Pneumonia and pleural effusion due to *Cryptococcus laurentii* in a clinically proven case of AIDS

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Non-neoformans cryptococci were previously considered to be saprophytes and nonpathogenic to humans. *Cryptococcus laurentii* is frequently used as a biological means to control fruit rot. Interestingly, *C laurentii* has recently been reported to be a rare cause of infection in humans. The authors report a case of pulmonary cryptococcosis caused by *C laurentii* in a diabetic AIDS patient who was on antituberculosis and antiretroviral treatments. The sputum smear revealed capsulated yeast cells that were identified as *C laurentii*. Repeated pleural fluid culture revealed growth of *C laurentii*. Both respiratory samples were negative for acid-fast bacilli. *Moraxella catarrhalis* and *Klebsiella pneumoniae* were also found in the sputum, but not in the pleural fluid. The patient had a good response to oral fluconazole therapy at 600 mg/day for five weeks and was then discharged. The present article is the first to report on the rare pulmonary involvement of *C laurentii* in the Indian HIV population. These unusual forms of cryptococci create a diagnostic predicament in the rapid diagnosis of pulmonary cryptococcosis. A high degree of suspicion and improvement of techniques for culture and identification will contribute to the early diagnosis and treatment of unusual fungal infections.

Key Words: *AIDS;* Cryptococcus laurentii; Klebsiella pneumoniae; Moraxella catarrhalis; *Non-neoformans cryptococcus*

Une pneumonie et une effusion pleurale attribuables au *Cryptococcus laurentii* dans un cas de sida démontré cliniquement

Auparavant, on croyait que le Cryptococcus non neoformans était saprophyte et non pathogène chez l'humain. Le *Cryptococcus laurentii* est souvent utilisé pour le contrôle biologique de la pourriture des fruits. Fait intéressant, le *C laurentii* a récemment été déclaré comme rare cause d'infection chez les humains. Les auteurs font état d'un cas de cryptococcose pulmonaire imputable au *C laurentii* chez un diabétique sidéen qui suivait un traitement antituberculeux et antirétroviral. Le frottis d'expectoration a révélé des cellules de levure encapsulées identifiées comme un *C laurentii*. Des cultures répétées du liquide pleural ont révélé la prolifération du *C laurentii*. Les deux échantillons respiratoires étaient négatifs aux bacilles acidorésistants. On a également trouvé du *Moraxella catarrhalis* et du *Klebsiella pneumoniae* dans les expectorations, mais pas dans le liquide pleural. Le patient a bien réagi à la thérapie orale de 600 mg/jour de fluconazole pendant cinq semaines et a obtenu son congé. Le présent article est le premier à rendre compte d'une rare atteinte pulmonaire au *C laurentii* au sein de la population sidéenne indienne. Ces formes inhabituelles de cryptococcose compliquent le diagnostic rapide de cryptococcose pulmonaire. Un fort degré de présomption et l'amélioration des techniques de culture et de dépistage contribueront au diagnostic et au traitement rapides d'infections fongiques inhabituelles.

Cryptococcosis, usually due to *Cryptococcus neoformans*, is considered to be one of the most serious fungal infections in immunocompromised patients. In the past, non-neoformans species have been generally regarded as nonpathogenic saprophytes. However, in recent years, opportunistic infections associated with *Cryptococcus albidus*, *Cryptococcus curvatus*, *Cryptococcus humicolus*, *Cryptococcus uniguttulatus* and *Cryptococcus laurentii* have been reported (1-6).

C laurentii, a basidiomycetous encapsulated yeast, is present in the droppings and cloacal samples of feral pigeons (7). *C laurentii* is used as a biopesticide and is efficient in controlling fruit rot in apples (8,9). *C laurentii* has recently been reported as a cause of pulmonary and cutaneous infections in humans. Interestingly, there are only 16 reported cases of disease caused by *C laurentii* infection. We report a case of pulmonary cryptococcosis resulting from *C laurentii*, along with *Moraxella catarrhalis* and *Klebsiella pneumoniae* infections, in a diabetic patient with AIDS, in whom complete clinical resolution occurred after oral fluconazole administration.

CASE PRESENTATION

A 35-year-old diabetic woman with clinically proven AIDS was admitted in September 2005 to the inpatient department of the YR Gaitonde Centre for AIDS Research and Education, a specialized AIDS care and research institution in Chennai, India. The patient presented with a febrile illness, breathlessness, dysphagia, odynophagia, vomiting, headache, cough and sputum, night sweats, malaise and left pleuritic chest pain for approximately one week. She was diagnosed with HIV infection in 2001 consequent to bouts of fever, diarrhea, aphthosis, rectal and genital ulcers, and weight loss. The patient complained of producing thick, mucopurulent sputum for the

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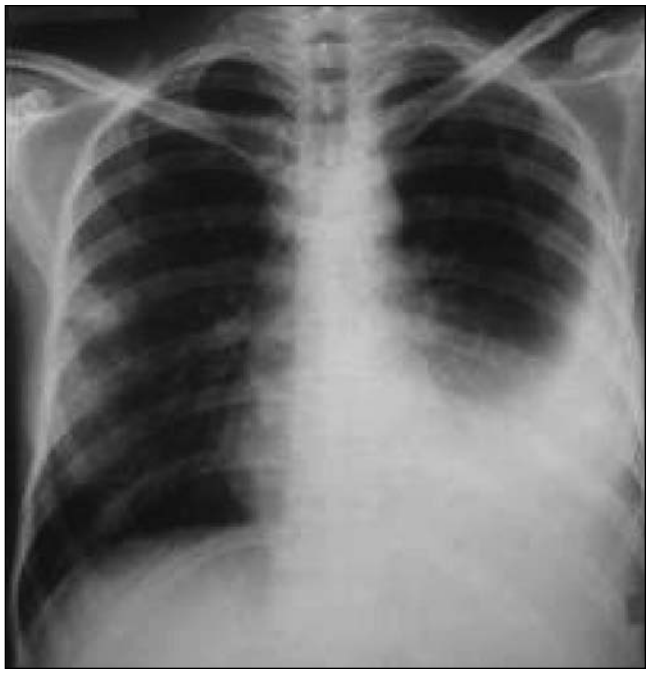

Figure 1) *Chest x-ray showing extensive left pleural effusion*

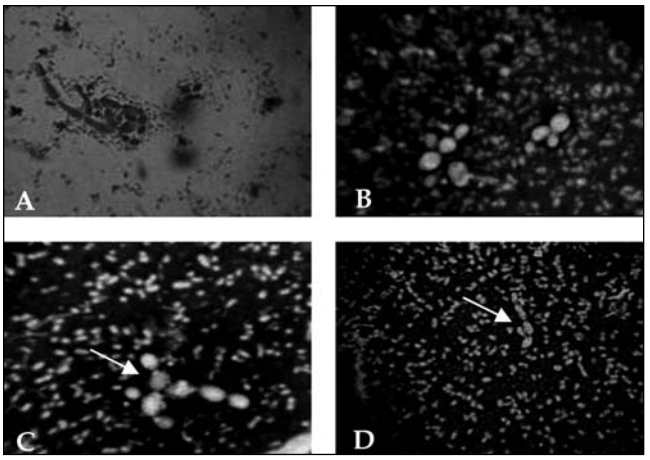

Figure 2) Cryptococcus laurentii. **A** *Direct examination of sputum revealing Gram-negative, round to oval, yeast-like fungus*. **B** *and* **C** *Negative staining with 0.5% nigrosin revealed encapsulated, round to oval, budding yeast cells with thick capsules*. **D** *Negative staining showing elongated budding yeast cells with capsules. The minute capsulated bacilli seen beside the yeasts were identified as* Klebsiella pneumoniae *(original magnification ×100, oil immersion). Arrows* (**C** *and* **D**) *point to the capsulated yeast* C laurentii

past few months and had been on some form of antiretroviral therapy with zidovudine for the past four years. At the time of admission, she was also on *Pneumocystis carinii* pneumonia prophylaxis with trimethoprim-sulfamethoxazole (160 mg/day trimethoprim and 800 mg/day sulfamethoxazole) and antituberculosis treatment with two months of daily isoniazid, rifampicin, ethambutol and pyrazinamide, followed by a seven-month continuation phase of daily isoniazid and rifampicin. On examination, the patient had a temperature of 38.5°C, a pulse of 106 beats/min, a blood pressure of 110/70 mmHg and blood oxygen saturation of 96%. She was thin, conscious, oriented and edema-free. A chest examination revealed reduced expansion on the left, quiet breath sounds, dullness to percussion in the left infrascapular region and tan-coloured, thick, mucopurulent sputum. She also presented with rales and coarse crepitations on auscultation. An abdominal examination revealed hepatosplenomegaly. A chest x-ray revealed extensive left pleural effusion (Figure 1). Laboratory examinations revealed that she had a random blood glucose of 9.8 mmol/L (normal values 4.4 mmol/L to 6.6 mmol/L), hemoglobin of 73 g/L (normal values 120 g/L to 150 g/L), total leukocyte count of 6.9×10^9/L (normal values 4×10^9/L to 11×10^9/L), total lymphocyte count of 0.2×10^9/L (normal values 0.8×10^9/L to 3.2×10^9/L), erythrocyte sedimentation rate of greater than 125 mm/h (normal values 0 mm/h to 30 mm/h) and a total platelet count of 161×10^9/L (normal values 150×10^9/L to 450×10^9/L). Her absolute CD4 lymphocyte count (Guava Technologies, USA) was 17 cells/µL (normal values 350 cells/µL to 1600 cells/µL) and her CD4 percentage was less than 14% (normal values 30% to 40%). Her liver function test revealed normal values, namely, alanine aminotransferase of 11 U/L (normal range 4 U/L to 36 U/L), total bilirubin of 6.8 µmol/L (normal values 2 µmol/L to 14 µmol/L) and conjugated bilirubin of 1.7 µmol/L (normal values 0 µmol/L to 4 µmol/L). Her urine creatinine was 8.84 mmol/day (variable). A serum electrolyte investigation revealed a chloride level of 110 mmol/L (normal values 96 mmol/L to 106 mmol/L) and bicarbonate of 15 mmol/L (normal values 22 mmol/L to 29 mmol/L). Serum sodium and potassium levels were normal. Her serum tested positive for HIV-1 antibodies by HIV-1/HIV-2 ELISA and Western blot assays (Immunetics Inc, USA), and was also positive for herpes simplex virus 2 immunoglobulin G by ELISA. The patient was negative for herpes simplex virus 2 immunoglobulin M and rapid plasma reagin antibodies for syphilis.

Pleural fluid drained on day 2 (200 mL) did not reveal any bacterial growth. A sputum examination showed Gram-negative, large, round to oval yeast cells (Figure 2A) that were initially misinterpreted as non-albicans *Candida*. Gram-negative intracellular diplococci and capsulated bacilli were also observed in the sputum, and were identified as *M catarrhalis* and *K pneumoniae*, respectively. The sputum and pleural fluid were negative for acid-fast bacilli (AFB) by Ziehl-Neelsen staining. The patient felt better after the pleural drainage and underwent blood transfusion on days 3 and 4. On day 5, she developed fever with a severe cough and dyspnea. Sputum smears were negative for AFB. Sputum and pleural fluid cultures on Sabouraud's dextrose agar with chloramphenicol (without cycloheximide) at 48 h revealed a few 1 mm to 2 mm in diameter, smooth, cream-coloured, poorly grown mucoid colonies and 2 mm to 3 mm in diameter mucoid colonies at 37°C and 25°C, respectively. No yeast cells were observed on pleural smear examination. Nigrosin staining revealed encapsulated, elongated, budding yeast cells with thickened cell walls and capsules; these cells were identified as *C laurentii* (Figure 2). The yeast was repeatedly encountered in pleural fluid culture. Antifungal therapy with oral fluconazole 600 mg/day for five weeks was started, along with oral ceftrioxone 1 g/day to 2 g/day for clearance of bacteria. Culture of both respiratory samples (BACTEC TB culture system, BD Biosciences, USA) did not reveal AFB. The patient responded well to the treatment and was discharged.

TABLE 1
Summary of data from cases of *Cryptococcus laurentii* infection in humans

Year (reference)	Age (years)	Sex	Underlying condition(s)	Prior steroid exposure	Prior catheter use	Prior neutropenia	Clinical diagnosis	Clinical presentation	Treatment	Outcome
1977 (2)	40	M	–	NR	NR	NR	Cutaneous infection	Cutaneous granuloma, regional lymph node enlargement	D-AmB	Resolved
1980 (12)	55	F	Adenocarcinoma, dermatomyositis	Yes	NR	NR	Lung abscess	Asymptomatic right upper lobe cavitary lesion	D-AmB	Resolved
1985 (16)	37	M	None known	NR	NR	NR	Pneumonia	NR	Surgery	Resolved
1989 (17)	13	F	ESRD, peritoneal dialysis	NR	NR	NR	Peritonitis	Fever, abdominal pain, cloudy dialysate fluid	Catheter removal, D-AmB	Resolved
1989 (18)	14	F	ESRD, peritoneal dialysis	NR	NR	NR	Peritonitis	Fever, abdominal pain, cloudy dialysate fluid	Catheter removal, peritoneal lavage with saline	Resolved
1995 (11)	61	F	Chronic uveitis	Yes	NR	NR	Endophthalmitis	Deteriorating vision	Fluconazole	Resolved
1997 (19)	17	M	Leukemia BMT	NR	Yes	Yes	Fungemia	Fever	Fluconazole	Resolved
1997 (4)	51	NR	Diabetes, wore contact lenses	NR	NR	NR	Keratitis	Central corneal ulceration, central descemetocele with trace aqueous leak	Enucleation, D-AmB, miconazole	Resolved
1997 (1)	<1	M	Premature birth	NR	Yes	NR	Fungemia	Hypotension, tachycardia	Catheter removal, D-AmB	Resolved
1997 (1)	27	F	Bacterial endocarditis	NR	Yes	NR	Fungemia	Fever, chills, painful cutaneous nodules	Catheter removal, fluconazole	Resolved
1998 (3)	34	M	AIDS	NR	NR	NR	Meningitis	Hypotension, fever, dyspnea, headache, dizziness, diplopia	D-AmB, flucytosine	Resolved
1998 (20)	26	M	Solid tumour	NR	Yes	Yes	Fungemia	NR	Catheter removal, fluconazole	Resolved
1998 (20)	50	M	Non-Hodgkin lymphoma	Yes	Yes	Yes	Fungemia	NR	Catheter removal, D-AmB	Death
1999 (20)	57	M	Acute myelogenous leukemia	Yes	Yes	Yes	Fungemia	NR	Catheter removal, D-AmB	Resolved
2000 (21)	<1	F	Premature birth	NR	Yes	No	Fungemia	Apnea, bradycardia, hypotension, hypothermia, abdominal distension	Catheter removal, D-AmB	Resolved
2005 (PR)	35	F	AIDS, diabetes	NR	NR	NR	Pneumonia	Pleural effusion, fever, chest pain, cough, rales, crepitation, hepatosplenomegaly	Pleural drainage, fluconazole	Resolved

BMT Bone marrow transplantation; D-AmB Deoxycholate amphotericin B; ESRD End-stage renal disease; F Female; M Male; NR Not reported; PR Present report

DISCUSSION

Cryptococci generally occur in soil contaminated with pigeon feces (10) and are transmitted to humans primarily through inhaled fomites. Species other than C *neoformans* have generally been thought to be nonpathogenic to humans (4,11,12). Although C *laurentii* has been reported as occurring worldwide, its natural habitat has not yet been thoroughly established. Cryptococcosis, an uncommon disease before the AIDS epidemic, has emerged as an important cause of illness and death in HIV-infected patients. However, there are little data on the isolation of C *laurentii* from the respiratory tract of AIDS patients; to date, only 16 cases, including the present report, have been published (these reports are summarized in Table 1). Thus, the present report is significant in that it potentially describes the first case of pneumonia resulting from C *laurentii* in the Indian HIV population.

Most patients with cryptococcosis suffer from substantial T cell dysfunction, as do patients with AIDS (13). Other immunological defects associated with cryptococcosis are lymphopenia and immune dysfunctions (14). Our patient was severely lymphopenic, as evidenced by laboratory results (total lymphotcyte count of 0.2×10^9/L). The repeated isolation of C *laurentii* from pleural fluid and sputum indicated that it was probably the cause of the pneumonic disease in our patient, although the pleural fluid did not reveal the yeast on direct examination. The involvement of AFB in the pleural fluid was also ruled out by negative AFB culture. The pleural fluid was bacteriologically sterile, ruling out other bacterial infections of the pleural space; M *catarrhalis* and K *pneumoniae* were isolated only from the sputum, not from the pleural fluid. The patient had initially felt better after pleural drainage, which could be the result of a possible reduction of the yeast

population in the fluid. There was no laboratory evidence of *P carinii*, possibly because the patient was on trimethoprim-sulfamethoxazole prophylaxis.

There is no validated standard treatment for *C laurentii* infection. Correlations between in vitro antifungal susceptibility test results and treatment outcomes do not exist for *C laurentii*. However, the patient tolerated oral fluconazole well and, with pleural drainage, the outcome was favourable. Feral pigeons may be carriers of *C laurentii* (7), but details on the patient's previous contact with pigeons were unavailable. She did, however, live in close proximity to agricultural areas and pastures. Because isolation of *C laurentii* from plants and soil has been previously reported (Fell and Statzell-Tallman [15]), a rural habitat and possible exposure to yeast, combined with underlying predisposing conditions, may have made our patient vulnerable to infection. Extensive surveillance of the patient's living environment could provide more insight into her acquisition of the yeast. A review of the literature showed only two reports pertaining to infection involving the respiratory tract: one involving a lung abscess (12) and the other involving pneumonia (16). Our patient is the second to be diagnosed with an AIDS and *C laurentii* coinfection; the first was a patient with meningitis (3).

CONCLUSIONS

The present report is the first to describe the rare pulmonary involvement of *C laurentii* in the Indian HIV population. The pulmonary symptoms may be noncharacteristic, but a high degree of suspicion and improvement of culture and identification techniques will contribute to the early diagnosis, treatment and management of unusual fungal infections in HIV/AIDS patients.

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DECLARATION OF ETHICS APPROVAL: This study was carried out after prior approval of the study protocols by the Institutional Review Board of the Voluntary Health Services – YR Gaitonde Centre for AIDS Research and Education Medical Centre, Taramani, Chennai, India. Written informed consent was obtained from the patient and her representatives before clinical specimen collection for the study.

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