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Prevalence and Susceptibility Testing of *Aspergillus* spp in Sudanese Patients Suffering from Uncontrolled Asthma and Cystic Fibrosis

SAMAR M. SAEED
HUMODI A. SAEED
KWATHAR A. M. SALIH
Microbiology Department
Sudan University of Science and Technology
WAFA I. ALHAJ
Microbiology Department, Al Neelain University
HISHAM N. ALTAYB
Microbiology Department
Sudan University of Science and Technology
EHSSAN H. MOGLAD
ESRAA KHALID
Department of Microbiology and Parasitology
Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan
MAZIN M. ALMOBASHAR
MWAHIB A. I. ISMAIL
Al shaab Teaching Hospital
Microbiology Department, Faculty of Medicine
University of Khartoum

Abstract

Background and objectives *Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder, occurring mostly in asthmatic and cystic fibrosis patients, due to an abnormal host T-helper 2 lymphocyte response to Aspergillus fumigatus antigens. Our aims were to detect the prevalence of Aspergillus spp in sputum specimens from asthmatic and cystic fibrosis patients*

Methods *This was descriptive cross- sectional study conducted during the period from February to August 2018 at El Shaab Teaching*

Hospital. Sputum specimens were collected, examined by direct microscopy using KOH and Gram's stain, and cultured on Sabouraud's Dextrose Agar. The isolated Aspergillus species were tested against Itraconazole and Voriconazole antifungals by E test Method.

Results *Out of a total of 100 asthmatic and cystic fibrosis patients, 10 (10%) Aspergillus spp were isolated, 7/10 (7%) , from asthmatic patients ,while 3/10 (3%) from cystic fibrosis patients . A. fumigtus 3 (30%) was the most frequent organism isolated from asthmatic patients while, 2(20%) A. flavus and 2(20%) A. terrus showed the same frequency among them. A. terreus 2 (20%) were isolated from cystic fibrosis patients followed by 1(10 %) A. niger.*

The minimum inhibitory concentrations (MICs) means of itraconazole was 1.9 ±2.46 (SD) and 0.5±0.49 (SD) for voriconazole. 4 (4%) Aspergillus isolates were sensitive to itraconazole , 2(2%) isolates were intermediate and 4(4%) isolates were resistant , while 9 (9%) isolates were sensitive to voriconazole and 1(1%) isolate was resistant .

Conclusion and recommendations *There is a significant prevalence of Aspergillus spp in Asthmatic and Cystic Fibrosis patients and this support the need to screen the asthmatic and Cystic fibrosis patients for fungal sensitization.*

Key words: Allergic pulmonary aspergillosis, Itraconazole, Voriconazole, Cystic fibrosis, Sudanese patients*, Aspergillus*

INTRODUCTION

Aspergillus species are saprophytic, thermo-tolerant fungi that survive and grow on organic debris and produce conidia, which humans inhale at the rate of hundreds per day without experiencing complications. *Aspergillus* species can produce a spectrum of diseases, including allergic bronchopulmonary Aspergillosis (ABPA), aspergilloma, chronic necrotizing aspergillosis , and life-threatening invasive aspergillosis (IA) [1].

Allergic bronchopulmonary aspergillosis (ABPA) is an immunological pulmonary disease resulting from a hypersensitivity to *Aspergillus* antigens, more specifically to *Aspergillus fumigatus* [2]. ABPA occurs mainly as a complication in patients affected by asthma or cystic fibrosis (CF) who have been sensitized to *A. fumigatus*[3], However, other Aspergillus species have been implicated including *A. niger, A. flavus, A. nidulans, A. oryzae and A. glaucus* [4].

The reported prevalence of ABPA is 1-2% in asthmatics, 7-14% in steroid-dependent asthmatics, and 2-15% in cystic fibrosis (CF) [5]. The fungal hyphae were commonly seen in mucoid plugs without evidence of tissue invasion due to hypersensitivity reaction (type I and III) to Aspergillus organisms that proliferate in the airway lumen, results in a constant supply of antigen with excessive mucus production and abnormal ciliary function [6] .

Clinical symptoms are recurrent wheezing, malaise with low-grade fever, cough, sputum production, and chest pain [4].This clinical diagnosis is confirmed by radiological and serological testing, mainly elevated total serum IgE, and sputum cultures [7].

Treatment of ABPA in asthmatic patients is the same as CF [8], It should consist of a combination of corticosteroids and itraconazole [9] . Oral corticosteroids therapy is used mainly to suppress the immunologic response to *Aspergillus* antigens [6] and antifungal therapy to lower the fungal antigen load in airways [10]. Ketoconazole has been used in ABPA, but has been replaced by the less toxic and more active agent, itraconazole [11]. Voriconazole and posaconazole are newer azoles whose efficacy in asthma- and CF-ABPA patients has been demonstrated in some studies [12, 13]. However these drugs showed higher toxicity compared to itraconazole due to their stronger inhibitory effect on hepatic cytochrome P450 enzymes [14].

To the best of our knowledge, there have been no studies in our country that has detailed the fungal biota in sputum from patients with asthma and cystic fibrosis.

MATERIALS AND METHODS

The study was a cross-sectional study conducted at Alshaab Teaching Hospital, Omdurman Chest Hospital and Omer Sawy Hospital Khartoum, Sudan. The study was carried out during period from June 2016 to April 2018, to determine the prevalence antifungal susceptibility of *Aspergillus* spp among Sudanese patients suffering from asthma and cystic fibrosis.

Early morning deep cough sputum specimens were collected in sterile clean dry containers.

All sputa were processed by adding one drop of 10% KOH and the slides were prepared and examined by low power field firstly and then by high power field .Then dry smears were done also for all specimens and stained by Gram s stain followed by examination with oil immersion lense.

Isolation and Identification

Sputa were inoculated by streaking on two tubes of Sabaroaud's dextrose agar with chloramphenicol and incubated at 25 C° and 37 C° up to one week with daily examination.

The isolates were identified by macroscopic appearance (Surface topography, texture and pigment) and microscopically by lacto phenol cotton blue preparation and slide culture as per standard recommended procedures. The microscopic characteristics for the identification were conidial heads, stipes, color and length, vesicles shape and seriation , metula covering, conidia size, shape and roughness [15].

Antifungal susceptibility testing

Inoculum was prepared according to M51-A and M38A2 CLSI guidelines. To induce conidium the isolates were grown on potato dextrose agar (PDA) slants at 35°C for 2–7 days before testing. The suspensions of conidial inocula were prepared from 2-7 days old cultures grown on PDA slants. The turbidity of the cell suspension was adjusted by spectrophotometry to an optical density of 0.09 to 0.13 for *Aspergillus* spp [16].

The plates were inoculated with conidial suspensions prepared in sterile saline and adjusted by swabbing the RPMI 1640 medium containing L-glutamine and buffered to pH 7.0 with 0.165 M MOPS in three directions. After excess moisture was absorbed into the agar and the surface was completely dry, E test strips (Itraconazole and voriconazole concentrations ranging from 0.002 to 32 g/ml) were applied to the RPMI dextrose agar and incubated at 35°C for 48 hrs.

The MICs were determined after 24 and 48 h of incubation at 35°C. The E test MIC was read as the drug concentration at which the border of the elliptical inhibition zone intersected the scale on the antifungal test strip, the obtained MICs for itraconazole and voriconazole interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUSAT) interpretation criteria.

Statistical analysis:

The collected data was analyzed using the computer program SPSS software version 25 for windows. Data were presented in form of tables and figures. Frequencies mean and standard deviation were calculated.

RESULTS

A total of 100 patients with asthma and cystic fibrosis diseases were participated in this study, Their age ranged from 19 to 86

years and mean ± STD (44 ±13.4 years). Out of them, 56 (56%) were males while 44 (44%) were females. The distribution of disease among study group gender was 41/30, (41% / 30%) asthmatic female/male and 15/14, (15% / 14%) female/male cystic fibrosis patients .figure (1).

The predominant age group was (19-49) years which represents (66%) of the study group. Female were 41(41%) , while male was 25(25%) in age group 19-49 years and 19(19%) female and 15 (15%) male for ≥ 50 years age group Table (1).

All sputum specimens examined directly with 10% potassium hydroxide wet mount and Gram's staining and then inoculated on SDA for isolation, ten *Aspergillus* spp were isolated, 7 (7%) from asthmatic patients (3 *A. fumigatus* , 2 *A. flavus* and 2 *A. terrus*) while 3(3%) isolates (2 *A. terreus* and 1 *A. niger*) isolated from cystic fibrosis patients. as shown on table (2).

E test results for Itraconazole against *Aspergillus* spp showed Itraconazole MIC mean was 1.9 ± 2.4 (SD) while voriconazole mean 0.5 ± 0.49 (SD) and 4 (40%) *Aspergillus* isolates were sensitive to itraconazole, 2(20%) Intermediate and 4(40%) isolates were resistant, while 9(90%) isolates were sensitive to voriconazole and 1(10%) isolate was intermediate as displayed in figure (3). Figure (4) and (5) displayed the results of itraconazole and voriconazole against *A.fumigatus, A. flavus , A. terreus* and *A. niger* by E test.

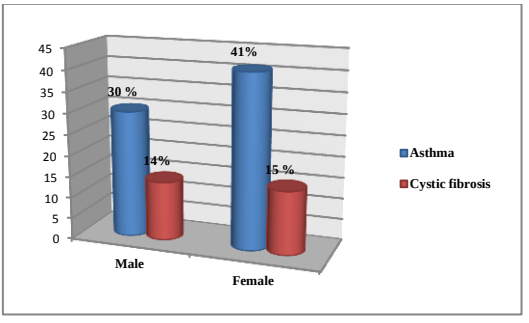

Figure (1): Frequency of disease type among study group

Table 1: Frequency of gender and age group among case group

Age group/years	Male	Female	Total
19-49	25(25%)	41(41%)	66(66%)
≥50	19(19%)	15(15%)	34(34%)
Total	44(44%)	56(56%)	100(100%)

Table 2: Frequency of *Aspergillus* spp in asthmatic and cystic fibrosis patients

Disease	*A. fumigatus*	*A. flavus*	*A. terrus*	*A. niger*
Asthma	3(30%)	2 (20%)	2(20%)	0 (0.0%)
Cystic fibrosis	0 (0.0%)	0 (0.0%)	2(20%)	1 (10%)
Total	3 (30%)	2 (20%)	4 (40%)	1 (10%)

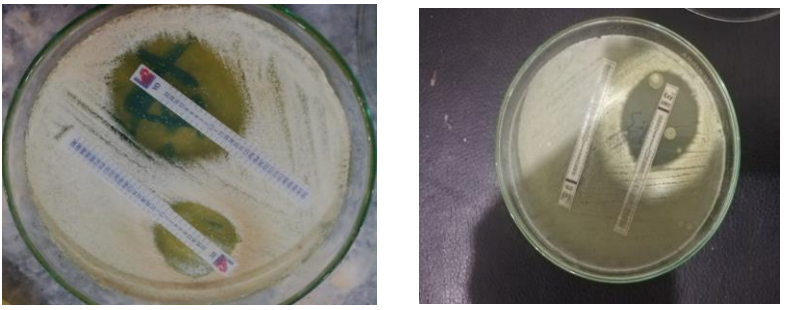

Figure (2): E-test gradient strips of Itraconazole and Voriconazole antifungal agents showing susceptibility of *A. terreus* (Left) and *A. fumigatus* (Right).

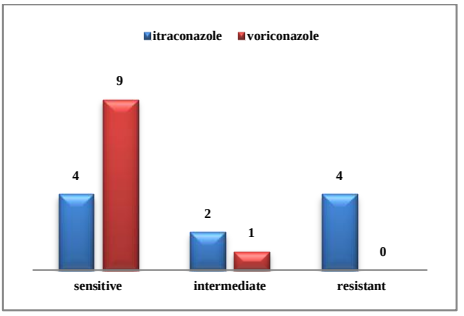

Figure (3): Minimum inhibitory concentrations of itraconazole and voriconazole

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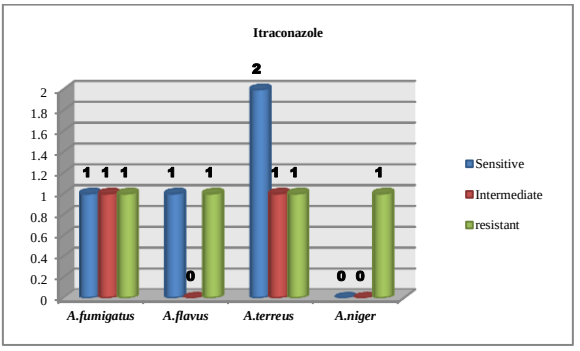

Figure (4): E test of itraconazole against different *Aspergillus* isolates.

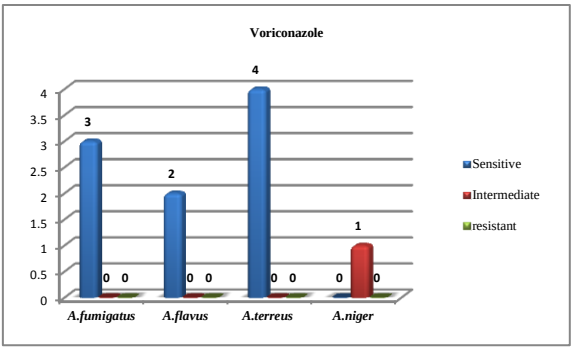

Figure (5): E test of voriconazole against different *Aspergillus* isolates

DISCUSSION

Aspergillus-related lung diseases are traditionally depend on the immunologic status of the host and the existence of an underlying lung disease [17]. little is known about the diagnostic significance of isolating *Aspergillus* spp. from respiratory cultures in immunocompetent patients with aspergillosis other than invasive pulmonary aspergillosis (IPA) [18]

In this study, the frequency of fungal infection and antifungal susceptibility of *Aspergillus* spp. were examined among Sudanese patients with cystic fibrosis and uncontrolled asthma.

The study population was 100 patients suffering from asthma and cystic fibrosis most of them were female with female/male ratio about 1.2:1. The frequency of asthmatic patients was 71(71%) and 29 (29%) was of cystic fibrosis patient.

Most of the isolated fungi were *A. terreus* followed by *A. fumigatus*, *A. flavus* and *A. niger*, this in consistent with Laham [19] *et al* who isolated *A. terrus* from asthmatic patient . *A. fumigatus* was predominant isolate among asthmatic patients followed by *A. terrus* and

A. flavus this may be due to small spore size that permits them to bypass the filtering system of the upper airways and continue deposition in the distal small airways also the thermo tolerant growth properties which allowing them to grow at body temperature, this finding in agreement with Shivananda [20] in his study in 1992 on 825 patients with pulmonary infections who found (15.39%) of isolates were *Aspergillus* spp of these, *A. fumigates was* (11.15%), *A. niger* was (3.2%) and *A. flavus* was(0.96%).

Similar results obtained by Kurhade [21] and his colleagues in India who documented that *A. fumigatus* was predominantly isolated from asthmatic patients , in contrast this finding was dis agree with Laham [19] *et al* who isolated *A. terreus* from asthmatic patient predominantly.

In the present study *A. terreus* was the most commonly isolated *Aspergillus* spp from cystic fibrosis patients followed by *A. niger*, this is different from study done by Tashiro [18] and his colleagues who reported *A. niger* (40%) was most frequently *Aspergillus* spp associated with patients diagnosed with ABPA. Mortensen [22] *et al* found that *A. fumigatus* was the most common species (37.2% and 33.2% of the cases) isolated from patients with cystic fibrosis followed by *A. flavus* (4.1% and 4.4% of the cases) and *A. terreus* (1.9% and 2.6% of the cases).

Many previous studies found the E-test a very simple method for determination of susceptibility profile of filamentous moulds to antifungals with few exceptions [22, 23].

In the present study the results of *in vitro* susceptibility testing of 10 isolates of *Aspergillus* species by E test showed that voriconazole (MIC mean 0.5 ± 0.49) was more active than itraconazole (MIC mean 1.9 ± 2.4) .This is in accordance with the study results of Verweij [25] *et al*(2002) in Netherland who evaluated *In vitro* activities of Itraconazole and voriconazole against *A. fumigatus* , the resistance to itraconazole was seen in (25%) of isolates, while (7.14%) of isolates were resistant to voriconazole, another study done by Lalitha [26] *et al* (2007) who reported that voriconazole and itraconazole had the lowest MICs against *Aspergillus* spp in compare with other fungi included in his study.

The present study demonstrates the excellent efficacy of voriconazole against all *Aspergillus* species and suggest that voriconazole may be the treatment of choice in pulmonary aspergillosis caused by these organisms ,Similar result was reported by Diekema[27] *et al* who documented the new triazoles posaconazole, ravuconazole, and voriconazole have excellent *in vitro* activity against *Aspergillus* spp.

CONCLUSION

In summary, Asthma and Cystic Fibrosis are major predisposing factors for *Aspergillus* spp. colonization and infection. For this reason, patients at risk, the antifungal treatment should be considered in the presence of clinical features and isolation of *Aspergillus* spp. from respiratory secretions. In contrast, antifungal treatment should not be initiated when *Aspergillus* spp. are recovered from respiratory specimens of patients without predisposing risk factors and in the absence of clinical and radiological signs of the disease.

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