/L was applied for susceptible (S) isolates was used.

In case of POS strains with MIC ≤ 1 mg/L were susceptible (S), and strains with MIC ≥ 8 mg/L were resistant (R).

For defining the MIC value of AMB resistant (R) isolates the threshold concentration > 1 mg/ L was used.

Time-kill methodolgy

The time-kill curves were plotted by using a Microsoft Excel 2003 software. According to the method description 99,9% or 3-log 10-unit reduction in CFU /mL from the starting

inoculum as a stringent criteria was used to define the fungicid activity of the tested antifungal agent. Less than 99.9% reduction in CFU/ml than or 3-log10-unit from the starting inoculum was used to define fungistatic activity of the antifungal agent. Paradoxical growth (PG) of the tested isolate was described when its slowing growth was observed (fungistatic effect) at low (below MIC) and at high (supra MIC) drug concentrations, but no effect was observed at the middle concentration of the drug (fungicidal effect).

IV. RESULTS

IV. 1. Results from identification experiments with MICRONAUT-Candida System and API ID 32C

API ID 32C correctly identified all tested ATCC strains after 48 hours incubation period. MICRONAUT-Candida misidentified *C. norvegensis* and *C. rugosa* ATCC strains, identifying both as *C. valida* according to the additional test proposed (microscopic morphology). As *C. pulcherrima* was not included in the MICRONAUT database, the *C. pulcherrima* ATCC strain was not identified. Other ATCC strain were correctly identified within 24 hours.

MICRONAUT-Candida correctly identified the more frequently occurring *Candida* species (*C. albicans*, *C. parapsilosis*, *C. tropicalis*) in clinical practice within 24 hours and results obtained were in accordance with results obtained by API ID 32C. Additionally, *Candida* species possessing primary (*C. krusei*) or secondary (*C. glabrata*) resistance to fluconazole were also identified within 1 workday.

MICRONAUT-Candida System correctly identified all tested *C. dubliniensis* isolates without additional tests.

C. famata, *C. guilliermondii*, *C. lusitaniae*, *C. kefyr* and *C. lypolitica* were correctly identifed by MICRONAUT-Candida System without extra tests within 24 hours.

MICRONAUT-Candida test system never misidentified *C. lusitaniae* strains as *C. famata* or *C. guilliermondii*. This is a great advantage of the new system, since the isolation and identification of these strains as well as from clinical and environmental samples means a challenge for mycologist. In some clinical situations *C. famata*, in contrast to *C. lusitaniae*, often shows high MICs to triazoles and caspofungin, while *C. lusitaniae* may be resistant to amphotericin B.

In the case of *C. rugosa* and *Rhodotorula glutinis* isolates, MICRONAUT-Candida needed

additional tests (microscopic morphology and nitrate assimilation, respectively) to accord with a API ID 32C. These additional tests are also very important as some *C. rugosa* isolates obtained from blood, may show AMB-, nystatin and FLU-resistance, too.

API ID 32C, even using an extra test (esculin hydrolisis) misidentified all tested *C. inconspicua* strains (n = 12) as *C. norvegensis* strain, as confirmed by PCR-ribotyping. These inherently fluconazole-resistant strains were correctly identified with MICRONAUT-Candida using an additional test (microscopic morphology). These identities were also confirmed by PCR-ribotyping.

As *C. pulcherrima* and *C. sake* is not involved in the database of the new test-system, MICRONAUT-Candida was unable to identify these isolates to species level. This is a deficiency of the new test-system. As some clinical isolates of *C. sake* proved to be cause vulvovaginitis, endocarditis and pericarditis, the identification to species level is not negligible.

Statistical analysis

MICRONAUT-Candida System without extra tests identified 241 (91.3%) of yeast isolates concordantly to API ID 32C. Only 2 (2/264, 0.76%) of clinical isolates were misidentified or unidentified by MICRONAUT-Candida, while 18 (18/264, 6.82%) were unidentified or misidentified by API ID 32C.

Using Fisher's-exact test for statistical analysis, the results showed that the new system MICRONAUT-Candida System performed more reliable identification of 264 yeast clinical isolates than API ID 32C (0.76% vs.6.82%, $p < 0.001$).

IV. 2. Experiments with C. inconspicua isolates

FLU and AMB MIC values for C. inconspicua isolates

In our experience FLU MIC for *C. inconspicua* clinical isolates as well as for the reference *C. inconspicua* ATCC 16783 were in the susceptible-dose dependent (S-DD, MIC: 16-32 mg/L and resistant category (R, MIC ≥ 64 mg/L). Amphotericin B MICs found were in a narrow range, slightly below or at the level of the *in vivo* available peak concentration (1 mg/L).

Time-kill studies with C. inconspicua isolates

Fluconazole after 24 hours incubation showed fungistatic effects against all of the tested *C. inconspicua* clinical isolates, as well as against the *C. inconspicua* ATCC 16783 strain at 1-16 x MIC values. However, all of the tested strains were killed by ≥ 4 x MIC fluconazole concentrations after 48 hour incubation regardless of their MIC values. Clinical relevance of the observed fungicidal effect is doubtful as such a high fluconazole level is difficult to achieve in human, therapeutic success with AUC/MIC (≥ 100) requiring a relatively high-dose regiment of FLU, which is not beneficial from the standpoint of the patient.

In time-kill tests AMB proved to be fungicidal against all of the tested *C. inconspicua* clinical strains, as well as against the *C. inconspicua* ATCC 16783 strain within 24 hours at a wide range of amphotericin B concentrations.

In these studies AMB showed excellent killing activity at 1-2 times the MIC (1 mg/L) against *C. inconspicua* strains. In most of the clinical isolates and ATCC 16783 reference strains, time to reach fungicidal endpoint varied between 2-24 hours.

However, at 0.5 mg/L AMB concentration only a fungistatic effect was observed for all tested strains.

Rapid killing by amphotericin B observed at clinically attainable concentrations, thus amphotericin B appears to be a good choice against *C. inconspicua*.

IV. 3. Experiments with „psilosis"- group isolates

Caspofungin MIC of „psilosis" -group isolates determined in RPMI-1640 and AM3 medium

The CAS concentration range was 0.015-8 mg/L. PG was observed neither in RPMI-1640 nor in AM3. For all three isolates MICs in AM3 were lower (MIC $_{C.\ parapsilosis}$: 0.25-0.5 mg/L, MIC $_{C.\ orthopsilosis}$: 0.06 mg/L, MIC $_{C.\ metapsilosis}$: 0.03-0.06 mg/L) than MICs in RPMI-1640 (MIC $_{C.\ parapsilosis}$: 0.5-2 mg/L, MIC $_{C.\ orthopsilosis}$: 0.12-0.25 mg/L, MIC $_{C.\ metapsilosis}$: 0.12-0.25 mg/L). In case of *C. orthopsilosis* and *C. metapsilosis* isolates we obtained lower MIC (2-3 dilution step) than in the case of *C. parapsilosis* isolates, thus indicating that the newly described species, regardless of the medium used, were more susceptible to caspofungin than *C. parapsilosis*. In both test medium CAS proved to be the most effective against *C. metapsilosis*.

Results obtained with „psilosis" group against caspofungin by using time-kill methodology

In the killing studies *C. orthopsilosis* isolates behaved similar to *C. parapsilosis*, fungistatic or paradoxical effect (PG) was observed regardless of the used test medium.
In the killing studies using RPMI-1640, caspofungin showed a fungistatic effect in the case of *C. parapsilosis* and *C. orthopsilosis* after 24 hours.
After 48 hours some of the *C. parapsilosis* and *C. orthopsilosis* isolates at 2 mg/L caspofungin concentration showed 99.9 % decrease in viable CFU numbers. This slow killing of the strains *C. parapsilosis* and *C. orthopsilosis* in RPMI-1640 against a

fungistatic effect it is probably due to a mutation occured in *Fks1*.

After 48 hours, caspofungin was fungicidal at concentrations of 1-8 mg/L (16-128 x MIC) almost against all *C. metapsilosis* isolates.

In killing studies with AM3, a fungistatic effect was observed after 24 hours against *C. parapsilosis* isolates and PG with *C. orthopsilosis* isolates. However, in AM3 after 48 hours, caspofugin proved to be clearly fungicidal against *C. orthopsilosis*, but not against *C. parapsilosis* isolates.

However, after 48 hours, all isolates were killed by caspofungin concentration 2-16 x MIC or higher. All *C. metapsilosis* strains were killed at caspofungin of ≥ 0.06 mg/L (≥ 1x MIC) after 48 but not 24 hours.

Paradoxical growth observed in test medium AM3 after 24-48 hours can be rather a consequence of stress response to the high caspofungin concentration than to the presence of genotype with decreased susceptibility.

IV. 4. Experiments with C. dubliniensis isolates

MIC values for C. dubliniensis isolates obtained from broth microdilution tests

The minimum inhibitory concentrations (MICs) of the tested isolates were susceptible to all triazoles and 5-fluorocytosin (MIC FLU: 0.12-0.25 mg/L, MIC POS: 0.015-0.12 mg/L, MIC VOR: 0.015-0.03 mg/L, MIC 5-FC: ≤ 0.12 mg/L). The most effective proved to be voriconazole. MICs obtained with AMB were at the level safely attainable in the serum (MIC AMB: 0.25-1 mg/L).

Time-kill studies with C. dubliniensis isolates

The results of the time-kill assay showed that all of the triazoles (POS, FLU, VOR) were

fungistatic against all of the *C. dubliniensis* isolates tested at a concentration easily attainable in the serum. However, all three drugs were fungistatic; only moderate decrease in viable cell numbers were observed and a 99.9 % reduction in colony numbers was not attained even at the highest concentration (4 mg /L, 8 mg/L, 4 mg/L) of the drugs.

AMB proved to be fungicidal against all clinical isolates after 48 hours; isolates were killed within 8 h by 1 mg/L. After 48 hours clinical isolate (with number 2) and reference strain CD 36 were killed even at 0.25 mg/L.

After 48 hours, 5-FC was fungistatic at 0.5-16 x MIC (0.06- 2 mg/L); however, at 32-64 x MIC (4-8 mg/L) fungicidal activity was observed against all *C. dubliniensis* isolates. Although strains with decreased susceptibility to 5-FC may occur, this antifungal agent can be use in the therapy against some FLU-resistant *C. dubliniensis* strains.

V. CONCLUSIONS

1.

a/. Comparative identification studies made with the MICRONAUT-Candida System and API ID 32C showed that in 24 hours the MICRONAUT-Candida System correctly identified the isolates of *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata* and *C. krusei* occurring most frequently in the clinical practice. The system also correctly identified the isolates of *C. dubliniensis.*

b./ The new test-system appears to result reliable data in routine mycology laboratories in the identification of rare but medically relevant yeast species with different antifungal profile. These include *C. famata, C. guilliermondii, C. lusitaniae.*

c./ While the system appears to be completely reliable in the identification of some certain FLU resistant species (*C. rugosa),* it gives at the least a good direction to identify other species (*C. inconspicua* and *C. norvegensis*), which help a lot in further tests to microbiologists .

d./ As a consequence of our studies the MICRONAUT test-system was introduced to the routine diagnostic laboratory of Clinical Microbiology Department, University of Debrecen, where it become possible to identify a great amount of *non-albicans Candida* isolates within 24 hours as well as to help the work in the laboratory.
(The current local practice is the following: If the identification is unambiguous according to the probabilities, the test, as the method shows, will be done simultaneously with the MICRONAUT-Candida and the ID 32C systems as well. With this method it is possible to do the identification in 24 hours and the ID 32C can support the laboratory work in problematic cases.)

2.

a./ Time-kill curves with *C. inconspicua* showed that high fluconazole concentrations are required to inhibit the growth of *C. inconspicua* isolates, thus it can not be recommended for therapy against invasive candidiasis caused by *C. inconspicua*.

b./ *In vitro* examinations with amphotericin B confirm previously gained *in vitro* experiences, which means that amphotericin B seems to be effective.

3.

a./ Within the genetically heterogenic *„psilosis"*- group the *C. metapsilosis* and *C. orthopsilosis* strains showed that according to time-kill methodology the caspofungin was more effective against *C. metapsilosis* and *C. parapsilosis* than *C. metapsilosis* isolates.

b./ According to the similarity of time-kill curves of *C. parapsilosis* and *C. orthopsilosis* species in RPMI-1640 and AM3 media the echinocandin therapy for candidiasis caused by *C. orthopsilosis* seems to be unsafe. Although against *C. metapsilosis* isolates the caspofungin showed a good *in vitro* activity, because of the proline-alanin swap in *Fks1* further *in vivo* tests can decide the ground of echinocandin therapy in the case of infections with *C. metapsilosis*.

4.

a./ By using time-kill methodology we concluded that both amphotericin B and 5-fluorocytosin as the oldest drugs in the therapy; the triazoles (fluconazole, voriconazole and posaconazole) all showed a good fungistatic or fungicid effect at concentrations easily attainable in serum against all tested *C. dubliniensis*. In case of a cross resistance with azoles the fungicidal amphotericin B suggested to use.

VI. Publications

Publications forming the basis of the Dissertation

1. **Szabó Zs**., Tóth B, Kovács M, Kardos G, Maráz A, Rozgonyi F, Majoros L. 2008. Evaluation of the new MICRONAUT-Candida system for yeast identification against the API ID32C method. J. Clin. Microbiol. 46: 1824-5. **IF: 3, 945**

2. **Szabó Z**., G. Sóczó**,** C. Miszti, P. Hermann, F. Rozgonyi. 2008. *In vitro* activity of fluconazole and amphotericin B against *Candida inconspicua* clinical isolates as determined by the time-kill method. Acta Microbiol. Immunol. Hung. 55: 53-61. **IF: 0,0**

3. Varga I, Sóczó G, Kardos G, Borbély Á, **Szabó Z**, Kemény-Beke Á, Majoros L**.** 2008. Comparison of killing activity of caspofungin against *Candida parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*. J. Antimicrob. Chemother. 62**:** 1466-68. **IF: 4, 328**

4**. Szabó Z**, Borbely A, Kardos G, Somogyvari F, Kemény-Beke A, Asztalos L, Rozgonyi F, Majoros L. 2009. *In vitro* efficacy of amphotericin B, 5-fluorocytosine, fluconazole, voriconazole and posaconazole against *Candida dubliniensis* isolates using time-kill methodology. Mycoses. Sep 16. **IF: 1, 402**

Other publications

1. **Szabó Z**, Szilágyi J, Tavanti A, Kardos G, Rozgonyi F, Bayegan S, Majoros L. *In vitro* efficacy of 5 antifungal agents against *Candida parapsilosis, Candida orthopsilosis,* and *Candida metapsilosis* as determined by time-kill methodology. 2009. Diagn. Microbiol. Infect. Dis. 64: 283-8. **IF: 2, 451**

Cummulative IF: 12, 126

Abstracts

1. Csukásné Bagoly E, **Szabó Z**, Széll M, Szabó J. Intenzív osztályok prevenciós vizsgálatai során izolált baktériumok előfordulási gyakoriságának és antibiotikum rezisztenciájának változásai. ÁNTSZ Hajdú-Bihar megyei Intézete, DEOEC Mikrobiológiai Intézete, Debrecen. MLDT 2002. évi Nagygyűlése, Gyula, 2002. augusztus 28-31(poszter: P20/01).

2**. Szabó Z**, Kristóf K, Cser V, Kardos Sz, Ghidán Á, Rozgonyi F. A 2001-2003 időszak klinikai mintáiból izolált MSSA és MRSA törzsek fenotípúsaiban mutatkozó eltérések a Semmelweis Egyetem Orvosi Mirkobiológiai Intézetében. Semmelweis Orvostudományi Egyetem, Budapest. A Magyar Mikrobiológiai Társaság 2004. évi Nagygyűlése, Keszthely, 2004. október 7-9 (előadás).

3. Majoros L, **Szabó Z**, Falusi E, Sóczó G, Kardos G. Comparison of the performance of the new commercial yeast susceptibility panel MICRONAUT –AM to the standard method 3rd Trends in Medical Microbiology. Poster N0: PO27. 28-31 October 2007. Turin, Italy. (**IF: 1, 342**).

4**. Szabó Z**, Földi R, Falusi E, Sóczó G, Kardos G, Rozgonyi F, Majoros L. Comparison of the performance of the new commercial yeast susceptibility panel MICRONAUT-AM to the standard method. Fourth Hungarian Conference of Mycology. May 29-31, 2008, Debrecen, Hungary.

5. Szilágyi J, Bayegan S, Gesztelyi R, Kardos G, Mózes J, Kemény-Beke Á, **Szabó Z**, Majoros L. *In vivo* efficiacy against *Candida orthopsilosis* in a neutropenic mouse model. 2nd Central Europen Forum for Microbiology (CEFORM). October 7-9. 2009, Keszthely, Hungary (előadás: ML-7).

6. Bayegan S, Szilágyi J, Gesztelyi R, Kardos G, Mózes J, Kemény-Beke Á, **Szabó Z**, Kovács R, Majoros L. Correlation between postantifungal effect and the efficacy of the single 5 and 10 mg/kg caspofungin doses for treatment of disseminated candidiasis caused by *Candida krusei* in a neutropenic mouse model. 2nd Central Europen Forum for Microbiology (CEFORM). October 7-9. 2009, Keszthely, Hungary (poszter: MP 19).