

Isolation and Identification of *Aspergillus* spp. from Human and Sheep Respiratory Infection in Al-Qadisiyah Province

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ABSTRACT

This study was conducted to isolate the most common *Aspergillus* spp. which cause dangerous diseases such as aspergillosis from respiratory infection in human and sheep, one hundred (100) human sputum sample collected from patients in respiratory diseases center of Al-diwanayah educational hospital during a period of two (2) months which were (November and December 2019), and one hundred (100) nasal swabs samples were collected from sheep which appeared clinical signs of respiratory infections from sheep flocks in rural in Al-Qadisiyah province, samples collected from sheep during the period of two months (2) which were January and February 2020. The isolated species in human appeared 74 out of 100 (74%) positive fungal isolate so *Aspergillus fumigatus* represents 12 isolates (16.21 %), *Aspergillus niger*, 8 isolates (10.81%), *Aspergillus flavus* 5 isolates (6.75%), *Aspergillus ochraceus* 2 isolates (2.7%), also another spp. of fungi were isolated which include *penicillium* spp. and *Alternaria* spp. 7 isolates (9.45%) for each one of them, *Geotrichum* spp. 4 isolates (5.4%), *Trichophyton mentagrophytes* 3 isolates (4.05%), *Cladosporium* spp. 2 isolates (2.7%), whereas Yeasts spp. constitute 22 isolates (29.72%). While the sheep revealed ninety six (96) fungal isolate out of (100) which include: *Aspergillus ochraceus* 9 isolates (9.37%), *Aspergillus niger* 6 isolates (6.25%), *Aspergillus flavus* 3 isolates (3.12%), *Aspergillus fumigatus* 2 isolates (2.08%), also other fungal species were isolated include: *Alternaria* spp. 9 isolates (9.37%), *Rhizopus* spp. 14 isolates (14.58%), *Trichophyton mentagrophytes* 13 isolates (13.54%), *penicillium* spp. 12 isolates (12.5%), *Trichophyton verrucosum* 10 isolates (10.41%), *Microsporium caninum* 6 isolates (6.25%), *Geotrichum* spp. and *Cladosporium* spp. 1 isolate (1.04%) for each one of them and Yeast spp. 10 isolates (10.41%).

Keywords: *Aspergillus* spp., human, sheep, respiratory infection

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INTRODUCTION

The genus *Aspergillus* contain more than two hundred species, a noteworthy effect on common health both useful, as vertebral column of many biotechnological aims domain from classical leavening to make of recombinant proteins, and reversely, as human and plant pathogens or as releaser mycotoxins (Gugnani, 2003; Scazzocchio, 2009). Amongst about twenty species pathogenic to human, *Aspergillus fumigatus*, is the main causative infection agent followed by *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus nidulans*, that is extremely used as type organism to examine many fundamental troubles of biology, like regulation of cell cycle (Morgan *et al.*, 2005; Scazzocchio, 2009). Although being most general found in decaying or growing vegetation, this fungus is wide prevalence in the climate distribution, it is can use many organic substrate also it have ability to adapt to various climate condition (McCormick *et al.*, 2010), like hypersaline waters (Butinar *et al.*, 2011). It is release great number of spores (a sexual spores) that readily spread in the air because their size is small 2-3 μ m in diameter ingrained hydrophobicity (O'Gorman, 2011). Also, these spores continue in the ambience for long times because the complex nature of the conidia wall, which preserve them from many chemical and physical distress (Latgé, 2007). The small size of conidia assists it to easily pass of the nasal cavity and upper respiratory system defense mechanisms and then reach to lung alveoli. In healthy person, the immune system prevents these fungal to produce infection, because alveolar macrophages readily recognize these inspired conidia, swallow and destroyed them (Brakhage *et al.*, 2010).

while, in immunocompromised individuals, *Aspergillus* species able to cause allergic diseases such as (asthma,

allergic sinusitis, alveolitis, an allergic bronchopulmonary aspergillosis and non-invasive aspergilloma) next exposure to conidia which spread in air (Osheroov, 2012).

MATERIALS AND METHODS

Sample collection

One hundred (100) human sputum samples were collected during period of two months which were (November and December 2019) from patients in respiratory diseases center of Al-diwanayah educational hospital which suffering from respiratory distress where as one hundred (100) nasal swabs specimens were collected from sheep suffering from cough, dyspnea, fever and nasal discharge in Al-Qadisiyah province (vet. hospital, vet. Clinics and rural area surrounding the city) during two months of winter 2020 (January and February) all specimens (human and sheep) where inoculated in brain heart infusion broth which acts as transport media and then in lab the samples inoculated on sabouraud dextrose agar (SDA) with chloramphenicol incubated in 25°C for 12 day for fungal isolation.

the colonies appeared were identified according to their color, shape, consistency and color of reverse plate and (Samson and Van Reenen Hokstra, 1988).

To confirm the diagnosis, wet mount smears prepare by take loop full from the fungal growth and mixed with 1-2 drop from lacto phenol cotton blue stain on the microscopic slide, that examine under power lense 10X and 40X to detect the structure which include hyphal and conidial elements that appeared to recognize the strains and after recognizing the fungus re inoculate in new plate for purification (Alexander and Street, 2001).

Statistical analysis:

Statistical analysis system was performed by use SPSS version 25 software. Data expressed in percentage.

Statistical tests like χ^2 -test, one and two ANOVA (F-test) and. The P-value less than 0.05 was considered significant (Field, 2005).

RESULTS

Isolation of Fungal from human sputum:

The isolation of fungal from (100) sputum sample revealed seventy-four (74%) fungal isolates. according to colour, texture, shape and the appearance of fungal structure under the microscope.

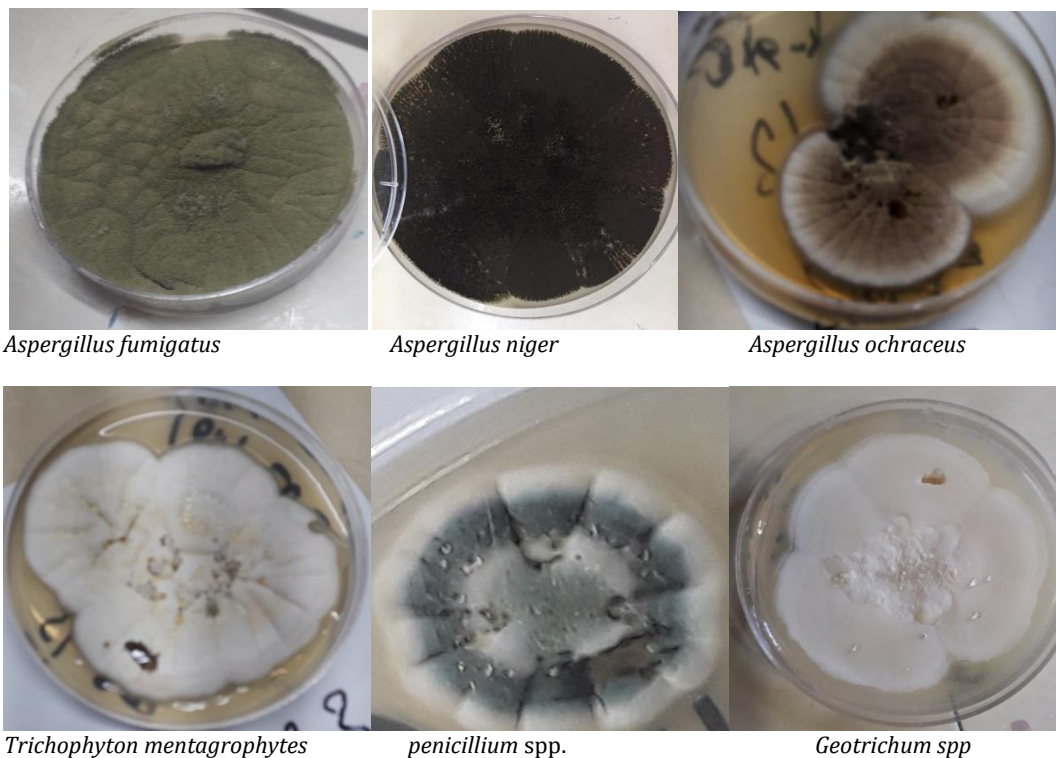


Figure show Morphological appearance of some isolated moulds cultures

There were eleven (11) molds species identified in human sputum in addition to twenty-two (22) isolates of the yeasts spp. show in the table (1).

Table 1. show fungal species (molds and yeast) isolated from sputum of human

Molds			Yeasts		
Species	Positive isolates	(%)	Species	Positive Isolates	(%)
<i>Aspergillus fumigatus</i>	12	16.21	Yeast spp.	22	29.72
<i>Aspergillus niger</i>	8	10.81			
<i>Alternaria</i> spp.	7	9.45			
<i>penicillium</i> spp.	7	9.45			
<i>Aspergillus flavus</i>	5	6.75			
<i>Geotrichum</i> spp.	4	5.4			
<i>Trichophyton mentagrophytes</i>	3	4.05			
<i>Aspergillus ochraceus</i>	2	2.7			
<i>Cladosporium</i> spp.	2	2.7			

<i>Trichophyton verrucosum</i>	1	1.35	
<i>Rhizopus spp.</i>	1	1.35	
<i>total</i>	74(100)		

Calculated X² = 69.64 p value = 0 (highly significant difference)

Isolation of Fungal from sheep nasal swabs:

The isolation of fungal from one hundred (100) nasal swap samples from sheep were recorded ninety-six (96) positive sample of fungal isolation and in proportion (96%).

There were twelve (12) molds species diagnosed in sheep nasal swab in addition to ten (10) isolates of the yeasts spp. as show in the table (2).

Table 2. shwos fungal species (molds and yeast) isolated from sheep nasal swabs

Molds			Yeasts		
Species	Positive isolates	%	Species	Positive Isolates	%
<i>Rhizopus spp.</i>	14	14.58	Yeast spp.	10	10.41
<i>Trichophyton mentagrophytes</i>	13	13.54			
<i>penicillium spp.</i>	12	12.5			
<i>Trichophyton verrucosum</i>	10	10.41			
<i>Alternaria spp.</i>	9	9.37			
<i>Aspergillus ochraceus</i>	9	9.37			
<i>Aspergillus niger</i>	6	6.25			
<i>Microsporum caninum</i>	6	6.25			
<i>Aspergillus flavus</i>	3	3.12			
<i>Aspergillus fumigatus</i>	2	2.08			
<i>Geotrichum spp.</i>	1	1.04			
<i>Cladosporium spp.</i>	1	1.04			
total	96(100)				

Calculated X² = 36.54 p value = 0 (Highly significant difference)

DISCUSSION

fungal isolated from human

There are twelve (12) fungal spp. Showed from seventy-four (74) fungal isolates obtained from sputum of human undergo from chronic disease in respiratory system. these results may because that the mainly of fungi are opportunistic pathogen in nature and may be found in healthy individuals and when the host immunity become lower, defects result from chronic respiratory infection in this case this fungi will cause disease, these evidence was confirming the idea showed by (Schwarz, P.; *et al.*, 2006) who showed that the increase pulmonary mycosis of the past decades because the increase use drug of broad-spectrum, chemotherapy and immunosuppressive agents as well as increased respiratory disease incidence, like lung cancer, tuberculosis and chronic obstructive pulmonary disease, also the result of current study was in agreement with Egyptian study (Ahmed M, *et al.*, 2019) who observed prevalence of fungal infection in respiratory tract was 66.6 %. While in contrast to (Jahromi SB, *et al.*, 2002) study in Tehran, Iran showed a prevalence of 36.6% for respiratory tract fungal isolates. Also, (Akhtar A, *ET AL.*, 2018) recorded 31.3 for pulmonary fungal isolates in Bhagalpur, India.

Aspergillus spp. Isolated as highly percentage of frequency (36.47%) where consist of *Aspergillus fumigatus* with (16.21%), *Aspergillus niger* (10.81), *Aspergillus flavus* (6.75%) and *Aspergillus ochreosus* (2.7%), while *Penicillium* spp. And *Alternaria* spp. recorded (9.45%) for each one, *Geotrichum* spp. recorded (5.4%), *Trichophyton mentagrophytes* (4.05%), *Cladosporium* spp. (2.7%), *Trichophyton verrucosum* and *Rhizopus* spp. recorded (1.35%) for each of them. The result of the current study in agreement with (Aziz, 2014) who mention that 28.08 % of total 381 isolates of the genus *Aspergillus*. Where record *Aspergillus fumigatus* have highest percentage (29.9%), then *A. niger* (28.9%), *Aspergillus flavus* (18.7%), *Aspergillus terreus* (12.14%) and *Aspergillus nidulans* (2.8%).

Several studies showed that *Aspergillus fumigatus* is more prevalence in the Western countries, whereas *Aspergillus flavus* is more prevalence in Asia and Middle East as the major cause of the pulmonary invasive aspergillosis (Singh and Paterson, 2005; Pfaller *et al.*, 2006). Also, Denis *et al.*, (2018) mentioned that the more common fungal was *A. fumigatus* (57.5%).

The features of the morphological appearance of *Aspergillus* spp. Isolated in the present study from human and sheep samples in agreement with Larone, (2011) and Refai *et al.*, (2014). In that the colonies of *A. fumigatus* ranging from white to green in color and with age turn to have gray color, or brown, and in microscopic appearance the vesicle is dome in shape while the conidia are oval-round, small, green tan that were similar to McClenny, (2005). Whereas the appearance of *A. niger* was woolly, originally appear as white yellow in color and due to conidia head turn to dark brown- black while in reverse white to tan, the vesicle under microscope appear as globular brown, black that were in agreement with Varga *et al.*, (2011). While *A. flavus* appear as yellow velvety to yellow-green and with age turn to green-brown while in reverse appear as white to red brown which in agreement with Rodrigues *et al.*, (2007). Finally, *A. ochraceus* colony have characteristic colour is yellow. The reverse is pale to brownish. In macroscopic appearance the conidiophores were powdery mass, the characteristic is chalky yellow to pale yellow brown. Microscopically,

finely roughen or smooth phialides appear as arrangement on the conidial heads in a biseriate. The conidial heads in culture appear globose at first, while with ageing adhere the conidial chains and develop into 2 or 3 divergent columns that agreement with Kwon-Chung *et al.*, (1992).

The current study appeared that *Aspergillus* species consider the main fungi respiratory tract mycosis and these findings are in agreement with (Latgeet *et al.*, 2001) who recorded that *Aspergillus* species may be considered responsible for significant morbidity and mortality so for important infection caused by saprophytic colonization of respiratory tract to rapidly invasive and disseminated diseases life-threatening, depending on the immune status for host. The lung persists to represent most frequent involved site, in *Aspergillus* infection. Lung infection is a phenotypic representation of the interaction between the virulence of the fungus and lowered the host's defense mechanism (Pagano *et al.*, 2008).

Fungal isolated from sheep

The fungal isolates from nasal swabs of sheep suffering from respiratory infection appeared high percentage of this study with 96% fungal isolates and There are thirteen (13) fungal spp. appeared in the present study, the fungi which isolated in this study from sheep belong to genera *Aspergillus* spp. (20.83%), this include *A. fumigatus* 2 (2.08 %), *A. ochraceus* 9 (9.37 %), *A. niger* 6 (6.25 %), *A. flavus* 3 (3.12 %), this result in contrast with Mona *et al.*, (2005) where showed the *A. fumigatus* isolate as the more common among *Aspergillus* spp. constituting 10 and 15 % in diseased goats and sheep and 10 and 12% in pneumonic lungs of goats and sheep respectively demonstrate that it was the main cause the aspergillosis in pulmonary. while Singh *et al.*, (1995) showed the aspergillosis incidence was 4.9% in both goats and sheep. other fungi in present study include *Alternaria* spp. 9 (9.37%), *Trichophyton mentagrophytes* 13 (13.54%), *penicillium* spp. 12 (12.5%), *Geotrichum* spp. 1 (1.04%), *Rhizopus* spp. 14 (14.58%), *Trichophyton verrucosum* 10 (10.41%), *Microsporum caninum* 6 (6.25%), *Cladosporium* spp. 1 (1.04%) and Yeast spp. 10 (10.41%).

CONCLUSION

The emergence of high rates of fungal infections within the respiratory infections in humans and sheep, with the emergence of clear respiratory symptoms represented in coughing, high temperature and nasal discharge, and the percentage of *Aspergillus* species were higher than other fungi isolated in this study.

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