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Research article

Isolation and identification of systemic mycological isolates from fishes samples that obtained from local markets in Baghdad, Iraq

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ABSTRACT

There are few researches concerning isolation and identification of yeasts and moulds from systemic infections in different kinds of fishes in Iraq. The objective of this study is to investigate the percentage of mycotic infection and evaluate the diversity of yeasts and moulds in the intestine of fish in different local markets in Baghdad. One hundred samples were collected from different parts of intestine that belong to 11 types of fish which included: *Ilisha* (28), *Liza abu* (25), *Cyprinu carpio* (24), *Barbus Luteus* (7), *Hypophthilminthys Molitrix* (5), *Aspius Varax* (4), *Barbus Sherpeyi* (2), *Mugil Cephitus* (2),*Barbus Xanthopterus* (1), *Barbus grypus* (1) and *Carrains auratus* (1). Many samples were taken from multiple portions in intestine and cultured on sabouraud dextrose agar (SDA). The results revealed that the percentage of systemic mycosis in fish was 62% which classified to 55% yeasts and 7% moulds. Divided to *Cryptococcus spp* (19%), *Candida krusei* (11%), *Candida quillermondii* (9%), *Blastomyces dermatitidis* (9%), *Candida pseudotropicalis* (7%), *Aspergillus flavus* (6%) and *Rizipus* (1%). The current study suggests that the fungal diseases appeared as a serious threat to the fresh water fishes and human through handling in infected fishes.

Keywords: Fish, Fungus, Yeast, Baghdad, Iraq.

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INTRODUCTION

Fish is one of the more important sources of animal protein available in the tropics and has widely accepted as good source of protein and other elements for maintenance of body health [1]. Fish proteins are noted for a high degree of digestibility and as a rich source of lysine and sulphur containing amino acids. Therefore it is suitable for complementing high carbohydrate diets especially in developing countries. Much attention is being directed at fresh water fish because of its health benefits, as a result of the presence of omega-3-fatty acids in the fish oil [2]. In many Asian countries, over 50% of protein intake comes from white fish. In Africa the proportion is 17%-50% and over 40% of animal protein consumed by Nigeria [3]. Fish is a good source of vitamine B12 and B6, moreover, fluorine and iodine which are needed for development of strong teeth and prevention of goiter in man [4]. The growth of fish

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farming has also raised the issue of fish disease. *Saprolegniasis, lernaeasis*, bacterial hemorrhagic and septicemia are the most common fish diseases in many countries. Also branchiomyosis which characterized by respiratory disorder [5]. Stressed and poorly feed fish are more susceptible to fungal infection. Indeed, every fresh water fish is exposed to at least one species of fungus during life time [6]. Fungal disease are second most serious cause of losses in aqua culture and fungal infection can attack all life stages of fish from egg to adult fish and cause serious diseases in fresh water fish throughout Asia [7]. The aim of this study is to investigate the percentage of mycotic infection and evaluate the diversity of yeasts and moulds in the intestine of fish in different local markets in Baghdad.

MATERIALS and METHODS

Sources of fish sample

A total of 100 fish samples weighing 700-1500 gm were purchased from randomly different shops in Baghdad and classified into eleven genera; *Ilisha* (28), *Barbus Xanthopterus* (1), *Barbus grypus* (1), *Cyprinu carpio* (24), *Liza abu* (25), *Aspius Varax* (4), *Barbus Luteus* (7), *Barbus Sherpeyi* (2), *Carrains auratus* (1), *Mugil Cephitus* (2) and *Hypophthilminthys Molitrix* (5) were collected in sterile polythene bags and directly transported to the laboratory in ice box [8].

Culture and identification of fungal isolates

Many swabs from different portions of intestine of fish were taken and cultured on sabauroud dextrose agar (BDH-England) plates and were incubated at 28±2 ºC for 3-7 days. Fungal isolates were diagnosed according to cultural characteristic, morphology of hyphae cells, spores and kind of fruiting bodies [9].

All yeast isolates were identified by direct microscopic examination using the lacto-phenol-cotton-blue stain (Fluka -Switzerland), several biochemical identification tests were used to diagnose these isolates such as sugar fermentation and assimilation tests. The isolates were identified with biochemical kits (API Candida, The API –yeast-IDENT system was used for identification of yeast isolates).

RESULTS

The current study reported high percentage of mycotic infections in fish that reached to 62% and classified into 2 species (7%) of moulds and 5 species (55%) of yeast (**Table 1**).

Tabla 1. Prevalence of mycotic infection in intestine of fish.

Types of fungi	No. of spp.	No. of isolates	Percentage (%)
Yeasts	5	55	55%
Moulds	2	7	7%

Present study revealed that the prevalence of mycotic infection caused by yeast is more than moulds (**Tables 2** and **3**) those shown wide diversity of yeast and mould species.

Table 2. Types of yeasts that isolated from infection of fish.

Type of yeast	No. of isolates	Percentage of yeast isolates from total isolates
Cryptococcus spp.	19	19%
Candida krusei	11	11%
Candida quillermondii	9	9%
Candida pseudotropicalis	7	7%
Blastomyces dermatitidis	9	9%

DISCUSSION

In present study, the ratio of mycotic infection (62%) is considered a very high percentage and constituted a big hazard to the public health in our country due to possibility of transmission most of these fungi to human being particularly fishermen, fishmonger and housewives through contamination of hands during their routine work in handling with the infected fishes, and on the other hand, effect of these fungi on the fish riches that causing economic losses in fish industry. There is very little or even no studies related with prevalence of systemic mycosis in fish in Baghdad, while this idea was taken from previous study [10] when the similar project went on in the prevalence of fish mycotic infection in Al – Deyalla, Iraq.

Table 3. Types of moulds isolated from intestine of fish.

Type of moulds	No. of isolates	Percentage of moulds isolates from total isolates
Aspergillus flavus	6	6%
Rizopus	1	1%

Fungi cause systemic disease in fish and induce pathological and histological changes in different internal organs like intestine, liver, kidney, spleen and heart [11]. Crowding fish breeding causes pollution and organic compound concentration in aquarium that enhanced bacterial, viral and mycotic infections. Temperature play an important role in spreading of diseases [12] because the collection of organic compounds in aquarium and pad draining of water lead to increasing temperature and faeces with decrease food conception from fish which lead to fungal contamination [13]. Here, the prevalence of mycotic infection caused by yeasts is more than mould infection due to the yeasts are more pathogenic from moulds and they are found on a wide variety of substances such as soil, plants, water, nectar of flowers, fruits, trees and exudates of animals [14]. Generally, the findings of this study agree with previous study [15] that reported moulds and yeasts were widely distributed in nature. The yeasts were usually implicated as the cause of mycotic infection in most animals. Many fungal genera have virulence factors which cause fish diseases and the favourable predisposing environment factors of ecology is important factor that affects on diversity of fungal genera in fish and their egg [16, 17]. The proportion of infection in this study with

varying species of fungi differed from other researches and this may be attributed to that the fungi have wide range of infections depending on the management of farm and environment in our country and other of the world. *Aspergillus spp* is considered widely spread fungi in nature because it found normally in soil and organic compounds, also it is important for carbon and nitrogen cycle in soil [18]. The current study reported that 6% of infection caused by *A. flavus*. This type of fungus has specific importance due to its ability to produce aflatoxin that causes serious problems in animals and human [19] and this investigation was coincided with previous study [20]. On the other hand, another study [21] revealed that Aspergillosis cause disease in fish and [22] found 54.7% out of 245 fish of carp was infected with *Aspergillus spp*. While in another study [23] 66% of *A. flavus* isolates were isolated from salted fish. It was reported that the causative agents of Aspergillomycosis in African fish are *A. flavus* through it responsible for contamination of fish feed [24]. In addition, other scientists found that 3 fungal spp. of *Aspergillus* were isolated from six organs of fish (gill, skin, buccal cavity, head, eye and abdomen) [25]. *Aspergillus spp*. was isolated from silver carp and gold fish [26]. Other type of *Aspergillus spp*. like *A. niger* was isolated from raw fish in Nigeria with 35% [3] and from smoked-dried fish samples [27]. One isolate of *Rizopus* spp. (1%) was isolated in this study that considered very low when compared with another study [3] reported that 15% of *R. Stolonifer*. Earlier study [26 and 27] found that *Rizopus* in fresh fish samples and isolated from carp and gold fish (*carassius auratus*), while in another study [22] recorded 25% of pathogenic *Rizopus* obtained from carp fishin Sulaimania Province. However, others found that *Rizopus spp.* is a normal mycoflora present in fish [28].

In concerning of yeast infection, Cryptococcosis is an important highly infectious mycozoonotic disease [29]. It has become a significant public global health problem worldwide. This disease caused by two species, *Cryptococcus neoformans* and *C. gattii*, this life-threatening infection afflicts not only immunocompromised individuals but also apparently immunocompetent subject [14]. In animals, Cryptococcosis is little different according to animal species, it mainly causes upper respiratory disease and cryptococcoma in central nervous system (CNS), meningoencephalitis / meningitis and pneumonia. The percentage of infection with this disease in present study is 19%. High percent and represents a big threatening to the public health in our country due to Cryptococcosis is an important highly infectious mycozoonotic disease. This study represented the first study on the isolaton of *Cryptococcus spp*. from fish in Baghdad. Other studies mentioned that the Dolphins have been also described as infected by *C. gattii* particularly, (*Tursiops truncatusand Stenella cuoeruleoalba*). This species developed pneumonia due to cryptococcosis [30].

Several species of genus Candida were isolated in present study, 27% from total yeast isolates (55) were found. *Candida* is prevalence with high ratio as compared with other types of yeasts. This ratio was higher than the ration of another study [22] that reported the percentage of isolation of *Candida spp* is 15%. from carp fish in Sulaimania Province. In another study the percentage was 70% of *Candida spp*. in different types of fish [23].

Our study documented the isolation of *Blastomyces dermatitidis* was 9% of total isolation in fish. Blastomycosis is zoonotic disease, which infects human and animals. It caused by *B. dermatitidis*, which is found as mould in the soil or at room temperatures and as yeast in tissues at body temperatures. This type of fungus found near river valleys or other waterways. It can infect many organs such as lung caused pneumonia, skin and central nervous system causing convulsion [31]. So, this type is very important due to its influence on public health. Also this ratio was similar to the result of another study [22] that found the percentage of isolation is 7.5% of *B. dermatitidis* in carp fish in Sulaimania Province.

In present study we isolated, identified and characterized of some pathogenic agents dealing with mycotic groups which act as the causative agents of systemic mycosis infection in fishes in Baghdad.

Conflict of interest

The authors declare that they have no conflict of interests.

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