Isolation and identification of some fungi from dogs and assessment of antifungal activity of Nystatin and Metronidazole in vitro

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ABSTRACT

The of mean of current study was to isolate and identify the moulds (fungi and yeast) from dogs infected by respiratory disorder by conventional methods. Also the assessment of antifungal activity of nystatin and metronidazole against *A. fumigatus, Ochracious, A. niger* in vitro. The total number of nose, mouth and ears swabs were 60 swabs (20 for each) collected from dogs during the period from the beginning of November 2019 to the end of December 2019in Diyala province. The mycological diagnosis were conducted by culture the specimens on Sabouraud dextrose agar, which represented by high percentage rate (65%) *A. fumigatus*, (50%) *A. niger* and low percentage rate (20%) *A. flavus* of nose isolates; and high percentage rate (50%) of *Candida sp.* and (20%) of each *A. niger, A. fumigates*, *A. flavus* from mouth swabs isolates. Whereas, the highest rate of isolate was (45%) *A. flavus*, *A. fumigates* and (30%) of Candida sp. from ear swabs isolates followed *by the* lowest percentage rate was (15%) *Rhizopus sp*. and *Ochracious sp*. form of each. The assessment of antifungal activity of nystatin is other dynamic in vitro than metronidazole against *Ochracious* sp. Also *Aspergillus* spp.

Keyword: Nystatin, metronidazole, assessment, fungi, dogs

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INTRODUCTION

In latest years, numerous opportunistic fungal infections like aspergillosis candidiasis,, zygomycosis cryptococcosis, pneumocystosis , geotrichosis, , trichosporonosis, rhodotoruliosis and fusariosis have been documented as an imperative cause of mortality and morbidity in developedin addition to developing nations [1, 2, 3,4]. These fungi are broadly common in environment and are recovered from air, plant, substrates , water, soil[1,3].Fungus can distress a lot of organs of body for example eye, ear, , sinus, lung, brain, bone, skin, kidney and heart [1].Fungi are widespread in both indoor and outdoor environments as mycelial fragmentation, spores or dissociated extracellular and intracellular components. They are generally saprophytic and not dangerous but under extra ordinary situation like lung diseases weakened immune systems or lung diseases, they can cause infections

indifferent spectrum of sickness like lung infections, allergic reactions and infections in different organs [5].

The majority common fungi that infected mucosal tissues as well as species like *Absidia*, *Rhizopus,* Aspergillus , *Rhizomucor* and *Mucor* but, *Fusarium & Bipolaris* are fewer widespread. These Fungi can reach to the sinonasal cavities via spores inhaled from animals [5, 6]. The fungi of Aspergillosis are a sickness that caused via *Aspergillus* species as *A. fumigatus*, *A. flavus*, *A. niger* and *A. Terreus* [7].As well as, these species have multiples virulence causes like phospholipase enzymes and gliotoxin, make this species to invading tissue of the host causing infections [7, 8].The oral cavity microbiota of animal and human is contain wide variety of yeasts and bacteria which are responsible for oral lesion [9]. They were reported the identification of the major microorganisms with a pathogenic potential in the oral cavity of animals also plays an important clinical role such as to provide support towards adequate diagnosis and therapy in veterinary medicine [10].

Nowadays, Nystatin remains the more available antifungal drugs in more developing countries. For treatment of fungal disease of the external auditory canal and of postoperative aural cavities studies have been due to the action of nystatin on the growth of various Aspergillus species[11].In recent years, people have become increasingly interested in dogs breeding; also few studies on fungal infections in dogs infected by respiratory disease. Therefore, the aim of present study was isolate and identify fungi from dogs infected by respiratory disorder in Diyala governorate.

MATERIALS AND METHODS

Isolation of fungi: The SDA were prepared aseptically and dispensed in sterile petri dishes. Each swabs collected from diseased dogs were inoculated onto the center and spread it on SDA plates which supplemented with 1% chloramphenicol and labeled each petri dish with the identification number given to respective samples after solidification of agar. Then incubation at 30°C for 5-7 days and checked daily for any growth before sub-culturing positive ones [5, 12].

Microscopic examination of cultures: Microscopical examination of cultures was performed by the selection of clean glass slide for each sample and added at least two drops of lactophenol cotton blue on the prepared slide. Then taken fungal colony growth by sterile inoculation pin of platinum, and placed on the above-mentioned stain, pressed on the slide by the use of sterile cover slip in a manner to ovoid the presence of air bubbles. Then examined by light microscope under X10 and X40 magnifications according to[5].According to[12]the fungi can be identified according to their general morphology, the microscopic properties of conidia, the hyphae and some of other structures.

Determination of Antifungal Sensitivity Pattern: The plates of each isolate were incubated at 25℃ and the nystatin and metronidazole as antifungal. These agents were done in triplicates under sterile conditions and results recorded after 24, 48, 72, 96 and 120 hours. The antifungal activity was determined by measuring clear inhibition zones diameters (including diameter of paper discs) formed using the caliper [12].

RESULT

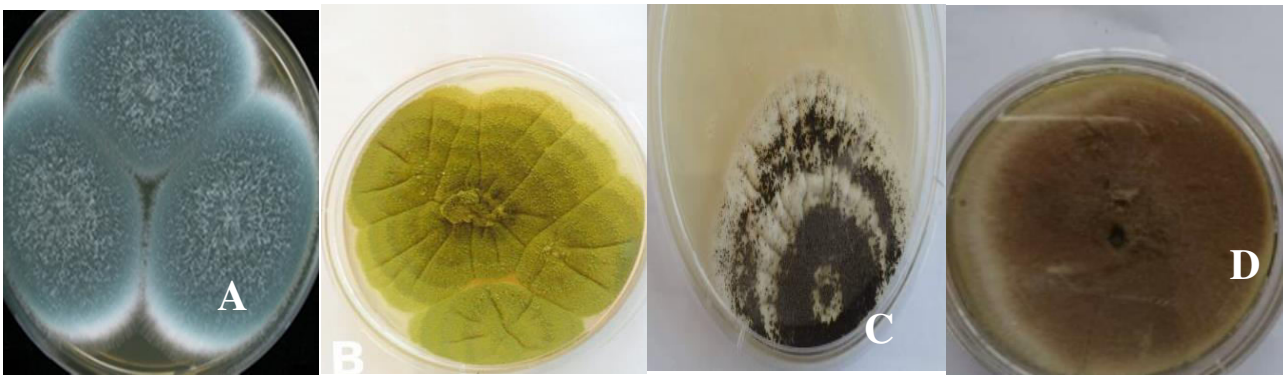
Clinically diseased dogs passing from depression, coughing, anorexia, and weakness laceration, nasal discharge, increase respiratory and pulse rate (Figure,1).

Figure (1): A Local breed dog (B) Terrier dog infected by respiratory infection characterized clinically by depression, and weakness nasal discharge

The total number of fungal isolates from nose, mouth and ear of diseased dogs were 31, 28, 33 isolates respectively. Therefore the *A. fumigatus A. niger* and *Candida sp.* were the most common fungi isolated from diseased dogs (Table 1) and (Figure 2).

(Table 1): Frequent and distribution fungi species isolated from nose, mouth and ear of diseased dogs.

Fungi species	Nose N. S (20) N I (%)		Mouth N. S (20) N I (%)		Ear N. S (20) N I (%)	
Aspergillus fumigatus	13	65%	6	30%	9	45%
Aspergillus niger	6	50 %	6	30 %	3	15%
Aspergillus flavus	4	20 %	6	30 %	9	45%
Candida sp.	8	40 %	10	50 %	6	30%
Rhizopus sp.	0	0	0	0	3	15 %
Ochracious sp.					3	15 %
Totale	31		28		33	
N. S= Number of swabs N I: Number of isoletes						



Fiqure (2): Pure culture of fungi isolated from diseased dogs (A) *Aspergillus fumigatus*(B) *Aspergillus flavus*(C) *Aspergillus niger* (D) *Ochracious sp.*

The result of assessment of antifungal activity of nystatin against *A. fumigatus, A.niger* (Fig. 3), *Ochracious i*n vitro by disc diffusion method was illustrated in(Table 2)

Table(2):Show diameter of zone inhibition of Nystatin against some fungi at3rd, 4th and 5th days

Isolates	Diameter of zone inhibition(mm)		
	at3rd day at 4th day at 5th day MM M		
A. fumigatus	38 mm	15 mm	11 mm
Ochracious spp	35 mm	25 mm	14mm
A.niger	21 mm	16 mm	11 mm
M= Mean			

Whereas, the assessment of antifungal activity of metronidazole against *A. fumigatus, Ochracious* show no inhibition effect of metronidazole at the first and second days of incubation while mild inhibition at 3rd day as in (Table, 3). Whereas *Aspergillus niger* show resistant toward metronidazole.

Table(5): Show Diameter of zone inhibition of metronidazole against some fungi at 3rd day

Name isolate	Diameter of zone inhibition(mm) at3rd day at 4th day at 5th day M M M		
A. fumigatus	12 mm	11 mm	10 mm
Ochracious spp	11 mm	10 mm	8 mm

A=Antifungal effect of nystatin against *Aspergillus fumigatus*

B=Antifungal effect of nystatin against *Aspergillus niger*

Discussion:

Aspergillosis especially in canine is more common to the upper respiratory tract, particularly in the nasal cavity, and the systemic infection is rare. Therefore clinically the infected dogs of the present study represented by severe depression, anorexia, and weakness, lacremation, nasal discharge, increase respiratory and pulse rate and coughing were agreement with other authors [13].

According to (Table 1) *A. fumigatus, A. niger* and *Candida sp.* were the most common fungi isolated from nose (65%), (50%), (40 %) respectively. While in mouth the highest rate of isolate was *Candida Spp* (50%), *A. niger*,*A. fumigatus* (20%).The high percentage of isolates nose and mouth is due to the infected of the higher region of the respiratory tract that started hot and moist in help for the molds reproduction. These results agreed with the researchers [14]. Also, they have been reported *Aspergillus fumigatus* is highly isolated, although various other species including *A. nidulans, A. niger,* , and *A. flavus* isolated from dogs [15].

On the other hands, *A. fumigatus, A. flavus* were isolated from ear of dogs than other fungal species. These finding agreed with [4]. Moreover, the results which are connected with [15,16] in his search who found previously that the sharing of fungal species in otomycosis was *A. niger* observed by *A. flavus* and *A. fumigatus.*

In addition, Aspergillus species isolated from animals study were consistent with the results of authors [17], they isolated *A. fumigatus*, and then followed by *A. flavus*; whereas, *A. niger* was isolated from ear followed by *A. fumigatus* and *A. flavus*.

Data in this study show that the assessment of antifungal activity of nystatin against *A. fumigatus, A. niger, Ochracious i*n vitro by disc diffusion method show restricted the inhibition growth of these species at 5th day of culture. These results confirm and extend those of earlier reports which indicated that nystatin is a broad spectrum antifungal agent which is active in vitro and in vivo against *Aspergillus* spp. *Candida* sp [18].

Whenever, the assessment of antifungal activity of metronidazole against, *A. fumigatus,* and *Ochracious* show no inhibition effect at the first and second days of incubation while mild inhibition at 3rd day, also *Aspergillus niger* show resistant toward metronidazole. These finding agrees with authors [19, 20] who reported that the metronidazole complexes are active against all the fungi species and

varies with the type of organism also have greater inhibitory activity on the organism compared to its free metronidazole.

CONCLUSION

A. fumigates, A. niger and *Candida sp.* were the most common fungi isolated from diseased dogs and nystatin is highly active than metronidazole in vitro against *Aspergillus* spp. and *Ochracious* sp.

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