

FIRST REPORT OF OCCURRENCE OF HUMAN PATHOGENIC FUNGUS, *BLASTOMYCES* (AJELLOMYCETACEAE; ONYGENALES) IN RAVI RIVER, LAHORE

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Received on: 11-09-2022; Reviewed on: 21-08-2023; Accepted on: 07-10-2023; Published on: 27-12-2023

Abstract

The Ravi River is considered as one of the eastern tributaries of the Indus River. The overall state of water quality is currently poor, owing mostly due to the direct discharge of household and industrial effluents into the river via a network of drains. Certain microorganisms, such as bacteria, viruses, and fungi are found in the water of this river. *Blastomyces dermatitidis* has been isolated twice, characterized, and studied from the water of Ravi River of Lahore. *Blastomyces dermatitidis* causes blastomycosis which primarily affects the lungs; it frequently disseminates and affects the skin, osteoarticular tissues, the genitourinary system, and other organs. Its conidia cause respiratory diseases i.e., Blastomycosis in humans and animals. One can easily get infected from this fungus if he even takes a bath from this water. As a result of the poor water quality, the river's ecosystem is not promising for the aquatic and human life and surrounding environment.

Keywords: Blastomycosis, filamentous fungi, first report, skin diseases, pneumonia-like infection.

Introduction

Blastomycosis is primarily a pulmonary infection but it frequently disseminates and affects the skin, osteoarticular tissues, the genitourinary system, and other organs as well (Smith and Kauffman, 2010). In the genus *Blastomyces*, thermally dimorphic fungi are included. This genus is mostly found in soil and water and also causes different diseases to human and animals. The transmission of blastomycosis is widespread throughout Africa (Saccante and Woods, 2010). In west Asia, a single case each in Israel and Saudi Arabia have been reported and five cases from India were confirmed to be of blastomycosis up till now (Randhawa *et al.*, 2013; Kumar *et al.*, 2014). According to a study of databases of Chinese literature, there were no more than twenty cases reported in China (Zhao *et al.*, 2011). The fungus produces small, light spores like bread mold that get into the air and can be inhaled deep into the lungs

(Baily *et al.*, 1991). The *Blastomyces* fungi grow best in wet areas such as riverbanks, lakeshores, along streams, and in swamps. Most dogs become infected with this by going into these areas. Signs of blastomycosis depend on the sites of infection. Common sites of infection are the eyes, bones, skin, and lymph nodes, but any tissue can become infected (Frean *et al.*, 1989).

On the other hand, investigators have previously hypothesized that African blastomycosis is caused by different *Blastomyces* strains which result in disseminated (cutaneous and bone) disease rather than localized pulmonary disease. Dissemination of the fungus from the lungs via the bloodstream is presumed to lead to skin and bone involvement (Carman *et al.*, 1989). From Pakistan, recently a case of blastomycosis has been reported (Chaudhry *et al.*, 2023).

The purpose of this research was to explore the diversity of filamentous fungi in water samples of various sites of Ravi River of Lahore by macroscopic and microscopic characterization. After performing isolation and characterization, *Blastomyces dermatitidis* was also found to occur in this river water whose conidia causes respiratory or other secondary diseases in humans and animals. So, the removal of *B. dermatitidis* from water is a major concern which can be addressed in future.

Materials and Methods:

In order to collect the water samples, three different sites of Ravi River were selected. And from each three sites, at least three replicates of water samples were collected. For isolation of filamentous fungi from Ravi River water, two culturing method were employed, the direct plate method and dilution plate method. During dilution plate method, several dilutions were employed to get better resolution of fungal colonies (Pereira *et al.*, 2009).

Culturing: About 20–25 ml molten cooled PDA and MEA were taken and poured into four 90 mm Petri plates. The media was allowed to harden for about 15 minutes. In direct plate method, about 5–7 drops of water were placed on media and then rotated gently to scatter water particles on media. In dilution method, 25%, 50% and 75% dilution of each Ravi River water sample were made, and 5–7 drops of these dilutions were poured on each Petri plate containing solidified PDA and MEA media. At that point, Petri dishes were then wrapped with parafilm particularly from the edges. Petri plates were incubated at $25\pm 2^{\circ}\text{C}$ for seven days in the dark (Kinsey *et al.*, 2010). Through microscopy, the growing culture was recognized. For slide preparation, a very little piece of the fungal strain was transferred to a glass slide with the use of a sterile needle. A drop of 5% KOH or Lactic acid was

used for colored structures. And a drop of Trypan Blue or 1% Congo red was used for hyaline structures. And they are used as a mounting medium over the slide. To ensure no trapping of air bubble, cover slip was placed very carefully.

Morphological and anatomical characteristics of colonies growing in PDA media were recorded. Illustrations were prepared and micrographs were taken. In order to identify the isolated filamentous fungal taxa, different literature was assessed such as Fungi of Pakistan (Ahmad *et al.*, 1997) and illustrated genera of imperfect fungi (Barnett *et al.*, 1986).

Results:

***Blastomyces dermatitidis* Gilchrist & W.R.**

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Macroscopic characterization:

Colony morphology: Pure culture light pink, powdery, fluffy, slightly bulged. Texture rough, powdery. Margins round, uneven. Growth rate slow. Reverse of colony light yellow having brown patches. Impure culture light pink, powdery, slightly raised.

Microscopic characterization: Vegetative mycelium hyaline in 5% KOH, pluriseptate, branched, narrow, conidiophore hyaline to light green, 2.85–4.28 μm , septate, once branched then produce small unbranched conidiophores, unbranched conidiophores are rather short, yet varying length extend from the basal hyphae and bear round to slightly pyriform (teardrop) shaped conidial head at the apex, conidial head light green, round, 11.4–14.25 μm , enclose cytoplasmic aggregations. conidia ovoid to pyriform, hyaline to light green, numerous, 8.5–11.4 \times 2.85 μm .

Material examined: *Blastomyces dermatitidis* was isolated twice from the water of Ravi River such as (SFB1) in 2018 and (SRB1) in 2021 (Fig. 1).

Comments:

Blastomyces dermatitidis is characterized due to the presence of hyaline to light green, septate and branched, up to 4.3 µm wide conidiophores bearing conidial head which is usually light green, round and up to 14.2 µm wide. Conidial head contains numerous conidia inside it. Conidia are ellipsoidal and hyaline. This species was matched with the description of *B. dermatitidis* provided in Sugar, 1992. *B. dermatitidis* is an ecologically mysterious pathogen causing sickness in humans and animals. Blastomycosis is a disease caused by this fungus, which is a respiratory disorder, and produces mild pneumonia-like infection in the beginning leading to a potentially fatal condition. Our lack of understanding of where and how this organism normally thrives in nature makes prediction of disease risk and prevention of sickness extremely difficult. Several other filamentous fungi are also reported from Pakistan which can cause potential diseases in humans and animals (Razaq and Yousaf, 2020).

Conclusion:

In efforts to record the biodiversity of filamentous fungi in river Ravi, *Blastomyces dermatitidis* was isolated, characterized and identified. This fungus is a causative agent for a potentially fatal disease, blastomycosis. Based on current findings, it can be inferred that the water of the River Ravi contains a variety of fungal isolates, some of which are important plant and human pathogens, therefore unfit for domestic use.

Conflict of interest: There is no conflict of interest.

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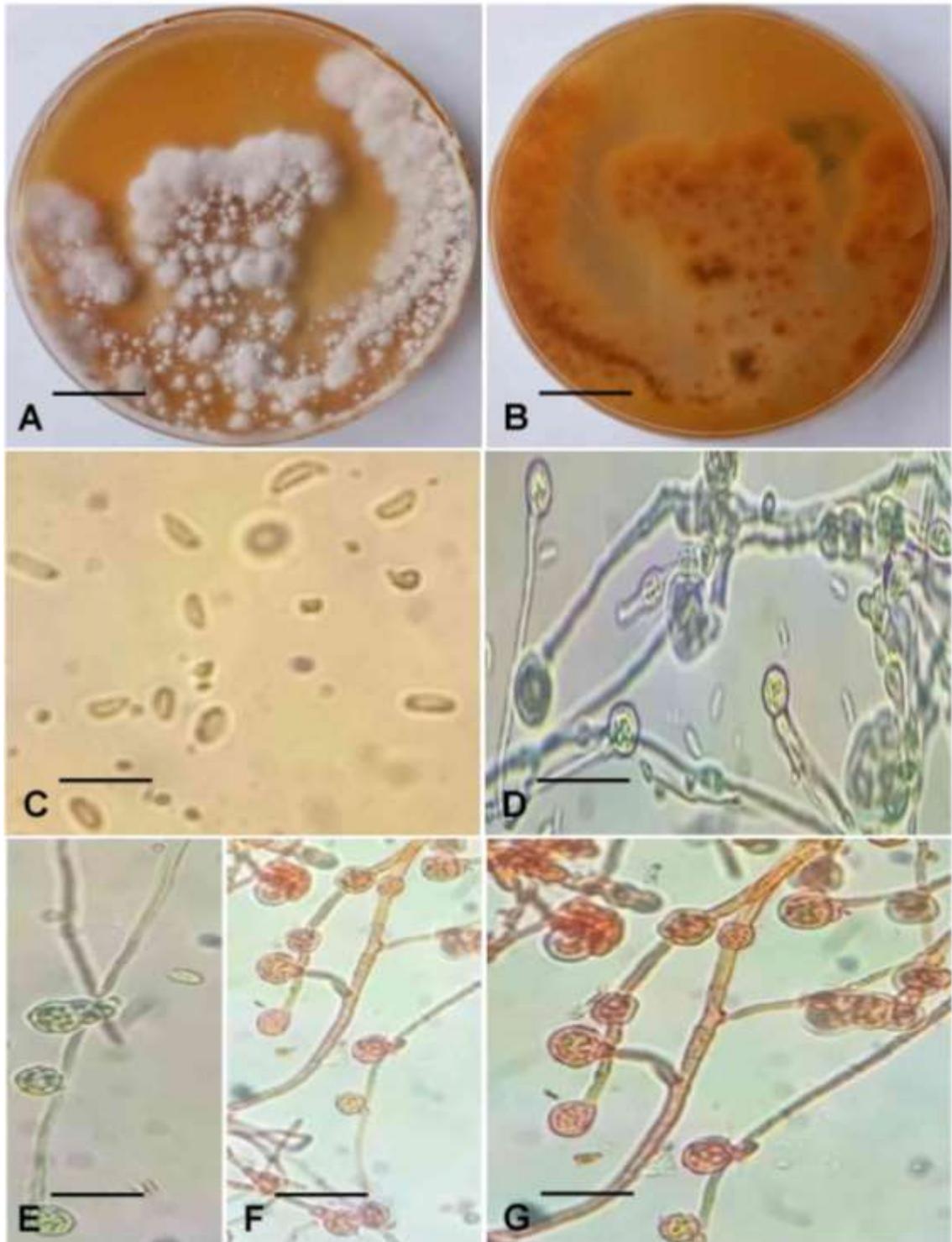


Fig. 1. A-G: Morphology of *Blastomyces dermatitidis* (SRB1). **A.** Pure colony. **B.** Reverse of colony. **C.** Light micrograph of conidia. **D&E.** Light micrographs of conidiophore. **F&G.** Light micrographs of hyphal structure include conidial head. **Scale bar: A& B= 1.3cm. C = 24µm. D = 35µm. E = 17µm. F = 21µm. G = 14µm.**