

Exhalation Air Fungal Culture of Mechanically Ventilated Aspergillosis Suspected Patients in Concordance with Other Conventional Diagnostic Techniques

M Bassam Aboul-Nasr^{1*}, Abdel-Nasser Zohri², Mohamed Adam³ and Enas Mahmoud Amer²

¹Department of Botany and Microbiology, Faculty of Science, Sohag University, Egypt

²Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt

³Department of Chest Diseases and Tuberculosis, Faculty of Medicine, Assiut University, Egypt

*Corresponding author: M Bassam Aboul-Nasr, Department of Botany and Microbiology, Faculty of Science, Sohag University, Egypt, Tel: 01007221923; E-mail: mbassamaboulnasr@yahoo.com

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Abstract

Aspergillosis remains one of the most challenging areas of illness. It has recently emerged as a world-wide health care problem, owing to extensive use of broad spectrum antibiotics, immunosuppressive agents and increasing population of terminally ill and debilitated patients. The ultimate aim of this investigation is to minimize morbidity and mortality of mechanically ventilated aspergillosis suspected patients, who have been notoriously difficult to be diagnosed and treated, by developing an accurate and easy diagnostic tool comparable to other conventional methods. Two hundred twenty seven suspected aspergillosis patients were screened for fungal infection using different conventional tools to evaluate different respiratory specimens sputum, bronchoalveolar lavage, plural fluid, blood, biopsy and ELISA technique for galactomannan and (1-3)- β -D-glucan antigens in serum and bronchoalveolar lavage. Four *Aspergillus* species (*A. niger*, *A. fumigatus*, *A. flavus* and *A. terreus*) were isolated and identified morphologically and genetically using the conventional diagnostic tools from suspected patients in all positive cultures. The results of the exhalation air of twenty three mechanically ventilated aspergillosis suspected patients came in accordance with those of the conventional methods used. Thus, exhalation air culture is an easily performed bedside tool for diagnosis of aspergillosis in ventilated patients.

Keywords: Aspergillosis; *Aspergillus* species; Diagnostic tools

Introduction

The problem of fungal infections became serious in 1960s, when wide-spectrum antibiotics and cytostatic agents entered widespread use [1]. Aspergilli cause a wide range of human ailments depending on the immune status of the host [2]. *Aspergillus* infection is the commonest invasive fungal infection which involves respiratory tract [3,4]. Among the human pathogenic species of *Aspergillus*, *A. fumigatus* is the primary causative agent of human infections, followed by *A. flavus*, *A. terreus*, *A. niger*, and the model organism, *A. nidulans* (*Emericella nidulans*) [2,5]. Aspergillosis developed widely in developing countries that are home to around five-sixth of the world's population and located in the tropical and sub-tropical regions of Asia, Africa and South and Central America where fungi thrive well in the hot and humid climate [6]. There was a 35.7% increase in mortality caused by invasive aspergillosis between 1980 and 1997. The clinical symptoms and signs associated with invasive pulmonary aspergillosis are notoriously vague. An accurate diagnosis of invasive aspergillosis is important for clinical reasons; an earlier diagnosis is associated with improved patient survival and tests with a high negative predictive value may allow expensive and potentially toxic antifungal drugs to be withheld [7]. Diagnosis is based on a combination of clinical risks, symptoms and signs, culture, histopathology and detection of the fungal components such as the antigen galactomannan and direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI) [8]. Radiology also plays an

important role in diagnosis and follow-up. Early findings on a computed tomography (CT) of invasive pulmonary aspergillosis are ground-glass attenuation surrounding a pulmonary nodule, the halo sign [9].

The ultimate aim of this study is to minimize morbidity and mortality in mechanically ventilated aspergillosis patients, which has been notoriously difficult to be diagnosed and treated, by developing an accurate and easy diagnostic tool for them comparable to other conventional tools.

Material and Methods

Mycological assay

Conventional methods:

1. Sputum specimens: Sputum specimens were collected from the patients in sterile cubs. Five ml of Sabouraud dextrose broth medium were added to each specimen and shaken for one minute on vortex and incubated at $37 \pm 2^\circ\text{C}$ for two hours to be liquefied.

2. Bronchoalveolar lavage (BAL): Bronchoscopy was done according to BTS guideline 2013 for diagnostic flexible bronchoscopy in adults.

3. Plural fluid: Plural fluid was aspirated by thoracentesis under aseptic condition.

One ml from each (prepared sputum, bronchoalveolar lavage, plural fluid) specimens were transferred aseptically into three sterilized nine

cm Petri dishes and 15-20 ml of pre-solidifying Sabouraud dextrose agar medium [10] were added to each plate. The plates were shaken slowly clockwise and anticlockwise to ensure homogenous distribution of the specimens in the agar media. The plates were transferred into sterilized plastic pages and incubated on $37 \pm 2^\circ\text{C}$ to distinguish opportunistic *Aspergillus spp* from other nonpathogenic environmental molds for 7-10 days [11].

4. Blood samples: One ml of blood sample was inoculated into triplicate sterilized Petri dishes containing pre-solidified 15-20 ml of Sabouraud dextrose agar medium. The plates were incubated on $37 \pm 2^\circ\text{C}$ for 7-10 days [12].

5. Biopsy: Biopsy sample was inoculated into pre-solidified Sabouraud's agar plates. Fungal growth was recorded after four days at $37 \pm 2^\circ\text{C}$. Plates with no growth after 7-10 days were considered sterile [13].

6. ELISA techniques: Uncentrifuged BAL and serum specimens were sent to El-Mokhtabar lab for galactomannan (GM) and (1-3)- β -D-glucan (BDG) enzyme immunoassay according to the manufacturer's specifications (*Platelia Aspergillus*, Bio-Rad, Marnes la Coquette, France) [14]. GM and BDG enzyme immunoassay test results were interpreted as positive when an optical density index of greater than or equal to 0.5 [15].

B. Exhalation air fungal culture technique:

Three Petri dishes containing Sabouraud dextrose agar medium in sterilized plastic pages opened and inverted were exposed to the exhalation air of the tracheal tube of the ventilator of the ventilated patients and incubated on $37 \pm 2^\circ\text{C}$ for 7-10 days. Number of colonies were estimated/cm³ of air/minute.

Results and Discussion

Aspergillosis is a serious infection which is difficult to diagnose due to the lack of definitive diagnostic criteria [16]. Number of methods for the diagnosis had been applied [17,18].

Two hundred and twenty seven patients were screened for aspergillosis suspected fungal species with different conventional methods. According to these methods, the results showed the presence of four species of *Aspergillus* which were *A. niger* (EN2 KY 6095), *A. fumigatus*, *A. flavus* (EN1 KY 609) and *A. terreus*. *Aspergillus niger* was the dominant species representing 32% of the total isolates

followed by *A. fumigatus* and *A. flavus* with almost the same percentage (29%) and finally *A. terreus* came with the least appearance between isolates (10%) (Figure 1). No significant differences in species appearance were noticed among the different conventional methods. Furthermore, sputum cultures weren't sensitive enough that gave only 27.3% positive cultures from the total specimens tested, while blood cultures gave 42.8% positive cultures and the bronchoalveolar lavage gives 75% positive cultures (Table 1). False negative or positive results might have been occurred in one or more of these methods. Fungal infections are undoubtedly rare causes of pleural effusions but it is still important to recognize them [19]. Only one patient was suspected with aspergillosis during our study period and had pleural effusions. Culture of his pleural fluid reflects a fungal infection with *A. niger* and *A. fumigatus* and the patient had a dramatic improvement after antifungal treatment depending upon our fungal culture results. Forty one samples of serum and eight of BAL samples which were subjected to conventional culture technique in this study and proved to be positive cultures were subjected to ELISA technique for both GM and BDG antigens. The results confirmed the results of cultures with 75% positive in BAL galactomannan and 51.21% and 60.97% positive in serum GM and BDG, respectively which in power with our results (Table 1). These results revealed that BAL galactomannan gave higher rate of positive results than serum galactomannan while no statistical differences were recorded between galactomannan and BDG in serum.

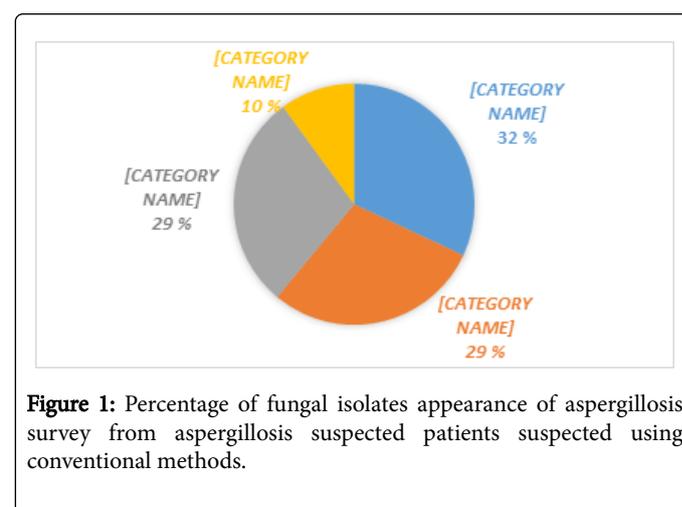


Figure 1: Percentage of fungal isolates appearance of aspergillosis survey from aspergillosis suspected patients suspected using conventional methods.

	Culture										ELISA technique					
	Sputum		Blood		BAL		Pleural fluid		Biopsy		BAL GM		Serum GM		Serum (1-3)- β -D-glucan	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Positive	41	27	21	43	8	32	1	100	1	50	6	75	21	51	25	61
Negative	109	73	28	47	25	68	0	0	1	50	2	25	20	49	16	30
Total	150	100	49	100	33	100	1	100	2	100	8	100	41	100	41	100

Table 1: Percentage of positive fungal specimens from aspergillosis suspected patients using conventional methods.

Still identification in culture is the gold standard for the diagnosing of aspergillosis (simple and low cost) but may not be feasible due to the long time required and difficulty in obtaining samples in some cases of

patients, lack of sensitivity and the difficulty in distinguishing between infection and colonization [20]. Also, sputum specimens are often contaminated with upper respiratory tract and mouth biota.

Additionally young children are unable to reliably expectorate (cough up) sputum [21]. For these reasons Robert et al. [22] concluded in their study that the use of BAL to identify potential pathogens in the lower respiratory tract of adult patients is usually well tolerated and provides useful information. Radha et al. [23] confirmed the advantages of using of BAL as an accurate and sensitive diagnostic tool for aspergillosis. Blood cultures were effective only in cases of fungemia. It is supposed that many cases of invasive mycoses remain undiagnosed, mostly because of the insufficient sensitivity of blood cultures, which has been estimated as 50% [24]. In contrast with Nawrot et al. [25] who showed that the culture of fungi from normally sterile tissue, including blood, remains an essential diagnostic test for invasive mycoses and the addition of a mycosis medium to the standard set of aerobic and anaerobic vials improves the sensitivity of the detection of fungi and reduces the detection time and a growing number of fungemia cases have been observed in recent decades. Shuwei et al. [26] said that nearly a quarter of fungemia may be missed if a fungal blood culture is not performed. The early detection of diagnostic markers of a fungal infection, such as fungal nucleic acids, antigens, antibodies or cell wall components, is essential. For example, galactomannan antigen (GM) is widely used in the diagnosis of invasive aspergillosis. Another serum marker for the presence of invasive aspergillosis is (1-3)- β -D-Glucan (BDG), which has been included in the relevant diagnostic criteria of the European Organization for Research and Treatment of Cancer/ Mycoses Study Group [27]. Galactomannan (GM) is a polysaccharide Galactomannan, a major component of the *Aspergillus* cell wall, is released during invasive disease, and the level of circulating serum or plasma GM is indicative of the fungal burden in the host. This antigen serves as a biomarker of *Aspergillus* disease [28]. It has been documented that serum GM can be detected at approximately 7-14 days before other diagnostic clues become apparent, and therefore, monitoring of GM may allow initiation of preemptive antifungal therapy before establishment of the corresponding life-threatening infection [29]. The GM assay appears to be more specific but may be less effective during the early stage of infection [27]. Detection of GM in bronchoalveolar lavage (BAL) fluid has been advocated as a sensitive test for diagnosing of invasive aspergillosis, both in hematology and non-hematology patients, including solid-organ transplant recipients and critically ill patients. probabilities [30]. The polysaccharide BDG is a component of the cell wall of many fungi. Measuring the serum or plasma BDG levels has a high level of accuracy in the discrimination of patients with and without invasive fungal infections, mainly invasive fungal infections due to *Candida* or *Aspergillus*. The use of the BDG assay in combination with the GM assay could be of great interest to clinicians who can use these assays to exclude or confirm suspected invasive fungal infections [31]. Positive results of serology support diagnosis of invasive aspergillosis, although those should be remarked in conjunction with other diagnostic procedures, including microbiologic culturing, histopathological examination, and radiographical testing [32].

Ventilated patients suspected with aspergillosis are difficult to be diagnosed in intensive care units specially patients with an endotracheal tube or a tracheostomy as it is associated with high mortality and morbidity rates. Bronchoscopy technique for ventilated patients is invasive, involves risks and has its limitations in patients with severe pulmonary disease, high respiratory support settings and coagulation abnormalities. Facing a rapid emergence and dissemination of multi-drug resistant microorganisms particularly in the ICU environment, strategies to reduce such general and non-targeted antifungal consumption have become very important. For all

the above obstacles in aspergillosis suspected patients with ventilation in ICU units we developed an easy method for diagnosis performance at the bedside, relatively simple, minimally invasive and inexpensive, have a proven acceptable accuracy and require minimal investment for the training of health professionals. Our method was culturing the exhaled air of the tracheal tube of the ventilator of ventilated patients and estimating the fungal causing agent of the disease. The results of twenty three of suspected aspergillosis patients tested proved eleven positive patients out of 23 (47.8%) whereas the rest 12 were void out of any growth of aspergilli (Table 2). Aspergilli total count in positive exhalation air cultures were found to be ranged between 2.42 to 0.71 fungal colony/cm³air/minute. Figure 2 showed that the dominant fungal species was *A. niger* (48%), followed by *A. fumigatus* (25%), *A. flavus* (17%) and finally *A. terreus* (10%) which came in accordance with the results of conventional methods of the 227 patients tested. Moreover serology of GM and BDG in serum powered the results of the culture of exhalation of ventilation air that 65.21% positive in GM and 73.91% positive in BDG. Although, there are four and six false positive results recorded in GM and BDG, however the BDG could indicate fungal infections other than *Aspergillus* (Table 2). These results showed that collecting exhaled aerosols is technically simple to perform and has the potential to change perspectives of diagnosis and therefore improves strategies for the prevention and transmission of fungal respiratory diseases.

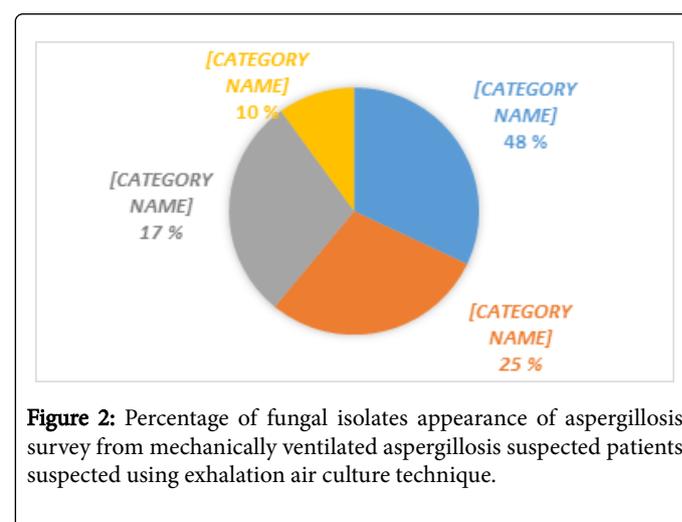


Figure 2: Percentage of fungal isolates appearance of aspergillosis survey from mechanically ventilated aspergillosis suspected patients suspected using exhalation air culture technique.

	Culture		Serology			
			Serum GM		(1-3)- β -D-glucan	
	No.	%	No.	%	No.	%
Positive	11	48	15	65.2	17	73.9
Negative	12	52	8	34.8	6	26.1
Total	23	100	23	100	23	100

Table 2: Percentage of positive fungal specimens from mechanically ventilated aspergillosis suspected patients using exhalation air culture technique.

Conclusion

An accurate diagnosis of invasive aspergillosis is important and may improve patient survival and allow expensive and potentially toxic antifungal drugs to be withheld. Combining all traditional methods is important for the diagnosis. From all the conventional tools of the diagnosis no clear winner among them and an appropriate tool must be chosen according to the condition of the patient and the disease. Thus exhalation air culture is the clue of diagnosis of the ventilated suspected aspergillosis patients in ICU. It is easily performed at the bedside, relatively simple, minimally invasive and inexpensive and has a proven acceptable accuracy and requires minimal investment for the training of health professionals.

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