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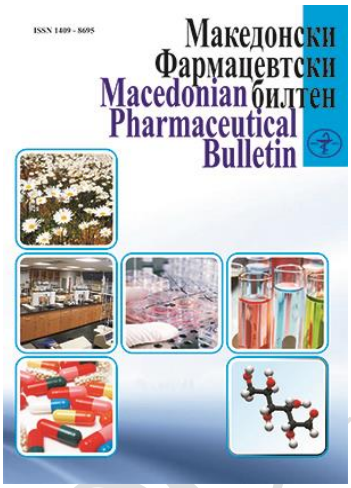

Title: Antifungal susceptibility profile of Aspergillus species from patients with increased risk for aspergillosis

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Antifungal susceptibility profile of *Aspergillus* species from patients with increased risk for aspergillosis

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Abstract

Aspergillosis is the most common fungal infection caused by molds, especially in high-risk patients. The treatment of these diseases is based on the use of polyene and azole antifungal drugs. Resistance rates of *Aspergillus* species to antifungal drugs vary widely across medical centers around the world. Antifungal susceptibility testing of *Aspergillus* species to antifungal agents could provide useful information for clinicians to make decision regarding the patient therapy. The aim of the study was to evaluate the antifungal susceptibility profile of *Aspergillus* species towards amphotericin B, itraconazole, voriconazole and caspofungin, isolated from patients with increased risk for aspergillosis. During a 2-year period, clinical specimens from 125 patients divided into 4 groups according to clinical diagnosis and EORTC/MSG criteria, were analysed at the Institute of Microbiology and parasitology, Faculty of Medicine, Skopje, Republic of North Macedonia. These groups included patients with primary immune deficiency, critically ill patients treated in intensive care units, patients with chronic aspergillosis and cystic

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fibrosis. All specimens (from respiratory tract and blood culture) were investigated with conventional mycological methods, by inoculation of specimens on media for support of fungal growth. Identification of *Aspergillus* was performed with macroscopic analysis of mold colonies and additional microscopic analysis of their conidia with lactophenol blue method. E-test strips of voriconazole, itraconazole, amphotericin B and caspofungin (AB *bioMerieux*, France) were used for determination of the antifungal susceptibility profile of *Aspergillus* species. Seventy-one isolates of *Aspergillus* species were confirmed in our patients. Four isolates of *A.fumigatus* (5.6%) were confirmed in blood cultures, from patients with primary immune deficiencies, and 67 isolates (94.4%) originated from respiratory specimens from patients with different underlying diseases. *A.flavus* was identified in 11 patients and *A.terreus* in 3 patients. Resistance to amphotericin B was detected in 6 isolates (2 isolates of *A.fumigatus*, 1 isolate of *A.flavus* and 3 isolates of *A.terreus*). Only one isolate of *A.fumigatus* showed resistance to itraconazole. All isolates of *Aspergillus* species were sensitive to voriconazole and caspofungin.

In vitro antifungal susceptibility testing with E-test demonstrated resistance to amphotericin B in 6 isolates of *Aspergillus* species. Only one isolate of *A.fumigatus* was resistant to itraconazole. All isolates of *Aspergillus* species showed sensitivity to voriconazole and caspofungin. Antifungal susceptibility testing of *Aspergillus* species with E-test provides useful information for clinicians for appropriate choice of antifungal agents for treatment of aspergillosis.

Keywords: *Aspergillus*, mold, infection, susceptibility, E-test

Introduction

Aspergillosis is an infection caused by *Aspergillus*, a cosmopolitan mold fungus which lives indoors and outdoors, and frequently can be found in airborne dust and air vents. Most people breathe in spores from these fungi every day, without being affected. But if the immune system is compromised, infection develops and can be displayed with wide spectrum of symptoms. Aspergillosis affects both immunocompetent and particularly immunocompromised patients undergoing steroid treatment, chemotherapy resulting in severe neutropenia, hematopoietic stem cell and solid organ transplantation (Mousavi et al., 2016). Disorders that

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affect the immune system like AIDS and some hereditary immune disorders, as well as cancer, can also contribute to development of this opportunistic fungal infection. Aspergillosis manifests as a broad-spectrum of diseases including aspergilloma, chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis and invasive aspergillosis, which is the most aggressive and rapidly spreading form of infection to the brain, heart, liver, and kidneys, with a very high mortality rate (Latgé and Chamilos, 2019).

The genus *Aspergillus* encompasses more than 250 species and is one of the largest genera of filamentous fungi causing human infections. *A.fumigatus* is the leading cause of human aspergillosis worldwide. In the last two decades, other non-*A.fumigatus* fungi, like *A.flavus, A.terreus* and *A.niger* have been recognized with increased frequency as causative agents of aspergillosis (Lass-Flörl and Cuenca-Estrella, 2017). This shift in the fungal epidemiology has been attributed to the increase of the percentage of the immunocompromised population, advances in mycological diagnosis, more accurate fungal identification, as well as the selective pressure caused by the extensive clinical use of broad-spectrum antifungal agents (Richardson and Lass-Flörl, 2008). Diagnosis of aspergillosis usually involves an x-ray or computed tomography and, if possible, culture of a sample of infected material, which is considered a gold standard (Raveendran and Lu, 2018).

The aim of the study was to evaluate the antifungal susceptibility profile of *Aspergillus* species isolated from patients with increased risk for development of aspergillosis, to amphotericin B, itraconazole, voriconazole and caspofungin, with E-test method.

Materials and methods

Study design

A retrospective diagnostic study was performed at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, Republic of North Macedonia, during a 2-year period (2014-2016).

Group of patients and mycological investigations

In this study, clinical specimens (from mucosal surfaces of respiratory tract and blood cultures) from 125 patients divided into 4 groups, according to clinical diagnosis and risk factors for invasive aspergillosis, were analysed at the Laboratory for diagnosis of mycoses, at the

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Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, Republic of North Macedonia. These groups included patients with primary immune deficiency, critically ill patients treated in intensive care units, patients with chronic aspergillosis and cystic fibrosis, using the EORTC/MSG criteria (European Organization for Researsh and Treatment of Cancer/Mycoses Study group) (Tsitsikas, et al., 2012).

The specimens were investigated with conventional mycological methods, by inoculation of specimens on media for support of fungal growth (Sabouraud and chromogenic CALB medium (Oxoid)). Blood cultures were analysed using the automated BacT/ALERT system (*bioMérieux*, France). Identification of *Aspergillus* on species level was performed with macroscopic analysis of mold colonies and further microscopic analysis of the reproductive elements (conidia). E-test strips of four antifungal drugs: itraconazole (0.002-32 μg/mL), voriconazole (0.002-32 μg/mL), amphotericin B (0.002-32 μg/mL) and caspofungin (0.004-32 μg/mL) (AB *bioMerieux*, France) were used for determination of the antifungal susceptibility profile of isolated *Aspergillus* species. RPMI 1640 (RPG, Remel) medium (150 mm) enriched with 2% glucose and buffered with MOPS buffer for testing of molds with E-test (Mirchevska and Bosshard, 2012) was used for antifungal susceptibility profile of isolated *Aspergillus* species. Inoculum of the mold was obtained with collection of mature conidia with sterile saline containing 0.5% Tween 20 (Sigma). The suspension was settled for about 15 minutes before transferring the supernatant into another tube to adjust the turbidity to 0.5 McF. RPMI 1640 agar containing MOPS and 2% glucose was smeared with the supernatant with cotton swabs in three directions. Finally, the antifungal agent strips were put onto the plates after the surface of the plates was dry. E-test MICs were read after incubation for 48 hours at 35 °C when the concentration at the border of the elliptical inhibition zone intercepted with the scale on the antifungal strip.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows. The results of our study are presented as numbers and percentages. Differences in distribution of proven, probable and possible fungal infections with *Aspergillus* were compared by Pearson Chi square test. P value less than 0.05 was considered statistically significant.

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Results

Specimens from mucosal surfaces of respiratory tract and blood cultures from 125 patients were divided in 4 groups (patients with primary immune deficiencies, critically ill patients treated in intensive care units, patients with chronic aspergillosis and cystic fibrosis) according to clinical diagnosis and EORTC/MSG (European Organization for Researsh and Treatment of Cancer/Mycoses Study group) criteria (Fig. 1) (Tsitsikas et al., 2012).

Fig. 1

Distribution of the examined participants in all four groups, according to clinical diagnosis for proven, probable and possible fungal infection, with EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study group) criteria, are presented in Table 1. According to EORTC/MSG criteria, only a small percentage of patients had proven infection with *Aspergillus*. Of these, 20% (7/35) patients with primary deficiency, and 10% (3/30) patients with prolonged stay in ICU.

Differences in distribution of proven, probable and possible fungal infection with *Aspergillus* were statistically significant between group I versus groups III and IV, and between group II versus groups III and IV (Table 1).

Table 1

Mycological investigation of blood cultures in our patients demonstrated positivity in 4 specimens only. All positive blood cultures were discovered from patients with primary immune deficiency. *A.fumigatus* was identified as an etiological agent in all positive blood cultures (Table 2).

Table 2

With cultural analysis of bronchoalveolar lavage (BAL), presence of *Aspergillus* was most frequently demonstrated in the group of chronic aspergillosis (63.33%), followed by

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56.67% in the CF group, 51.43% in the group with primary immune deficiency, and 43.33% in patients hospitalized in ICU. Still, differences in positivity of blood cultures was insufficient for analysis of the statistical significance (*p=0.46*).

Regarding the fungal presence in positive BAL specimens, the most frequently identified species (79%) was *A.fumigatus* (53/67). Thirty two percent of the isolates (17/53) of *A.fumigatus* originated from the specimens of the patients with chronic aspergillosis, and 26% (14/53) of the isolates were identified in the specimens from patients both in the group with primary deficiency and cystic fibrosis (Table 3).

Table 3

Analysis of the antifungal susceptibility profile of *Aspergillus* species towards the antifungal agents demonstrated that MIC of amphotericin B was in the range from 0.19 to higher than 16 μg/mL, for all species. Regarding the antifungal susceptibility pattern of *A.fumigatus*, MIC of one isolate of *A.fumigatus* was 6 μg/mL, for another isolate the MIC was 4 μg/mL, and for all other 55 isolates of *A. fumigatus*, MIC was in the range from 0.19 to less than 2 μg/mL. Regarding the antifungal susceptibility profile of isolates of *A.flavus*, the MIC of amphotericin B was 0.19-8 μg/mL, and for 3 isolates of *A.terreus*, the MIC of amphotericin B was higher than 16 μg/mL.

Most isolates in our study demonstrated susceptible antifungal pattern to amphotericin B, except isolates of *A.terreus* which showed high MIC values towards amphotericin B (Table 4).

Table 4

Analysis of the antifungal susceptibility profile of *Aspergillus* species to itraconazole demonstrated that MICs of itraconazole were in the range 0.19-0.75 μg/mL, except for one isolate of *A.fumigatus*, with MIC higher than 4 μg/mL (Table 5).

Table 5

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Analysis of the antifungal susceptibility profile of *Aspergillus* species to voriconazole demonstrated that MICs of voriconazole were in the range 0.064-0.25 μg/mL for *A.fumigatus*, 0.25-0.38 μg/mL for *A.flavus* and 0.094-0.5 μg/mL for *A.terreus*. Cross resistance was not registered (Table 6).

Table 6

Analysis of the antifungal susceptibility profile of *Aspergillus* species to caspofungin demonstrated that MICs of caspofungin were in the range 0.094-0.125 μg/mL for *A.fumigatus*, 0.094-0.125 μg/mL, for *A.flavus*, and 0.125-0.19 μg/mL for *A.terreus* (Table 7).

Table 7

In table 8 are summarized MIC ranges of Amphotericin B, itraconazole, voriconazole and caspofungin towards different isolates of *Aspergillus* species.

Table 8

CLSI (Clinical and Laboratory Standards Institute) breakpoint values for amphotericin B, itraconazole and voriconazole for *A.fumigatus* are the following: R>2 mg/L (resistant), S≤1 mg/L (sensitive) (Bassetti et al., 2018). CLSI breakpoint values for amphotericin B for *A.flavus* are considered one concentration higher than the values for *A.fumigatus*. CLSI breakpoint values for *A.flavus* and *A.terreus* for itraconazole are R>2 mg/L (resistant), S≤1 mg/L (sensitive). CLSI breakpoint values for voriconazole for *A.flavus* and *A.terreus* are always considered one concentration higher. For caspofungin, testing of antifungal susceptibility is still not standardised (Bassetti et al., 2018).

In table 9, the antifungal susceptibility profile of isolates of *Aspergillus* to antifungal drugs is presented (amphotericin B, itraconazole, voriconazole and caspofungin).

Table 9

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Discussion

Aspergillosis is defined as an infection or disease caused by fungi from the genus *Aspergillus*, and can be manifested from allergic reactions to disseminated invasive disease in immunocompromised patients. Aspergillosis usually affects the respiratory system and manifests with wide spectrum of clinical symptoms and signs. *Aspergillus fumigatus* are common agents of invasive fungal infections worldwide (85 to 90%) (Latgé and Chamilos, 2019; Mousavi et al., 2016). However, other *Aspergillus* species have also become important emerging pathogens in the recent years (Richardson and Lass-Flörl, 2008). Criteria for the identification of IA have greatly benefited from the European Organisation for the Research and Treatment of Cancer (EORTC) and Mycoses Study Group (MSG) criteria for defining invasive fungal infections including IA (Tsitsikas et al., 2012). Due to high mortality (80-90%) of invasive aspergillosis, early diagnosis and antifungal treatment are key factors for favorable outcome. Still, diagnosis of invasive aspergillosis is a big diagnostic problem, and presents a big laboratory challenge because clinical symptoms and signs, as well as radiological signs are often non-specific, and conventional methods have low sensitivity (Bassetti et al., 2018). In the recent years, there is a progress in the development of new antifungal agents, which have potent activity against different mold species. Because the number of invasive infections caused by *Aspergillus* species has increased and resistance to established antifungal agents has been documented in many studies, determination of the *in vitro* antifungal susceptibility profile of *Aspergillus* species to established antifungal agents is necessary (Lass-Flörl et al., 2006).

In our study, the antifungal susceptibility profile of *Aspergillus* species towards amphotericin B, itraconazole, voriconazole and caspofungin, isolated from patients with increased risk for aspergillosis, was evaluated with E-test method. Specimens originated from patients that were classified into 4 groups according to EORTC/MSG (European Organization for Researsh and Treatment of Cancer/Mycoses Study group) criteria (Tsitsikas et al., 2012). The prevalence of resistance of *Aspergillus* towards antifungal agents is not very well known, since antifungal susceptibility testing in clinical laboratories is not performed routinely. In our study, MIC of Amphotericin B was in the range 0.19-16 μg/ml for all species. Most of the isolates of *A.fumigatus* demonstrated susceptible profile to Amphotericin B (MIC was in the range from 0.19 to less than 2 μg/mL), except 2 isolates which demonstrated resistance (MICs of both

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isolates were 4 and 6 μg/mL respectively). For *A.flavus*, the MIC of Amphotericin B was 0.19-8 μg/mL, and for 3 isolates of *A.terrus*, MIC for Amphotericin B was higher than ≥16 μg/mL. Similar results for antifungal susceptibility profile of *A.fumigatus* and *A.flavus* were obtained in the study of Denardi, where the MICs for Amphotericin B ranged from 0.06 to 1.0 μg/mL for *A.fumigatus*, but were much higher (0.5–8.0 μg/mL) for *A. flavus* (Denardi et al., 2018). In the study of Shi and coworkers, high MICs of Amphotericin B in isolates of *A.terrus* were also registered (Shi et al., 2010). Most of the isolates of *Aspergillus* in our study demonstrated a sensitive profile towards Amphotericin B, except the isolates of *A.terreus* which demonstrated higher MICs, probably reflecting the intrinsic resistance to Amphotericin B (Iwen et al., 1998).

In our study, most isolates of *Aspergillus* demonstrated relatively low MICs for itraconazole (range 0.19-0.75 μg/mL), except one isolate of *A.fumigatus*, which demonstrated resistance towards itraconazole with MIC >4 μg/mL. The first case of triazole resistant *A.fumigatus* have been reported in 1997 in patients previously receiving itraconazole (Denning et al., 1997). Although estimates of resistance in these fungi to azoles is not investigated enough, still, the wide use of azoles in patients with haematological conditions, especially for antifungal prophylaxis, could additionally contribute to resistance to azoles in these microbes. In the study of Howard and coworkers, resistance to azoles was detected in 6% of *A.fumigatus* isolates, mainly from patients with chronic pulmonary aspergillosis or immunodeficiencies, who have recently received azoles (Howard and Arendrup, 2011). A French study demonstrate prevalence of resistance towards azoles which was 0.85%, among 118 isolates of *A.fumigatus*, in a prospective study with 89 haematological patients, probably due to short exposition to azoles (Alanio et al., 2011). Recently, an epidemiological analysis of resistance to azoles, in *A.fumigatus* isolates of 762 patients with haematological patients, receiving transplantation of allogenic stem cells in two German centers (Steinmann et al., 2015). *A.fumigatus* was identified in 27 recipients haematopoetic stem cells, and 8 of them (30%) had invasive aspergillosis caused by azole-resistant *A.fumigatus*. Snelders and coworkers reported detection of 8 isolates of *A.fumigatus* resistant to itraconazole in haematological patients (Snelders et al., 2008). The occurrence of azoles resistant isolates of *A.fumigatus* varies worldwide, from 2.1–20% in the UK, 10–12% in the Europe, 10% in Asia, Africa, America and Australia to 1.75% in India, probably due to varying usage of azoles that may select for antifungal resistance (Shishodia et al., 2019). In our study, no resistance of *Aspergillus* species to itraconazole was demonstrated in

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isolates of *Aspergillus* among ICU patients. Similar results were demonstrated in the study of Rozaliyani and his co-workers, where they showed that all isolates from patients hospitalized in intensive care units were susceptible to amphotericin B, azoles and micafungin (Rozaliyani et al., 2021). Analysis of antifungal susceptibility testing of *A.fumigatus* from our CF patients, showed resistance to itraconazole in only one isolates, probably due to previous antifungal prophylaxis (MIC was higher than 4 μg/mL). An extensive Danish study about the resistance of *A.fumigatus* to azoles, from 159 totally examined patients with cystic fibrosis demonstrates an overall azole-resistance rate of 7.3% (Risum et al., 2020).

The risk for cross resistance between azoles is high. High percentage of itraconazole resistant isolates of *A.fumigatus* are cross resistant to voriconazole and posaconazole. Howard demonstrated that 74% and 65% itraconazole resistant isolates were cross resistant to posaconazole and voriconazole respectively (Denning et al., 1997). MIC of voriconazole among isolates of *A.fumigatus* in our study were in the range 0.064-0.125 μg/mL, 0.25-0.38 μg/mL for *A.flavus* and 0.094-0.5 μg/mL for *A.terreus*, and cross resistance was not detected. MIC of caspofungin was in the range 0.094-0.125 μg/mL for *A.fumigatus*, 0.094-0.125 μg/mL for *A.flavus*, 0.125-0.19 μg/mL for *A.terreus.*

Caspofungin is used as a second line treatment for invasive aspergillosis, due to its safe pharmacological profile. Resistance to this antifungal agent is rarely reported among isolates of *A.fumigatus* (Arendrup et al., 2009). The antifungal susceptibility pattern of *Aspergillus* species to caspofungin in our patients, demonstrated low MIC values (range 0.094-0.125 μg/mL for *A.fumigatus*, 0.094-0.125 μg/ml, for *A.flavus*, and 0.125-0.19 μg/mL for *A.terreus*). The antifungal susceptibility testing of caspofungin showed good antifungal activity against all isolates of *Aspergillus*, and similar results were obtained in the study of Shi et al. (2010).

Conclusions

Aspergillosis affects both immunocompetent and particularly immunocompromised patients. Accurate identification at the species level is of a crucial importance, since patient treatment and outcome depend on different antifungal susceptibility patterns of *Aspergillus* species. We identified 71 isolates of *Aspergillus* species, and the most frequent etiological agent was *A.fumigatus.* *In vitro* antifungal susceptibility testing demonstrated resistance in *Aspergillus*

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to amphotericin B in 6 patients (8.5%). Only one isolate of *A.fumigatus* was resistant to itraconazole. All isolates of *Aspergillus* species showed sensitivity to voriconazole and caspofungin.

In vitro antifungal susceptibility testing of *Aspergillus* species against antifungal drugs commonly used for treatment of invasive fungal infections, provides useful information for clinicians for appropriate choice of antifungal agents for the care and successful treatment of patients with aspergillosis.

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Резиме

Профил на антифунгална осетливост на *Aspergillus* species од пациенти со зголемен ризик за аспергилоза

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Клучни зборови: *Aspergillus*, мувли, инфекција, осетливост, Е-тест

Аспергилозата е најчестата габична инфекција предизвикана од мувли, особено кај лица со висок ризик. Терапијата на овие заболувања се заснова на примена на антифунгални средства од групата на полиени и азоли. Стапките на резистенција на *Aspergillus* species кон антифунгални средства варира значајно во различни медицински центри низ светот. Затоа, тестирањето на осетливоста на *Aspergillus* species на антифунгални средства може да обезбеди корисна информација за клиничарите за донесување на одлука во однос на терапијата на пациентот. Целта на оваа студија беше да го евалуира профилот на антифунгална осетливост на *Aspergillus* species кон амфотерицин

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Б, итраконазол, вориконазол и каспофунгин, изолирани од пациенти со зголемен ризик за аспергилоза.

Во тек на 2-годишен период, клинички примероци од 125 пациенти, поделени во 4 групи, според клиничката дијагноза и EORTC/MSG критериумите, беа анализирани на Институтот за микробиологија и паразитологија, на Медицинскиот факултет во Скопје. Овие групи вклучуваа пациенти со примарна имунодефициенција, критично болни лица лекувани на одделите за интензивно лекување, пациенти со хронична аспергилоза и цистична фиброза. Сите примероци (од респираторен тракт и хемокултури) беа анализирани со конвенционални миколошки методи, со инокулација на примероците на подлоги за подршка на раст на фунги. Идентификацијата на *Aspergillus* беше изведувана со макроскопска анализа на колониите на мувлите, и со дополнителна микроскопска анализа на нивните конидии со методот на лактофенол сино. Стрипови на Е-тест на вориконазол, итраконазол, амфотерицин Б и каспофунгин (АВ *bioMerieux*, Франција) беа користени за одредување на профилот на антифунгална осетливост на *Aspergillus* species кон овие антифунгални средства.

Кај нашите испитаници беа докажани 71 изолат на *Aspergillus* species. Четири изолати на *A.fumigatus* (5,6%) беа потврдени во позитивни хемокултури, од пациенти со примарен имун дефицит, а 67 изолати (94,4%) потекнуваа од примероци од респираторен тракт, од лица со различни претходни заболувања. *A.flavus* беше идентифициран кај 11 пациенти, а *A.terreus* кај 3 пациенти. Резистенција кон амфотерицин Б беше детектирана кај 6 изолати (2 изолати на *A.fumigatus*, 1 изолат на *A.flavus* и 3 изолати на *A.terreus*). Само еден изолат на *A.fumigatus* демонстрираше резистенција кон итраконазол. Сите изолати на *Aspergillus* species беа осетливи на вориконазол и каспофунгин.

Тестирањето на антифунгалната осетливост на *Aspergillus* species со Е-тест обезбедува корисна информација за клиничарите за соодветен избор на антифунгални лекови за терапија на аспергилозата.

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Table 1. Distribution of proven, probable and possible fungal infections according to EORTC/MSG criteria

	group I N=35	group II N=30	group III N=30	group IV N=30
n (%)	n (%)	n (%)	n (%)	n (%)
proven 10 (8%)	7 (20%)	3 (10%)	0	0
probable 68 (54.4%)	21 (60%)	19 (63.33%)	22 (73.33%)	6 (20%)
possible 47 (37.6%)	7 (20%)	8 (26.67%)	8 (26.67%)	24 (80%)
		[b]p < 0.001 I vs II *p=0.3* II vs III *p=0.345* III vs IV *p<0.001* I vs III *p=0.03** II vs IV *p<0.001* I vs IV *p<0.001*		

[a]p(Chi-square test) [b](Fisher exact test) **p<0.05 **p<0.01*

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Table 2. Positive blood cultures in four groups of patients

	group I N=35	group II N=30	group III N=30	group IV N=30
Blood cultures	n (%)	n (%)	n (%)	n (%)
Negative 121 (96.8%)	31 (88.57%)	30 (100%)	30 (100%)	30 (100%)
Positive 4 (3.2%)	4 (11.43%)	0	0	0
Blood cultures – species				
A. fumigatus n=4	4	0	0	0

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Table 3. Bronchoalveolar lavage (BAL) culture and identified fungal species

	group I N=35	group II N=30	group III N=30	group IV N=30
BAL culture	n (%)	n (%)	n (%)	n (%)
negative 58 (46.4%)	17 (48.57%)	17 (56.67%)	11 (36.67%)	13 (43.33%)
positive 67 (53.6%)	18 (51.43%)	13 (43.33%)	19 (63.33%)	17 (56.67%)
	Chi-square: 2.59 *p=0.46*			
Identified mold species in BAL				
A. fumigatus n=53	14	8	17	14
A. flavus n=11	2	4	2	3
A. terreus n=3	2	1	0	0
p(Chi-square test)				

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Table 4. Antifungal susceptibility profile (in MIC values) of *Aspergillus* species to amphotericin B

MIC values (μg/mL)	0.19	0.25	0.38	0.75	1.5	2	4	6	8	16
A.fumigatus (57)	11	21	9	12	2		1	1		
A.flavus (11)	3	1	1	2	2	1			1	
A.terreus (3)										3

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Table 5. Antifungal susceptibility profile (in MIC values) of *Aspergillus* species to itraconazole

MIC values (μg/ml)	0.19	0.25	0.38	0.75	1.5	2	4	8	16
A.fumigatus (57)	11	10	18	17			1		
A.flavus (11)			5	6					
A.terreus (3)			1	2					

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Table 6. Antifungal susceptibility profile (in MIC values) of *Aspergillus* species to voriconazole

MIC values (μg/mL)	0.064	0.125	0.25	0.38	1.5	2	4	8	16
A.fumigatus (57)	14	29	14						
A.flavus (11)			1	10					
A.terreus (3)	2			1					

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Table 7. Antifungal susceptibility profile (in MIC values) of *Aspergillus* species to caspofungin

MIC values (μg/mL)	0.094	0.125	0.19	0.25	0.38	1.5	2	4	8	16
A.fumigatus (57)	42	15								
A.flavus (11)	9	2								
A.terreus (3)		1	2							

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Table 8. MIC range of antifungal agents toward different *Aspergillus* species

Species No isolates (71)	MIC (μg/mL) min. – max.			
	Amphotericin B	Itraconazole	Voriconazole	Caspofungin
A.fumigatus (57)	0.19 – 6.0	0.19 – 4.0	0.064 – 0.125	0.094 – 0.125
A.flavus (11)	0.19 – 8.0	0.38 – 0.75	0.25 – 0.38	0.094 – 0.125
A.terreus (3)	≥16	0.38 – 0.75	0.094 – 0.5	0.125 – 0.19

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Table 9. Antifungal susceptibility profile of *Aspergillus* species towards amphotericin B, itraconazole, voriconazole and caspofungin

Species	amphotericin B		itraconazole		voriconazole		caspofungin	
N^{o} isolates (71)	S	R	S	R	S	R	S	R
A.fumigatus (57)	55	2	56	1	57	0	57	0
A.flavus (11)	10	1	11	0	11	0	11	0
A.terreus (3)	0	3	3	0	3	0	3	0

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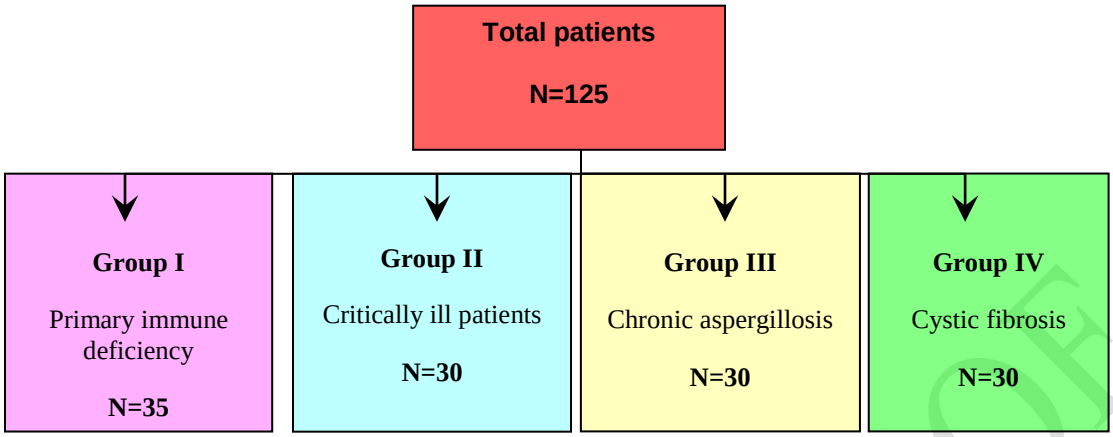

Fig. 1. Classification of patient groups according to clinical diagnosis and EORTC/MSG (European Organization for Researsh and Treatment of Cancer/Mycoses Study group) criteria.

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