Antifungal Susceptibility of Aspergillus sp. that causes Pneumonia in Cancer Patients

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Abstract

Aspergillosis is a fungal disease caused by *Aspergillus* sp., a filamentous fungus that is found in the air. Invasive pulmonary aspergillosis is known to affect immunocompromised patients.80 sputum sample collect from cancer patient infected with pneumonia. This sample cultured on SDA for isolation and identification *Aspergillus spp.* And test the sensitivity of Aspergillus to antifungal. diagnosis by macroscopic and microscopic examination. The result show the most *Aspergillus sp.* causes pneumonia, A.*fumigatus* represent 12 (40 %) following by *A.niger* 8 (26.7%), *A.terrus* 4(13.3%) and *A.flavus* 2 (6.7%). *Alternaria sp.* 2(6.7%) and *Pencillum sp.* 2 (6.7%) also isolated. antifungal test shows that 8 isolates A. niger resistance to itraconazole and all other Aspergillus sensitive. Two isolated Aspergillus flavus resistance to nystatin and other Aspergillus are sensitive.

Key Words: Aspergillus sp., Pneumonia, Cancer.

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Introduction

Aspergillosis is a fungal disease caused by *Aspergillus*, a filamentous fungus that is saprophytic and can be found in the air. (Chabi, M.L et al., 2015). *Aspergillus spp.* infections continue to be associated with substantial morbidity and death. (Jenks, J.D., & Hoenigl, M., 2018). These can be classified into 3 distinct clinical categories, *viz*. allergic aspergillosis, saprophytic colonization, and invasive aspergillosis (Shah, A., & Panjabi, C., 2016). *Aspergillus* is the most common fungal causes pneumonia in neutropenic patients, with *A. fumigatus* being the most common cultured of this genus, although *A. flavus*, *A. niger*, and amphotericin B-resistant *A. terreus* have also identified as important pathogens (Limper, A.H et al., 2011). Polyenes, azoles, echinocandins, and flucytosine, which are grouped into four categories, are currently accessible antifungal drugs (Van Daele *et al*., 2019). Sterols are compounds found in fungi that influence physiological as well as cellular functions. Because the majority of these sterols are the same as sterols found in humans, an antifungal drug must have a specific fungal target (that is not present in the human). Ergosterol, a sterol found only in fungal and not in human, is one of the major common targets for antifungal medicines (Onyewu *et al*., 2003). Antifungals that are azole are first-line alternatives for preventing and treating invasive fungal infections (Benitez & Carver, 2019). polyene which are fungicidal rather than fungistatic like azole. Amphotericin B, nystatin, and natamycin are popular polyenes. (Brajtburg *et al*., 1990; Moen *et al*., 1961). Azole fungal resistance has arisen as a major public health issue in medicine in recent years. (Azevedo *et al*., 2015). Resistance mechanisms unrelated to the cyp51A gene ATP Fungi have MFS transporters and ABC transporters, both of which are efflux pumps. Fungal cells are able to expel toxic substances, such as azole molecules, through the membrane-bound proteins. Increasing their expression causes an increase in resistance to azole antifungals because the concentration of the fungicide in the cell decreases (Berger *et al*., 2017).

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Materials and Methods

All 80 sample were collected from cancer patient with symptom of pneumonia, these sputum sample were obtain from patients treated in Euphrates hospital of cancer in alnajaf city. early morning sputum before eating any think, and use mouth rinse and brush perior to collection, the sample collected in sterile wide mouthed container, the sample transport to laboratory as soon as possible.

Identification of Fungal Isolates

Fungal isolates were diagnosed depending on the culture, microscopic characteristics. For fungal isolation, samples were inoculated on sabouraud dextrose agar (SDA) with chloramphenicol and incubated at 25°C for 6 days. The color, form, consistency, and reverse plate color of the colonies were used to identify them according to the Pitt and Hocking (1997) and Ellis (1971).
Microscopic characteristics Conidial heads, stipes, color and length, vesicles form and seriation, metula covering, conidia size, shape, and roughness, as well as colony parameters such as diameter after 7 days, color of conidia, mycelia, exudates and reversal, colony texture and shape were used for identification.

Antifungal disk Diffusion Susceptibility Testing

Use itraconazole disk and nyststin disk for test the sensitivity to *Aspergillus sp.*

1. Inoculum Preparation

According to Petrikkou, et al., (2001) prepare inoculum.
To preparation suspension of sporangiospore cover the sporulating colonies with (1ml) sterile salin (0.85%). Then Transfer conidia, hypha fragment, and sporangiospores mixture into a sterile tube. wait (3-5) minutes to heavy particles settled, transfer top homogenous suspension to sterile tube and close tightly and mixed by vortex for 15 s. Measure the optical density of suspension by spectrophotometer at (530 nm) wavelength ,and adjusted the suspension at rang (0.09- 0.13). The suspension used directly.

2. Inoculation of Test Plates

After (15 min) of preparation inoculum suspension, dip sterile cotton swab and Inoculate the surface agar by streaking the swab entire surface agar, ensure streaking entire agar by rotate the plate.

3. Application of Disks to Inoculated Agar Plates

After inoculation entire agar plate put antifungal disk on surface agar and pressed it down to good contact with agar surface .after ending invert the plate wait for (15min) befor incubation it on (35 °C).

4. Reading Plates

After (20 -24) hr of incubation if the inoculum was correct, the inhibition zone should be equally around it. The plat put on black non reflect surface measure the diameter of inhibition zone around disk.

Results

Table 1. Frequency of *Aspergillus sp.*

Culture Results	Frequency	Percent
Aspergillus fumigatus	12	40 %
Aspergillus. niger	8	26.7 %
Aspergillus. terrus	4	13.3 %
Aspergillus. flavus	2	6.7 %
Penicillium	2	6.7 %
Alterneria	2	6.7 %
Total isolate	30	100.0

Table 2. Sensitivity of Aspergillus spp. To itraconazole and nystatin

Fungi Species		Nystatin				
		Sens		Resist	Inter	P-value
	A-fumigatus	No.	2	0	10	0.001 H
		% of Total	7.7%	0.0%	38.5%	
	A.niger	No.	8	0	0	
		% of Total	30.8%	0.0%	0.0%	
	A.terrus	No.	0	0	4	
		% of Total	0.0%	0.0%	15.4%	
	A.flavus	No.	0	2	0	
		% of Total	0.0%	7.7%	0.0%	
Total		No.	10	2	14	
		% of Total	38.5%	7.7%	53.8%	
				Itraconazole		
				Sens	Resist	P-value
	A-fumigatus	No.		12	0	0.001 H
		% of Total		46.2%	0.0%	
	A.niger	No.		0	8	
		% of Total		0.0%	30.8%	
	A. terrus	No.		4	0	
		% of Total		15.4%	0.0%	
	A. flavus	No.		2	0	
		% of Total		7.7%	0.0%	
Total		No.		18	8	
		% of Total		69.2%	30.8%	

Chi-Square at significant at level 0.05, H: Highly significant

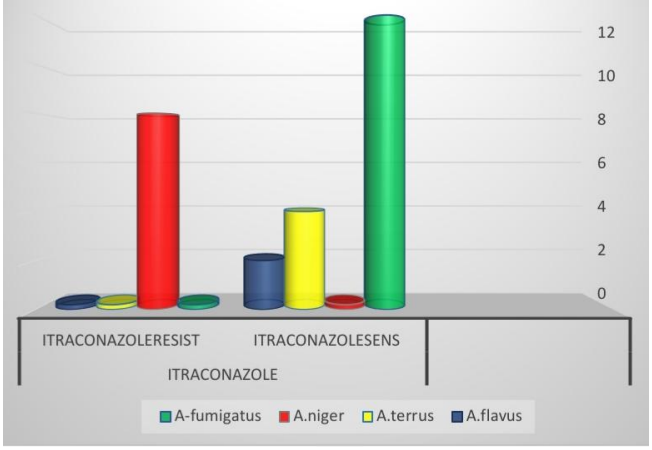

Figure 1. Sensitivity of Aspergillus sp. To itraconazole

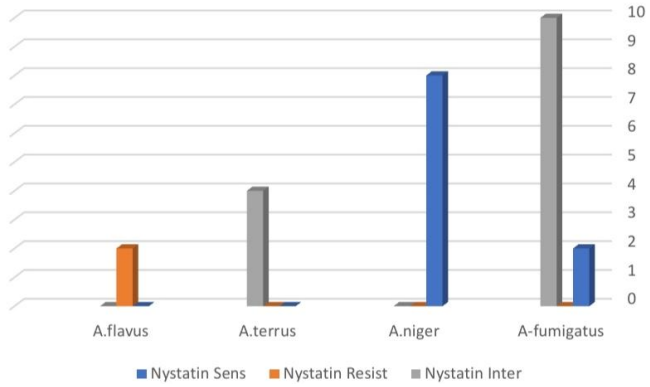

Figure 2. Sensitivity of Aspergillus sp. To nystatin

Discussion

The *A.fumigatus* is the most species isolate 12 (40%). This agree with (Gautier, M et al., 2016) that found *A.fumigatus* is the most common species recovered from cases of invasive aspergillosis. Following by *A.niger* 8(26.7 %), *A.terrus* 4 (13.3%) and *A. flavus* 2 (6.7 %) and this agree with (Limper, A.H et al., 2011) *A. fumigatus* being the most common cultured of this genus, although *A. niger, A. terrus and A. flavus*, have also identified as important pathogens. other then *Aspergillus* spp. *Alterneria* sp. 2 (6.7%) *Pencillum* sp. 2 (6.7%) also isolate.

There are 12 isolate from *Aspergillus fumigatus* all this isolated is sensitive to itraconazole and only 2 isolate sensitive to nystatin where as 10 isolate is intermediate to nystatin.

And 8 isolate *Aspergillus niger* all this isolate resistance to itraconazole where as all this isolate is sensitive to nystatin and this agreement with found in Poulsen *et al*., (2021) that increased in resistance *A.niger* due to decrease of peroxidative enzyme expression, increased of an ATP-binding cassette (ABC) transporter expression and inhibition of synthesis ergosterol. And this resistance acquired due to triazole such as (itraconazole) antifungal is the first tine treatment against IA (Spiess *et al*., 2014) and tong term used to human therapies (Pérez-Cantero *et al*., 2021) and also azole used to treatment crops causes increase exposure of fungal to this azole in environment that might influence on human health (Berger *et al*., 2017) Ther are 4 isolate of *Aspergillus terrus* all isolate is sensitive to itaconazole and all this isolate is intermediate to nystatine.

There are 2 isolate of *Aspergillus flavus* all isolate is sensitive to itraconazole and resistance to nystatine and this agree with found in (Ramanuj *et al*., 2015) that *A. flavus* is resistance to nystatin by used disk diffusion method and also agree with what found in Khamees & Ahmed, 2019) the result notice all isolated *A. flavus* is resistance to nystatin for this reason it is more virulence to human *Aspergillus flavus* as resistance to nystain anti-fungal agent while *A. niger* remained sensitive tonystatin (Al Azad *et al*., 2016) .for this used itraconazole for treatment *A.flavus* infection while use nystatin to treatment *A. niger* infection.

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