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STUDY SOME VIRULENCE FACTORS OF ASPERGILLUS SPP THAT ISOLATED FROM CANCER PATIENTS WHO ARE SUFFERING FROM CHEST RESPIRATORY DISEASE

Faiza Majid Najm1 ; Sanaa Hussein Mohammed1
1. Biology Department, College of Science, Kirkuk University, Iraq
fayzanajm970@gmail.com

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Abstract:

The present study was designed to Isolation and diagnosis of Aspergillus species from cancer patients. A total of two hundred fifty-six (256) were collected from patients (sputum sample), aged between (11-<61 years) who visited the tumor center in Kirkuk city at a period from November 2020 to November 2021. In the current study, the sputum specimens were collected from patients with respiratory tract cancer and infection. 256 samples were directly examined by using microscopic with 10% KOH solution from which a total of 143 samples were found positive while other, 113 samples were negative. *A. fumigatus* showed a percentage of 100%, followed by *A. niger* with a percentage of 92.3%. While, the lowest percentage of urease production was by *A. parvisclerotigenus*, that reached 50%. *A. fumigatus* showed a percentage of 70.3%, followed by *A. niger* with a percentage of 53.8%. While, the lowest percentage of protease production was by *A. oryzae*, that reached 20%. *A. fumigatus* showed a percentage of 100%, followed by A. *flavus* with a percentage of 94.7. While, the lowest percentage of hemolysin production was by *A. parvisclerotigenus*, that reached 0%. it is concluded that the fungus of Aspergillus species has different abilities to produce virulence factors, and the most dangerous species is A. fumigatus.

Keywords: Aspergillus, protease, urease, virulence factors.

INTRODUCTION

Isolation of Candida spp. and, at a lower rate, Aspergillus spp. from respiratory samples in patients admitted in the Intensive Care Unit (ICU) is frequent [1]. Isolation of other filamentous fungi as *Mucorales, Scedosporium Fusarium* is by far less frequent, but these fungi are associated with high mortality in critically ill patients. Fungal pulmonary involvement presents particular characteristics that complicate the patient's management. Presence of fungi may represent a true infection although frequently it only implies colonization of the respiratory tract, leading to a very different management and prognosis. Discrimination between colonization and infection is not easy and, frequently, antifungal treatment is initiated associated with an increase in adverse events and costs [2]. On the other hand, fungal infections are associated with high mortality in those cases where treatment initiation is delayed. In last years, new antifungals and new routes of administration for some of them have been introduced in the clinical practice, thus complicating treatment election by treating clinicians. Lastly, most available information regarding diagnosis and treatment of fungal respiratory infections (especially in the case of *Aspergillus spp.*) was referred to neutropenic onco-hematological patients and cannot always be extrapolated to the critically ill patient. Approaches to critical issues as epidemiology, diagnosis, discrimination between colonization and infection, treatment and prevention of fungal respiratory infections were addressed [3-4]. Each participant presented a review of a critical issue, which afterwards was discussed by all participants that approved the consensus recommendation. No grades of the quality of the evidence or strength of recommendations were used. The objective was to elaborate practical recommendations based on scientific evidence, when available, or on expert opinions for the management of fungal respiratory infections caused by *Candida spp., Aspergillus spp. and Zygomycetes* in the critically ill patient, including solid organ transplant recipients [3-6]. Invasive pulmonary aspergillosis (IPA) is a severe disease, and can be found not only in severely immunocompromised patients, but also in critically ill patients and those with chronic obstructive pulmonary disease (COPD) [7]. IPA was first described in 1953 [8]. Due to widespread use of chemotherapy and immunosuppressive agents, its incidence has increased

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over the past two decades [9]. Of all autopsies performed between 1978 and 1992, the rate of invasive mycoses increased from 0.4% to 3.1%, as documented by Groll et al. [10]. IPA increased from 17% to 60% of all mycoses found on autopsy over the course of the study. The mortality rate of IPA exceeds 50% in neutropenic patients and reaches 90% in haematopoietic stem-cell transplantation (HSCT) recipients (Fukuda et al., 2003).

MATERIALS & METHODS

Sample collection

A total of two hundred fifty-six (256) were collected from patients (sputum sample), aged between (11-<61 years) who visited the tumor center in Kirkuk city at a period from November 2020 to November 2021. The collection was started first by sterilizing the mouth and gargling with saline solution in the early morning and the sample was taken from each patient and placed in sterile glass bottles, and the investigation was conducted the initial examination for the presence of fungi was followed by direct microscopy using an amount of potassium hydroxide 10% KOH, at the same time the samples were cultured using Swabs by passing them on the surface of sterile plastic or glass Petri dishes containing Dextrose Sabouraud Agar , after which it was incubated at 37 0C for 24–72 hours.

Direct examination:

Specimens were placed on microscopic slide, with few drops of 10% KOH, (cover slip added and warmed up over a light flame just under boiling point). The slide was examined under the low power 40x and high power 100x objectives to detect fungi and their septet hyphae [12].

Sample Culturing

Sputum Samples were cultured on SDA supplemented with 0.04 mg/mL chloromphenicol to inhibit the growth of bacteria, then incubated at 28 °C and 37°C and examined for 10 days [13].

Laboratory diagnosis of fungal isolates

The diagnosis was based on the phenotypic characteristics of the colonies, including the colony's shape, color, size, and texture. As for the microscopic characteristics, they included the shape and color of the fungal thread and the coids. This was done by transferring part of the fungal colony using a sterile vector on a glass slide and using the cotton blue lactophenol dye, then examined under the microscope for observation Microscopic traits of mycelium [14].

Tests used to detect virulence factors

Urease test

The urea medium was prepared according to the manufacturer's instructions, and this medium was used to test the ability of molding to produce the urea-dissolving enzyme urease, and the change of the color of the medium from yellow to pink is evidence of urease production.

Protease Test

The ability of the isolated fungi to produce protease enzyme was tested using milk media.

Hemolysin test

This test was used to identify the ability of fungi to hemolysis. after incubated for (1-) 5) days and at a temperature of 37 °C, the appearance of transparent halos around the implantation area is a positive result.

RESULTS AND DISCUSSION

4.1. Isolation of fungi from the lower respiratory tract (LRT) of infected cancer patients

In the current study, the sputum specimens were collected from cancer patients with respiratory tract infection. 256 samples were directly examined by using microscopic with 10% KOH solution from which a total of 143 samples were found positive while other, 113 samples were negative (table 1). In the absence of metulae, hyphae and dichotomous were observed as branching, conidial heads and chains basipetally from phialides, whilst chains of conidia and vesicles were born directly. Using the above Potassium hydroxide alkaline solutions (KOH) the fungus samples would remain unaffected and easily recognizable from other mixed substances [12]. Both, microscopic examination and culturing methods had produced same results.

Table (1): Distribution of positive and negative cultured cases according to both diagnosing procedure adopted.

Procedures	Samples	Positive samples		P value
		No.	%	
Direct examined by 10% KOH	256	143	55.85	0.183
Culturing Procedure	256	143	55.85	0.152

Virulence factors

Urease production

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Table (2) shows the ability of all Aspergillus isolates to produce urease enzyme, but in different proportions. A. fumigatus showed a percentage of 100%, followed by A. niger with a percentage of 92.3%. While, the lowest percentage of urease production was by A. parvisclerotigenus, that reached 50%.

Table (2): The ability of isolated samples to produce urease enzyme

Isolate	Urease production				Total
	Positive		Negative		
	Number	%	Number	%	
A. fumigatus	37	100	0	0	37
A. flavus	17	89.5	2	10.5	19
A. niger	12	92.3	1	7.7	13
A. terreus	6	75	2	25	8
A. oryzae	4	80	1	20	5
*A. **tropicalis***	2	50	2	50	4
Total	**78**	**90.7**	**8**	**9.3**	**86**

The results of the current study agree with the study of Zohri et al., [15], which showed that most of the isolated species during the study were urease producers, and all isolates of A. fumigatus and A. flavus produced urease, and the type A. niger followed in terms of percentage of Percentage of urease results. Many microbes that cause different diseases of the human body have the ability to use urea as a source of nitrogen through their production and secretion of the enzyme urease, which converts urea to ammonia and carbamic acid, which in turn decomposes spontaneously to form ammonia [16].

Protease production

Table (3) shows the ability of all Aspergillus isolates to produce protease enzyme, but in different proportions. A. fumigatus showed a percentage of 70.3%, followed by A. niger with a percentage of 53.8%. While, the lowest percentage of protease production was by *A. oryzae*, that reached 20%.

Table (3): The ability of isolated samples to produce protease enzyme

Isolate	Protease production				Total
	Positive		Negative		
	Number	%	Number	%	
A. fumigatus	26	70.3	11	29.7	37
A. flavus	10	52.6	7	47.8	19
A. niger	7	53.8	6	46.2	13
A. terreus	3	37.5	5	62.5	8
A. oryzae	1	20	4	80	5
*A. **tropicalis***	2	50	2	50	4
Total	**78**	**90.7**	**8**	**9.3**	**86**

Pathogenesis of aspergillosis is dependent on various factors of the host (immune status) and virulence factors of the pathogen. Some putative virulence factors have been identified for different

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Aspergillus species. These include adhesions e.g., biofilm production and haemolysin, pigments hydrolytic enzymes such as proteases, proteinase, lipase, phospholipases, α-amylase, low-molecular-weight, non-protein metabolites [17-18]. The current study is agrre with Singh and Urhekar [19] who referred that A. fumigatus showed proteinase 76.7% (23/30) and phospholipase activity 93.3% (28/30).

Hemolysin production

Table (4) shows the ability of all Aspergillus isolates to produce hemolysin enzyme, but in different proportions. A. fumigatus showed a percentage of 100%, followed by A. *flavus* with a percentage of 94.7. While, the lowest percentage of hemolysin production was by A. parvisclerotigenus, that reached 0% as howen in figure (1).

Table (4): The ability of isolated samples to produce Hemolysin

Isolate	**Hemolysin** production				Total
	Positive		Negative		
	Number	%	Number	%	
A. fumigatus	37	100	0	0	37
A. flavus	18	94.7	1	5.3	19
A. niger	10	76.9	3	23.1	13
A. terreus	5	62.5	37.5	25	8
A. oryzae	4	80	1	20	5
A. tropicalis	0	0	4	100	4
Total	**78**	**90.7**	**8**	**9.3**	**86**

The ability of some fungi to hemolyes in vitro may explain the presence of factors of virulence such as enzymes or toxins capable to damage the tissues and causes various diseases to humans [20]. Hemolysis is the ability of microbes to breakdown the red blood cells by the action of hemolysins, erythrocyte-lysing enzymes that it produces. Colonies to induce hemolysis when grown on blood agar is used to classify certain microorganisms. Blood hemolysis detection requires a culture medium for bacterial growth and a source of blood as a culture medium supplement. In this study formulated media were used instead of blood base agar and supplemented with blood to obtain hemolysis.

The results of the current study agreed with the study of Zarrin [21], which confirmed that all isolates of A. fumigatus that were grown on sheep blood medium produced hemolysin, and this was confirmed in the same study by bimolecular detection of the gene. Mezher et al. [22] indicated that A. fumigatus produces hemolysin with a percentage of 62.5%.

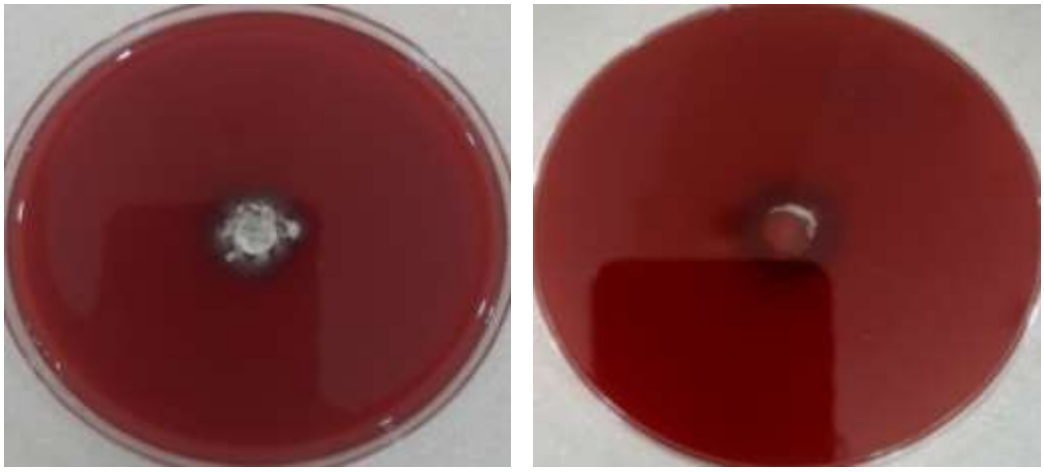

Figure (2): Hemolysis test results of Aspergillus.

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CONCLUSION

Based on the results of the current study, it is concluded that the fungus of Aspergillus species has different abilities to produce virulence factors, and the most dangerous species is A. fumigatu.

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