



Short Communication

Detection of Mycoflora from Marine Fishes of Karachi Fish Harbour and Ormara Region (Pakistan)

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ABSTRACT

In the present study we have isolated 5 different species of fungi *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium* sp. and *Fusarium* sp. from 14 different genera of commercially important marine fishes *Acanthopagrus* sp., *Parastromateus niger*, *Nemipterus* sp., *Pampus argenteus*, *Ilisha* sp., *Alepes djedaba*, *Epinephelus* sp., *Teraponjarbua*, *Terapon puta*, *Scomberomorus koreanus*, *Epinephelus coioides*, *Lutjanus* sp., *Pomadasys* sp. and *Lutjanus johnii* procured from Kemari fish harbor (Pakistan). Our study shows that fresh fishes were not contaminated by incidence of fungi. *Aspergillus flavus* was the most dominant fungus as compared to other species of fungi but it represents the common mycoflora of this region.

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Authors' Contributions

NS and NI designed the study, performed the experiments, analyzed the data and wrote the article.

Key words

Fungi, Fish, *Aspergillus niger*, *Penicillium*, Ormara.

Pathogenic microorganisms mostly attack on frozen sea food during storage. Fungal contamination during storage reduces the quality of food for human consumption due to unpleasant taste, lipolysis and sliminess (Abd El-Fattah, 2002; Hassan and Abdel Dayem, 2004). Different fungi produce mycotoxin and reduce the reproduction of fish in aquaculture (Ali *et al.*, 2011). Mycotoxins in fungi are harmful for human health and in large amount cause many carcinogenic diseases (Hassan *et al.*, 2009). Vandersea and Litaker (2007) isolated six fungi from gills of Channel catfish from which 3 genera belongs to *Aphanomyces*, *Achlya* and *Phialemonium*. Some common fungi such as *Aspergillus* sp., *Penicillium* sp. and *Absidia* sp. were isolated from fish feed (Alinezhad *et al.*, 2011). Fifteen different species of fungi were identified from *Coregonus lavaretus holsatus* (Czeczuga *et al.*, 2004). *Saprolegnia parasitica* is the most common fungal species which mostly causes infection in fishes and eggs of Salmon (Ebrahimzadeh *et al.*, 2007). *Aspergillus niger* is a most common pathogen which causes external and internal infection in fishes and also served as human pathogen (Alinezhad *et al.*, 2011; Firoozbakhsh *et al.*, 2005; Ebrahimzadeh *et al.*, 2007). Fish served as a better source of high nutritional values due to its amino acids, particularly lysine, vitamins A, D and E, thiamin, riboflavin and niacin (vitamins B₁, B₂ and B₃) as well as minerals including iron, calcium, zinc,

iodine, phosphorus, selenium and fluorine (Sankar, *et al.*, 2013). The present study aims to isolate and identify the fungi of marine fishes from Ormara and Karachi harbor.

Materials and method

Economically important marine fishes *Acanthopagrus* sp., *Parastromateus niger*, *Nemipterus* sp., *Pampus argenteus*, *Ilisha* sp., *Alepes djedaba*, *Epinephelus* sp., *Teraponjarbua*, *Terapon puta*, *Scomberomorus koreanus*, *Epinephelus coioides*, *Lutjanus* sp., *Pomadasys* sp. and *Lutjanus johnii* were collected randomly from Kemari harbour of Pakistan.

Marine fishes of economically important *Sardinella longiceps*, *Scomberoides commersonniana*, *Otolithus ruber*, *Opisthopterus tardoore*, *Thryssa vitrirostris* and *Thryssa setirostris* were collected randomly from Ormara station of Pakistan.

Fishes were identified from the FAO published books (Fischer and Bianchi, 1984). Potato Dextrose Agar (PDA) media containing antibiotics was used for the isolation of fungus. After dissection small pieces of muscles were removed and 3 small pieces of muscles placed into culture media petri dishes. Then petri plates were incubated at 25°C for 5 days. After growth of fungus small portion of fungi was taken out with the help of needle and put on a slide containing 2 drops of lacto phenol then examined under a compound microscope. Isolated fungi on fish identified with the help of Barnett and Hunter (1998). Statistical analysis was performed using Microsoft excel software. Data are presented as means $\pm$ standard deviation of means.

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Results and discussion

Only 4 genera of fungi viz., *A. niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium* sp. and *Fusarium* sp. were isolated from 14 fishes viz., *Acanthopagrus* sp., *P. niger*, *Nemipterus* sp., *P. argenteus*, *Ilisha* sp., *A. djedaba*, *Epinephelus* sp., *Terapon jarbua*, *T. puta*, *S. koreanus*, *E. coioides*, *Lutjanus* sp., *Pomadasys* sp. and *L. johnii* from Kemari harbour which represent that freshly catch fishes less susceptible for fungal contamination. *Aspergillus flavus* was the most dominant fungus as compared to other species of fungi but it represents the common mycoflora of this region (Table I). *Penicillium* sp. and *Fusarium* sp. was recorded in very less percentage as compared to *Aspergillus* sp. and *Rhizopus stolonifer*. *S. koreanus* contain *Aspergillus flavus*, and *Rhizopus stolonifer*. *Ilisha* sp., *A. djedaba*, *Epinephelus* sp., *E. coioides*, *Terapon jarbua*, *T. puta* and *Lutjanus* sp. infected with *Aspergillus flavus* only. *A. niger* and *Aspergillus flavus* isolated from *P. argenteus* and *Pomadasys* sp. *Fusarium* sp. identified from *Acanthopagrus* sp. whereas *Penicillium* sp. isolated from *L. johnii*. *Rhizopus stolonifer* isolated from three genera of fishes *S. koreanus*, *Nemipterus* sp. and *L. johnii*.

Seven genera and 9 species of fungi were recorded from fishes of Ormara region. The most dominant isolated fungal sp. in the examined fishes was *A. niger*. *A. niger* and *Penicillium* sp. were isolated from *O. ruber*. *A. niger*, *A. fumigatus*, *Rhizopus* sp. and *Rhizoctonia solani* were examined from tissues of *S. commersonniaus*. *A. niger*

and *Fusarium* sp. were recorded from *O. tardoore*. *A. niger* was observed in *T. vitirostris*. *A. niger*, *A. flavus* and *Penicillium* were identified from *T. setirostris*. 3 genera of fungi *A. niger*, *Cladosporium* sp. and *Mucor* sp. were associated with *S. longiceps* (Table II). Our results represent that very less number of fungi isolated from *T. vitirostris*.

We isolate 4 genera of fungi from different variety of fishes from Kemari harbour similar studies carried out by Refai *et al.* (2010) reported healthy and diseased fish sample contains 2081 fungal species among which *Saprolegnia*, *Scopulariopsis*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Paecilomyces*, *Curvularia*, *Fusarium* and *Mucor* from *Oreochromis* sp. and *Clarias gariepinus*. Shahbazian *et al.* (2010) reported *Fusarium oxysporum*, *F. npoa*, *penicillium citrinum*, *Aspergillus treuse*, *Alternaria* sp., *Aspergillus clavatus*, *Cladosporium* sp., *Helmintosporium* sp. and *Mucor* sp. and 5 fungi belongs to saprolegniaceae family from eggs of trout. Eight genera of fungi *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *Acremonium* sp., *Alternaria* sp., *Fusarium solani*, *Saprolegnia* sp. and *Cladosporium* sp. were isolated from eggs and brood stock of trout fish (Fadaeifard *et al.*, 2011).

In our studies we found that very less number of fungi was isolated from marine fishes whereas similar studies carried out by Siddique *et al.* (2009) but they found that fresh water culturable fishes more susceptible with fungal infection as compared to non culturable fresh water fishes.

Table I: Fungi isolated (%) from fishes of Karachi (Pakistan) fish harbor.

Name of fishes	Name of fungi				
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium</i> sp.	<i>Fusarium</i> sp.
<i>Ilisha</i> sp.	0±0	55.55±1.0	0±0	0±0	0±0
<i>Alepes djedaba</i>	0±0	44.44±0.57	0±0	0±0	0±0
<i>Epinephelus</i> sp.	0±0	22.22±1.15	0±0	0±0	0±0
<i>Scomberomorus oreanus</i>	0±0	33.33±1.0	33.33±1.0	0±0	0±0
<i>Epinephelus coioides</i>	0±0	11.11±0.57	0±0	0±0	0±0
<i>Terapon jarbua</i>	0±0	44.44±1.15	0±0	0±0	0±0
<i>Terapon puta</i>	0±0	44.44±1.52	0±0	0±0	0±0
<i>Pampus argenteus</i>	11.11±0.57	11.11±0.57	0±0	0±0	0±0
<i>Parastromateus niger</i>	22.22±1.15	0±0	0±0	0±0	0±0
<i>Nemipterus</i> sp.	0±0	0±0	22.22±0.57	0±0	0±0
<i>Acanthopagrus</i> sp.	0±0	0±0	0±0	0±0	11.11±0.57
<i>Pomadasys</i> sp.	22.22±0.57	11.11±0.57	0±0	0±0	0±0
<i>Lutjanus johnii</i>	0±0	0±0	11.11±0.57	11.11±0.57	0±0
<i>Lutjanus</i> sp.	0±0	11.11±0.57	0±0	0±0	0±0

Table II: Fungi isolated from economically important fishes of Ormara region.

Name of fungi	Name of fishes					
	<i>Otolithus ruber</i>	<i>Scomberoides commersonnia</i>	<i>Opisthopterus tardoore</i>	<i>Thryssa vitirostris</i>	<i>Thryssa setirostris</i>	<i>Sardinella longiceps</i>
<i>Aspergillus niger</i>	55.55±2.08	44.44±0.577	44.44±0.577	22.22±1.154	33.33±0.0	11.11±0.577
<i>A. flavus</i>	0±0	0±0	0±0	0±0	11.11±0.577	0±0
<i>A. fumigates</i>	0±0	22.22±1.154	0±0	0±0	0±0	0±0
<i>Penicillium</i> sp.	44.44±1.154	0±0	0±0	0±0	33.33±1.00	0±0
<i>Rhizopus stolonifera</i>	0±0	11.11±0.577	0±0	0±0	0±0	0±0
<i>Fusarium solani</i>	0±0	0±0	11.11±0.577	0±0	0±0	0±0
<i>Cladosporium</i> sp.	0±0	0±0	0±0	0±0	0±0	11.11±0.577
<i>Mucor</i> sp.	0±0	0±0	0±0	0±0	0±0	11.11±0.577
<i>Rhizoctonia solani</i>	0±0	22.22±0.577	0±0	0±0	0±0	0±0

Iqbal *et al.* (2012) isolated *Saprolegnia* and *Achyla* from *Ctenopharyngodon idella*, and *Catla catla*. Refai *et al.* (2010) reported that *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Blastomyces* spp. and *Rhizopus* spp. are normal mycoflora. Many pathogenic fungi cause diseases such as *Saprolegnia* sp. cause Saprolegniasis, *Aspergillus* sp. cause Aspergillosis and *Paecilomyces* cause Paecilomycosis. Surrounding environment mostly effect the population of marine fungi (Fadaeifard *et al.*, 2011).

We found that *Aspergillus* sp. was a most dominant fungus in both Kemari harbour and Ormara region as compared to other fungi different scientist also observed that as compared to *Penicillium* spp., *Candida* spp., *Rhodotorula* spp., these fungi *Aspergillus* sp. was a most dominant fungi (Ammar, 2001; El-Ahl, 2010). *Aspergillus flavus* was a most dominant fungus isolated from fish feed (Ibrahim, 2000). From different samples of shellfishes, *Penicillium corylophilum* was isolated from them (Nofiani *et al.*, 2010). Hassan (2003) observed that fishes exposed with fungal contamination during handling, storage and transportation process. Marine environment contain large amount of salt so marine fishes less susceptible for fungal contamination but in culturable fishes contaminated feeds by worker hands and poor water supply play an important role in fungal infection (Hassan and Abdel Dayem, 2004; Hassan *et al.*, 2007).

Isolation of 4 genera of fungi viz., *A. niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium* sp. and *Fusarium* sp. from Kemari harbour comparable to the study of Iqbal *et al.* (2014) isolated four genera of fungi *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus* from head, eyes, buccal cavity, operculum, gills, skin of fresh water silver carpfish, *Hypophthalmichthys molitrix*. Jalilpoor (2006) isolated 4 genera of fungi *Fusarium* sp., *Saprolegnia* sp., *Penicillium*

sp. and *Mucor* sp. from eggs of *Aspencer percicus*. Infection by *Fusarium* sp. in fresh water fishes is reported to increase in recent years (Shahbazain, 2010). In Iran 16 species of fungi were isolated from infected eggs of rainbow trout *Oncorhynchus mykiss* (Das *et al.*, 2012). Haroon *et al.* (2014) isolated 5 genera of *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium* and *Alternaria* from freshwater ornamental fishes *Carassius auratus* (L.), *Xiphophorus maculatus* and *Poecilia reticulata*.

In the present study we isolate nine species of fungi from six different variety of fishes from Ormara region, similar studies carried out by Hassan *et al.* (2011) isolated seven genera of fungi and two genera of yeast from different types of fish whereas *Alternaria* spp., and *Penicillium* spp. were the most dominant genera as compared to other fungi. Siddique *et al.* (2009) found that 15 fish species were infected with three genera of fungi viz., *Branchiomyces* sp., *Saprolegnia* sp. and *Aphanomyces* sp. Eli *et al.*, (2011) reported 3 diseases of fungi viz., Saprolegniasis, Branchiomyces and Dermocystidium from culturist fish of Africa. Hashem (2011) isolated 15 species of fungi from African catfish whereas 6 number of fungal species isolated from bony bream. Iqbal and Saleemi (2013) isolated three genera of fungi such as *Aspergillus* sp., *Blastomyces* sp., *Penicillium* sp. and unidentified mycelium from eyes, head, gills, buccal cavity and operculum of *Catla catla* fish.

In the present study we found that very less percentage of common mycoflora isolated from fish meat of Ormara region and Kemari harbour that cannot disturb the nutritional value of fish.

Conclusion

The foregoing study has indicated that Kemari harbour and Ormara region is less polluted and very less

percentage of fungi was isolated from this region. The present investigation is a significant work to study the mycological quality of marine fishes of Pakistan coast. It is suggested that further study is required to study the mycological quality of marine fishes of Pakistan from different regions.

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Statement of conflict of interest

The authors in the paper showed no potential conflicts of interest.

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