Original Article

Isolation of *Histoplasma capsulatum* and *Blastomyces dermatitidis* from Iraqi Patients with Lower Respiratory Tract Infections

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Abstract

One hundred and fifty immunocompetent and 150 presumably immunocompromised patients suffering from lower respiratory tract infections were enrolled in this study. The clinical specimens were collected from April 2007 to June 2008 and included sputum (247), bronchial wash (80), and blood (300) samples. The identification process employed direct examination, culture, conversion test, and serological study. Among 218 fungal isolates only six were categorized as true pathogenic fungi; two *Histoplasma capsulatum*, and four *Blastomyces dermatitidis*. The former isolates were detected in two immunocompromised patients, while the latter isolates were detected in two immunocompetent and two immunocompromised patients.

Key words: *Histoplasma capsulatum*, *Blastomyces dermatitidis*, respiratory tract infection.

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Introduction

"The clinical presentations of fungal respiratory infections are nonspecific and often overlap with other infectious and noninfectious processes. The specific diagnosis is often delayed."[1,2]

Fungal respiratory diseases consist of fungal colonization, allergic reaction, and invasive infections of the respiratory tract and lungs.[3] With colonization, the fungal pathogens can be isolated in the absence of any signs or symptoms of clinical infection.[4,5] However, patients with fungal infection of the respiratory tract usually exhibit clinical manifestations.[6]

Considerable variations exist in the pathogenicity of fungi in the respiratory tract. Some are highly pathogenic and capable of establishing an infection in all exposed individuals, while others cause diseases only in immunocompromised hosts.[1] For example, the endemic fungal pathogens *Histoplasma capsulatum*, *Blastomyces dermatitidis, Coccidioides immitis,* and *Paracoccidioides brasiliensis* cause infection in both healthy and immunocompromised hosts in defined geographic locations of North America and around the world.[7]

Histoplasma capsulatum is a dimorphic fungus found in the temperate zones of the world.[8] The spores of the fungus are inhaled into the lung and transformed into yeast form within a few days. Histoplasmosis is the most common pulmonary and systemic mycosis in humans.[9] Because of the similarity in the symptoms, it is sometimes mistaken for tuberculosis.[10] Infection with *B. dermatitidis* occurs by the inhalation of the conidia into the lung followed by its transformation at body temperature to the yeast phase and reproduction by budding.[11]

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Pulmonary blastomycosis has been reported to mimic bacterial pneumonia or bronchogenic carcinoma, which results in either delayed therapy or the performance of unnecessary diagnostic procedures.[12]

Fungal pneumoni occurs most often in immunocompromised hosts. However, infection due to the endemic fungi may either be asymptomatic or present with systemic signs often confused with bacterial and viral infections.[10] The present study identifies *H. capsulatum* and *B. dermatitidis* among Iraqi immunocompetent and immunocompromised patients suffering from lower respiratory tract infections.

Materials and Methods

Patients

The present study was conducted from April 2007 to June 2008 on 300 patients suffering from lower respiratory tract (LRT) infections. There were 175 male patients (58.3%), and 125 female patients (41.7%). Their ages ranged from 1 to 89 (mean ± SD 55.4±17.9) years. The patients included 150 (50%) immunocompetent and 150 (50%) presumably immunocompromised individuals. The immunocompromised patients had different primary or underlying diseases that could compromise their immunological status, including carcinoma and leukemia (n=69, 46%), uncontrolled diabetes mellitus for more than five years (n=38, 25.3%), old tuberculosis with negative acid-fast bacillus (AFB) examination (n=16, 10.7%), and long-term (>3 years) corticosteroid therapy (n=27, 18.0%).

Studied Samples

A total of 627 samples were collected from patients admitted to the respiratory care units (RCUs), bronchoscopy units, and medical wards of both the Teaching Hospital and the Oncology and Nuclear Medicine Hospital in Mosul, Iraq. The samples consisted of 247 sputum and 80 bronchial wash (both sputum and bronchial wash were taken from 27 patients). Blood samples were taken from all 300 patients for serological study.

Each sputum sample was shaken by a vortex for 3-5 minutes for homogenization. The bronchial wash samples were centrifuged for five minutes, and then the sediment was used for culture and direct microscopic examination. Blood was centrifuged at 3000 rpm/min for three minutes. The serum was transferred into tubes and stored at -20 ^{o}C until use.

Isolation of Histoplasma and Blastomyces

Each bronchial wash or sputum specimen was inoculated onto brain heart infusion (BHI) blood agar, yeast extract phosphate agar, and modified Sabouraud agar with chloramphenicol (40 mg/L) and gentamicin (25 mg/L). All media used were purchased from Oxoid, United Kingdom. The inoculated media were incubated at 28-30^{o}C, examined periodically after the fifth day of incubation, and considered negative after 4-6 weeks.[7,13] At the time of doing the culturing, four slides were prepared from each clinical specimen. Wet-mounted slides were prepared by mixing a loopful of each specimen with a drop of 20% KOH solution and then examination under the high power of a light microscope. The second wet-mounted slide was prepared by mounting each specimen with a drop of calcofluor (BBL, U.S.A.) and a drop of 20% KOH solution and examined under a fluorescent microscope.[13] The third heat fixed smear was stained with Gram stain and examined under oil immersion lens. Lastly, alcohol-fixed smears were stained with Giemsa stain and also examined under oil immersion lens.[14] Lactophenol mounting preparation was also done from the growth after culturing.

The conversion (dimorphism) test was done by inoculating a portion of the mold colony of suspected *Histoplasma* or *Blastomyces* on BHI-blood agar, and incubating at 37^{o}C for 10-15 days for the appearance of yeast form of the isolates.[7]

Serum of patients with *H. capsulatum* isolates were tested by double immunodiffusion technique (Ouchterlony) to detect anti-Histoplasma antibody. The antigen (REF # 100201) and antibody (REF # 100601) used were purchased from Meridian Bioscience, Inc., Cincinnati, Ohio. Positive and negative controls were included with the test.

Results

Two species of true pathogenic fungi were identified in six out of 218 fungal isolates from 300 patients with LRT infections. Two isolates of *H. capsulatum* were detected only in immunocompromised patients, and four isolates of *B. dermatitidis* were detected in both immunocompetent and immunocompromised patients (two isolates in each group) (Table 1). These isolates were identified according to

Table 1. The true pathogenic fungi detected out of 218 isolates from immunocompetent and immunocompromised patients.

Fungi	Isolates		Patients			
			Immunocompetent (N=150)		Immunocompromised (N= 150)	
	No.	%	No.	%	No.	%
True Pathogenic	6	2.7	2	0.9	4	1.8
H. capsulatum	2	0.9	-	-	2	0.9
B. dermatitidis	4	1.8	2	0.9	2	0.9
Opportunistic	212	97.3	80	36.7	132	60.6
Total isolates	218	100.0	82	37.6	136	62.4

their morphological features by direct examination and culture.

The two isolates of *H. capsulatum* were detected from cases of pneumonia from elderly (85 and 65 years) immunocompromised patients. One of these patients (male) suffered from underlying bronchial carcinoma, and the other (female) had underlying old tuberculosis. The main symptoms present in these patients were fever, cough, dyspnea, and weight loss. Both patients were treated with antibiotics for several months.

H. capsulatum was identified by the appearance of small, oval budding yeast cells inside macrophages or mononuclear cells on direct Giemsa-stained smears (Figure 1a) Calcofluor/KOH staining did not reveal the presence of intracellular yeast form in any of the samples. Colonies on different types of media, initially appeared as glabrous or wrinkled and creamy in color within 10-15 days, and white or light brown aerial hyphae developed on modified Sabouraud's agar at 28°C 3-4 weeks after culture (Figure 1b). Microscopical appearance with lactophenol mounting revealed filamentous, septated hyphae with characteristic echinulate macrospores (macroconidia) and finger-like projections, in addition to the microconidia (Figure 1c). Upon conversion of mold to yeast form at 37°C granular, mucoid cream-colored colonies became brown in color (Figure 1d), which microscopically showed oval to round small budding yeast cells (Figure 1e). The Ouchterlony test was negative for anti-Histoplasma antibodies in sera from both patients.

Four isolates of *B. dermatitides* were detected. Two were isolated from sputum of immunocompetent patients, while the other two were from bronchial wash of immunocompromised patients. Their ages ranged from 32 to 75 years. The younger immunocompetent patient (male) was a smoker, suffering from chronic bronchitis with fever, cough, hemoptysis, and productive sputum. The older immunocompetent patient (male) was also a smoker for 50 years with cough, dyspnea, and hemoptysis. Chest X-ray showed cavitation and a mass in the right lung. The two immunocompromised patients, one female and one male smoker, had pneumonia. The major symptoms present in both patients were cough, fever, dyspnea, anorexia, and chest pain. The three youngest patients received antibiotics for several months.

The specimens of sputum or bronchial wash in wet preparation with 20% KOH solution and calcoflour stain revealed large budding yeast cells under light and fluorescent microscopes respectively (Figure 2a). In addition Gram and Giemsa stained smears were examined under oil immersion lens. Growth of *B. dermatitidis* on different types of media appeared after 18-20 days at 28°C. Colonies first appeared on modified Saboraud's agar as yeast-like growth, followed by the development of hyphal projections on the surface, and finally the entire surface became downy or fluffy white (Figure 2b). Microscopical appearance on lactophenol-mounted slides showed mycelial elements and numerous round, one-celled conidia attached to hyphae under high-power magnification (Figure 2c). The conver-

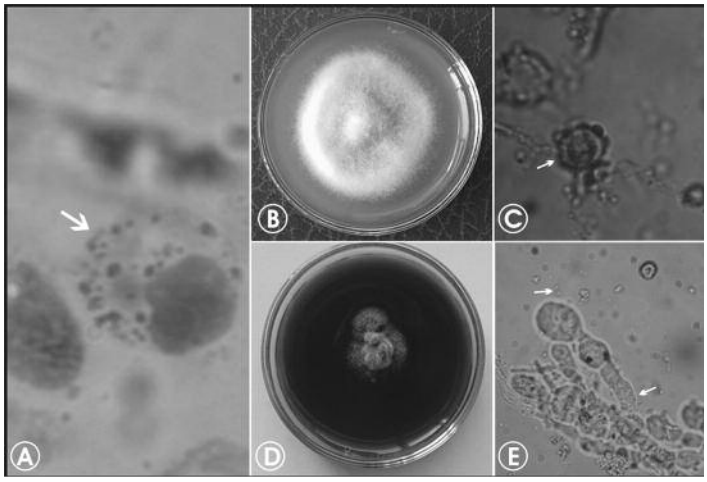

Figure 1. *Histoplasma capsulatum*. a. Direct examination of a Giemsa stained smear of bronchial wash showing small, oval budding yeast cells within monocyte (100X). b. Colony on modified Sabouraud's agar, showing white to brown velvety appearance. c. Lactophenol mount, showing the characteristic macroconidia and microconidia (arrowed), 40X. d. Colony on BHI blood agar, showing yeast – like appearance. e. Lactophenol mount, showing small oval budding yeast cells with mycelial elements in stage of conversion to yeast cells (arrowed), 40X.

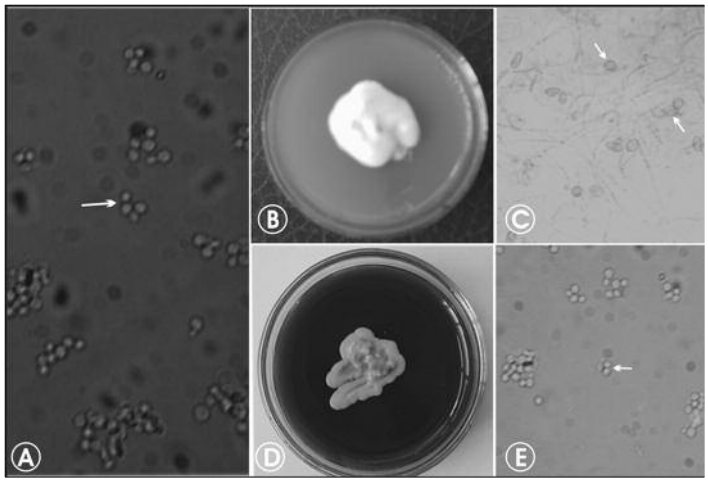

Figure 2. *Blasatomyces dermatitidis.* a. Direct examination of clinical specimens mounted in calco fluor and 20% KOH solution, showing large budding yeast cells with broad based budding under fluorescent microscope, 40X. b. Colony on modified Sabouraud's agar, showing white, fluffy mold. c. Lactophenol mount, showing mycelial elements and smooth conidia produced on conidiophore (arrowed), 40X. d. Colony on BHI blood agar showing smooth yeast. e. Lactophenol mount, showing large budding yeast cells with thick walls and broad base of buds (arrowed), 40X.

sion of mold to yeast colonies on enriched medium at 37°C appeared after 10-12 days. The yeast colony was creamy to tan in color and soft (Figure 2d). Microscopically, the colonies showed large, round, single budding, thick-walled cells with broad-based budding at 40X magnification (Figure 2e).

Discussion

The diagnosis of fungal infections depends on the selection and collection of the clinical specimens for laboratory studies. In addition, clinical history and presentation may add to the identification process because fungal infections manifest similarly to bacterial and other lung infections.[6]

Two true pathogenic fungi, *H. capsulatum* and *B. dermatitidis,* were isolated in the present study. Even though these species are endemic worldwide,[15] this is the first report of these fungi being isolated from clinical samples from Mosul, Iraq. These species were detected in immunocompetent and immunocompromised patients. Previous studies showed that the true pathogenic fungi were primary pathogens in immunocompetent individuals, but also caused secondary infections in immunocompromised patients.[1,16] The isolated *H. capsulatum* were detected in two elderly immunocompromised patients. Chronic pulmonary histoplasmosis was reported to occur mostly in old patients with underlying diseases and represents 10% of symptomatic cases.[9] It is known that the main differential diagnosis of pulmonary histoplasmosis is tuberculosis.[17,18] On the other hand, cancer patients receiving cancer chemotherapy and patients on a high dose, long-term steroid therapy are at risk for developing histoplasmosis.[19,20] Reactivation of latent disease can occur in elderly and immunocompromised individuals years after infection with *H. capsulatum*.[18] The patients with *H. capsulatum* isolates suffered from pneumonia and were not responsive to antibiotics and/or antimycobacterium therapy. Such a history is usually encountered in cases of chronic pulmonary histoplasmosis[21] and may be associated with increased mortality, as happened in the elderly tuberculosis patient in this study.

Intracellular budding yeast in sputum and/or bronchial wash were apparent with Giemsa staining and not by wet mounting with KOH and calcofluor solution. This confirmed that Giemsa stain is more

likely to show the intracellular yeast cells than KOH or calcofluor.[7] Furthermore, the microscopic characteristic macroconidia helped in the identification. Another investigator also reported that the appearance of macroconidia allows a presumptive diagnosis of histoplasmosis.[17] However, the genera *Sepedonium* and *Chryosporium* form similar macroconidia, therefore conversion of mold to yeast is necessary to ensure that the fungus is *H. capsulatum*.[22]

Antibodies against *Histoplasma* were not detected by immunodiffusion in the patients with histoplasmosis in our study. This may be related to the immunocompromised status of the studied patients.[18]

Pulmonary blastomycosis can present in a manner indistinguishable from bacterial pneumonia, tuberculosis, or bronchogenic carcinoma.[23] The relatively low isolation rate noted in the present study and other reports may be a result of blastomycosis being misdiagnosed and difficult to identify in the diagnostic laboratories. The lungs are considered the most common site of fungal infection and can present with a cavity and large masses.[17] Moreover, patients with blastomycosis mostly are elderly, but the disease also occurs in infants and the very elderly.[11]

Out of the four *Blastomyces* isolates, three were detected in male smokers. This may indicate a potential association between blastomycosis and environmental conditions surrounding male-dominated professions and recreational activities.[17]

The use of KOH solution with or without calcoflour to detect the large budding yeast cells of *Blastomyces* in clinical specimens is considered a simple test that has a high diagnostic yield.[11] Furthermore, sputum has a high recovery yield in culture.[24,25] Other studies stated that out of the clinical samples, sputum and tracheal secretions were positive in 86% of cases, and specimens from bronchoscopy were positive in 92% of cases.[23] The specimens from the four patients in our study with blastomycosis were positive for *Blastomyces* both by direct smear and culture, in addition to the conversion test, which ensures that the fungus is *B. dermatitidis*.[12]

Conclusion

Histoplasma capsulatum and *B. dermatitidis* are important uncommon pathogens in LRT infections mainly in immunocompromised patients. The immunodiffustion test is of a limited value for the detection of specific antibody of *H. capsulatum*.

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