Title: Human blastomycosis in South Africa caused by *Blastomyces percursus* and *Blastomyces africanus* sp. nov., 1967 – 2014

Authors: Tsidiso G. Maphanga*, Monica Birkhead, José F. Muñoz, Mushal Allam, Thokozile G. Zulu, Christina A. Cuomo, Ilan S. Schwartz, Arshad Ismail, Serisha D. Naicker, Ruth S. Mpembe, Craig Corcoran, Sybren de Hoog, Chris Kenyon, Andrew M. Borman, John A. Frean, and Nelesh P. Govender

Affiliations: National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa (Tsidiso G. Maphanga, Monica Birkhead, Mushal Allam, Thokozile G. Zulu, Arshad Ismail, Serisha D. Naicker, Ruth S. Mpembe, John A. Frean, Nelesh P. Govender); University of the Free State, Bloemfontein, South Africa (Tsidiso G. Maphanga); Broad Institute of MIT and Harvard, Cambridge, MA, USA (José F. Muñoz, Christina A. Cuomo); University of Alberta, Edmonton, Alberta, Canada (Ilan S. Schwartz); University of the Witwatersrand, Johannesburg, South Africa (Serisha D. Naicker, John A. Frean, Nelesh P. Govender); Ampath National Laboratory Service, Johannesburg, South Africa (Craig Corcoran); Westerdijk Fungal Biodiversity Institute, Utrecht and Nijmegen, The Netherlands (Sybren de Hoog); Center of Expertise in Mycology of RadboudUMC/ Canisius Wilhelmina Hospital, Nijmegen, The Netherlands (Sybren de Hoog); Institute of Tropical Medicine, Antwerp, Belgium (Chris Kenyon); UK National Mycology Reference Laboratory, Public Health England, Bristol, United Kingdom (Andrew M. Borman)
Human blastomycosis in South Africa

*Corresponding author*: Miss Tsidiso G. Maphanga, National Institute for Communicable Diseases – Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses, Private Bag X4, Sandringham, 2132, South Africa. Phone: +27 11 555 0323. Fax: +27 11 555 0435. E-mail: tsidisom@nicd.ac.za

Running Title: Human blastomycosis in South Africa

Keywords: Blastomycosis, mycoses, South Africa, Blastomyces, emeromycosis, histoplasmosis, tuberculosis

Word count (text): 3400

Conflicts of interest: None declared
Human blastomycosis in South Africa

Abstract (257 words)

We re-evaluated 20 cases of blastomycosis diagnosed in South Africa between 1967 and 2014, with *Blastomyces dermatitidis* considered to be the etiological agent, in light of newly-described species and use of more advanced technologies. In addition to histopathological and/or culture-based methods, all 20 isolates were phenotypically and genotypically characterised, including multilocus typing of five genes and whole genome sequencing. Antifungal susceptibility testing was performed as outlined in Clinical and Laboratory Standards Institute M27-A3 and M38-A2. We merged laboratory and corresponding clinical case data, where available. Morphological characteristics and phylogenetic analyses of five-gene and whole-genome sequences revealed two groups, both of which were closely related to but distinct from *B. dermatitidis*, *Blastomyces Gilchristii* and *Blastomyces parvus*. The first group (n=12) corresponded to the recently-described *Blastomyces percursus* and the other (n=8) is described here as *Blastomyces emzantsi* sp. nov. Both species exhibited incomplete conversion to the yeast phase at 37°C and were heterothallic for mating types. All eight *B. emzantsi* isolates belonged to the α mating type. Whole genome sequencing confirmed distinct species identities, as well as the absence of a full orthologue of the *BAD-1* gene.

Extrapulmonary (skin or bone) disease, probably resulting from hematogenous spread from a primary lung infection, was more common than pulmonary disease alone. Voriconazole, posaconazole, itraconazole, amphotericin B and micafungin had the most potent in vitro activity. Over the 5 decades, South African cases of blastomycosis were caused by species that are distinct from *B. dermatitidis*. Increasing clinical awareness and access to simple rapid diagnostics may improve diagnosis of blastomycosis in resource-limited countries.
Introduction

The first human case of blastomycosis was described by Gilchrist in 1894 from skin tissue [1]. Blastomycosis was initially believed to be restricted to North America [2]. However, since the first case was reported from Tunisia in 1952, cases of blastomycosis have also been reported throughout Africa, and less commonly from India, the Middle East, and occasionally Europe [3,4]. Differences have been noted between North American and African cases of blastomycosis, both in the isolates and the clinical presentation of disease. For instance, blastomycosis in North America primarily involves the lungs, while more African cases have involved skin and bone [3].

The genus Blastomyces belongs within the family Ajellomycetaceae and order Onygenales which includes other thermally-dimorphic fungi such as Histoplasma, Emmonsia, Emmonsiellopsis, Paracoccidioides, and a newly-described genus, Emergomyces [5]. Initially, the Blastomyces genus was thought to comprise a single species, Blastomyces dermatitidis [6]. Three other species have since been described within Blastomyces, including B. gilchristii (a cryptic species phenotypically indistinguishable from B. dermatitidis), B. percursus and B. silverae [5, 7, 8]. In addition, Emmonsia parva (Blastomyces parvus) and Emmonsia helica (Blastomyces helicus) have been assigned to the genus [8]. B. dermatitidis produces abundant large broad-based budding yeasts at 37°C, B. percursus produces large yeast-like cells from fragmented, swollen hyphal cells, B. helicus produces variably-shaped yeast cells in short chains, while thin-walled giant cells and occasional broad-based budding yeast-like cells are seen in B. parvus and B. silverae strains [5, 8, 9].

In prior studies, mycological differences were noted between North American and African Blastomyces strains [10-16]. African strains were more difficult to convert from the mould to
the yeast phase [10-12]. While North American and African isolates shared the K antigen, North American strains additionally possessed an A antigen, which is absent from most African strains [11]. When tested for sexual compatibility, African strains failed to mate with North American strains of *B. dermatitidis* [10]. Furthermore, DNA melting curve analyses separated the North American and African strains into groups that differed sexually, histologically, epidemiologically and clinically [15].

In view of the recent description of new pathogenic species within the genus *Blastomyces* and the potential molecular epidemiological, clinical and public health implications, we re-examined South African *Blastomyces* human clinical isolates archived over five decades.
Materials and methods

Case detection and isolate archive

We conducted passive laboratory-based surveillance for thermally dimorphic fungi associated with human disease from 2008 through 2014 at nine South African diagnostic pathology laboratories in the public- and private-sector. We defined a case of blastomycosis as a person with an isolate cultured from any specimen, initially identified as *B. dermatitidis* by standard phenotypic culture-based or histological methods. In addition, we included 17 clinical isolates from South Africa, which had been cultured from 1967 through to 1999, previously identified as *B. dermatitidis* by phenotypic methods and archived at the National Institute for Communicable Diseases (NICD), South Africa or in the National Collection of Pathogenic Fungi (NCPF), United Kingdom. For ten cases, we obtained detailed information on patient demographics, medical conditions including HIV and co-infections, details of the patient’s clinical presentation, diagnostic investigations, management and outcomes from previously-published case reports [3-5,17-21]. Limited information was available for the other ten archived isolates. In total, we studied 20 *Blastomyces* isolates. Examination of isolates included morphological characterisation by light and electron microscopy, determination of minimum inhibitory concentrations (MIC) for nine antifungal agents and phylogenetic analyses using the sequences of five genes [22]. We also performed phylogenetic analysis using whole-genome single nucleotide polymorphisms (SNPs) for 18 isolates.

Morphological description and electron microscopy

All isolates were sub-cultured onto Sabouraud agar or potato dextrose agar (DMP), Sandringham, South Africa and incubated at 25°C and 30°C for up to 4 weeks for the mycelial phase. Isolates were transferred onto brain heart
infusion (BHI) agar with or without 5% sheep blood (DMP) and incubated for 1-3 weeks at 37°C and 40°C for conversion to the yeast phase. Mycelial growth was also inoculated onto a urea agar slope (DMP) and incubated at 37°C for 6 days. Yeast and mycelial isolates were examined using light microscopy and electron microscopic (Supplementary). The descriptive terminology used follows that of Jiang et al. (2018) [8].

Antifungal susceptibility testing

Susceptibility testing was performed for both yeast- and mould-phase isolates using a reference broth microdilution (BMD) method and the commercial Etest method (bioMérieux, Marcy, l’Etoile, France). The BMD method was performed according to Clinical and Laboratory Standards Institute (CLSI) M27-A3 and M38-A2 with a modified inoculum size for the latter as described by Maphanga et al. 2017 (Supplementary) [23-25]. Quality control (QC) strains were included in each test run: Candida parapsilosis American Type Culture Collection [ATCC] 22019, Candida krusei ATCC 6258 and B. dermatitidis ATCC 10225 for yeast-phase tests and Aspergillus fumigatus National Collection of Pathogenic Fungi [NCPF] 7097, A. fumigatus NCPF 7100 and B. dermatitidis ATCC 10225 for mycelial-phase tests. The MICs for C. parapsilosis ATCC 22019, C. krusei ATCC 6258 were within the CLSI-recommended ranges. We calculated a geometric mean (GM) for each MIC distribution using Stata version 14.0 (StataCorp Limited, College Station, Texas, USA).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 20 isolates using the Zymo ZR fungal/bacterial DNA MiniPrep Kit (Zymo Research Corp., USA) according to the manufacturer’s instructions. PCR amplification of five genes (internal transcribed spacer [ITS], large subunit [LSU], actin, β-tubulin and intein PRP8) and sequencing PCR was performed as described previously.
Human blastomycosis in South Africa

(Supplementary) [22]. The samples were sequenced in a 3130 Sequencer (Applied Biosystems, Life Technologies Corporation, USA). Sequences were subjected to BLAST analyses in GenBank (www.ncbi.nlm.nih.gov) for identification.

MLST phylogenetic analysis

DNA sequences were aligned with MAFFT version 7 and datasets for the five-gene MLST loci were trimmed with BioEdit version 7 [26, 27]. The phylogenetic tree was generated by a maximum likelihood statistical method using 1,000 bootstrap replications on MEGA version 6 from the concatenated dataset and individual genes (Supplementary) [28]. A clade was defined as isolates from different patients which shared a common ancestor with >80% support values in the maximum likelihood analyses.

Whole genome sequencing and de novo assembly

Paired-end libraries were prepared using the Nextera XT DNA library kit, followed by 2 × 300-bp sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). The sequenced paired-end reads were quality trimmed and de novo assembled using Qiagen CLC Genomics Workbench version 10 (Qiagen, The Netherlands). All contigs were then submitted to GenBank (QGQE00000000=SA-NICD-12, QGQF00000000=SA-NICD-09, QGQG00000000=SA-NICD-10, QGQH00000000=SA-NICD-11, QGQI00000000=SA-NICD-15, QGQJ00000000=SA-NICD-10, QGQK00000000=SA-NICD-13, QGQ00000000=SA-NICD-14, QGQL00000000=SA-NICD-16, QGQM00000000=SA-NICD-01, QGQN00000000=SA-NICD-07, QGQP00000000=SA-NICD-02, QGQR00000000=SA-NICD-08, QGQS00000000=SA-NICD-05, QGQT00000000=SA-NICD-06 and QKWI00000000=NCPF4091).
Phylogenetic analysis using whole-genome single nucleotide polymorphisms

Reads were aligned to the *B. percurus* assembly strain BP222 (GenBank accession GCA_003206225.1_ASM320622v1) using BWA-MEM version 0.7.12 [5,29]. Variants were then identified using GATK version 3-7 (Supplementary) [30]. For phylogenetic analysis, the 1,712,033 sites with an unambiguous SNP in at least one isolate and with ambiguity in at most 10% of isolates were concatenated; insertions or deletions at these sites were treated as ambiguous to maintain the alignment. Maximum likelihood phylogenies were constructed using RAxML version 8.2.4 using the GTRCAT nucleotide substitution model and bootstrap analysis based on 1,000 replicates [31].

Determination of nucleotide diversity using whole genome

Genome-wide nucleotide diversity (π) was computed for *B. emzantsi, B. percurus* and the *Blastomyces* genus using VCFtools v0.1.12 [32]. The average nucleotide diversity (π) was computed for non-overlapping sliding windows of 10 kb.

Identification of the mating types from WGS data

The reverse and forward primer pairs of *Blastomyces MAT1-1* and *MAT1-2* obtained from Li *et al.* (2013) were BLASTed with our *Blastomyces* contigs [29]. For the *Blastomyces adhesion-1 (BAD-1)* gene determination, the genome of a reference strain of *Ajellomyces dermatitidis* (ATCC 26199: U37772) was mapped to those of our *Blastomyces* strains. Obtained sequences were visually analysed and subjected to BLAST analyses in GenBank (www.ncbi.nlm.nih.gov) for confirmation of mating types and *BAD-1* sequences.

Ethics and data availability
Human blastomycosis in South Africa

Ethics clearance for the study was obtained from the ethics committees of the University of the Free State (13/2016), the University of Cape Town (704/2013 and 138/2014) and the University of the Witwatersrand (M140112), South Africa. The authors declare that the data supporting the findings of this study are available within the paper, e.g. NCBI accession numbers and supplementary information files.
Results

Isolates and cases

Isolates were available from 20 cases of blastomycosis diagnosed from 1967 through 2014.

Only the year of isolation was available for nine archived Blastomyces isolates: 1975 (n=6), 1992 (n=2), 1999 (n=1). For one isolate, no information was available at all. Clinical data were available for the ten remaining cases. Nine patients were adult males, with a median age of 37 years (interquartile range (IQR), 32.5 – 52 years). Isolates were cultured from skin biopsy (n=4), pus from a thoracic vertebral abscess (n=2) (Figure 1), pus from a subcutaneous abscess (n=2), brain tissue (n=1) and biopsy of a tongue ulcer (n=1). For the nine patients for whom this information was available, B. dermatitidis was initially identified as the causative pathogen by histopathological examination (Table 1). All 20 isolates had previously been phenotypically identified in culture as B. dermatitidis.

Mycological examination of the 20 isolates

Based on examination of cultures grown on Sabouraud agar (at 25°C) and BHI agar (at 37°C), we distinguished two morphological groups of isolates. Twelve isolates (group 1) resembled B. percreusus morphologically. The remaining 8 isolates (group 2), here named B. emzantsi, resembled B. parvus in some aspects but were morphologically distinguishable (Figure 2, 3). The morphological characteristics of B. emzantsi were similar to those described for B. percreusus except that we observed clavate, complanate cells during conidiogenesis, with occasional helical hyphae at 25°C, while at 37°C, there were adiaspore-like cells, hyphal fragments and infrequent broad-based, budding cells on culture (Figure 2). No growth at 40°C was observed for any isolate from either group. Group one isolates were urease-negative within 24 hours of incubation, while group two isolates were urease-positive within 24 hours at 37°C.
Antifungal susceptibility testing of isolates

All isolates grew for MIC determination after five to six days of incubation. Tables 2 and 3 summarize the distribution, range and GM MIC/minimum effective concentration (MEC) for nine antifungal agents for the yeast- and mould-phases of 17 isolates. There was no difference between the BMD and Etest GM MICs for voriconazole, posaconazole and itraconazole for the yeast- and mould-phases of *B. percursus* and *B. emzantsi* (Table 2 and 3). The yeast- and mould-phase GM MICs of fluconazole was higher for both *B. percursus* and *B. emzantsi* (BMD GM MIC range: 1.41 mg/L to 2.18 mg/L) than the other tested azoles. The amphotericin B Etest GM MICs for *B. percursus* mould phase was lower (0.01 mg/L) than the MIC for *B. emzantsi* mould phase (0.26 mg/L). The BMD GM MICs for anidulafungin were higher (range: 0.27 mg/L to 1 mg/L) than those for miconafungin (range: 0.03 mg/L to 0.13 mg/L) for both yeast- and mould-phases of *B. percursus* and *B. emzantsi*.

Multilocus and whole genome phylogenetic analyses

Based on ITS sequences only, the 12 strains of group one were confirmed as *B. percursus*, while the eight strains of group two were very similar to *B. parvus* (97%) and *B. helicus* (97%). A phylogenetic tree of the ITS sequences separated the two groups from *B. dermatitidis*/*B. gilchristii*, *B. parvus*, *B. silverae*, *B. helicus* and *Emmonsia sola* (Figure 4A). Multilocus phylogenetic analysis of the concatenated sequences of ITS-LSU-PRP8-β-tubulin-actin confirmed the separation and clearly demonstrated that group 1 (*B. percursus*), and group 2 (*B. emzantsi*) isolates were distinct species (Figure 4B). Phylogenetic analysis of each gene (ITS, LSU, intein PRP8, beta tubulin and actin) sequences also revealed 2 distinct groups (Figure 2 supplementary). *B. dermatitidis* and *B. gilchristii* did not separate into two different clades. Whole genome phylogenetic analysis of 1,712,033 variant sites identified
from aligning whole genome reads to *B. percursus* strain BP222 strongly supported these phylogenetic relationships, also confirming that isolates from group 1 clustered with *B. percursus* (100% bootstrap support) and that isolates from group 2 conformed to an intermediate monophyletic clade between *B. parvus* and *B. percursus/deformaticus* that represents a novel *Blastomyces* species (100% bootstrap support; Figure 4C). Large genetic diversity was found in sympatric *B. emzantsi* and *B. percursus* species with an average of 900,000 SNPs between species.

Clinical presentation and treatment of ten cases

*B. percursus*

Four patients (cases 2-5) presented with a two- to seven-month history of symptoms including weight loss, productive cough with hemoptysis, cutaneous lesions including chronic ulcers and headache. Most of these patients had multi-system involvement. All seven patients (cases 1-7) had extrapulmonary disease, and three (case 2, 3, 6) additional patients had pulmonary disease. Four patients (case 1, 3, 4, 6) had cutaneous involvement. One patient presented with vertebral disease (case 7). *B. percursus* was isolated in Gauteng (case 1, 4, 5, 7), Western Cape (case 1, 2) and Free State provinces (case 6) of South Africa (Figure 4D).

*B. emzantsi*

Patient 10 had an underlying severe combined immune deficiency, with a subcutaneous abscess, but other clinical data were lacking. Patients 8 and 9 presented with cough, weight loss, night sweats and dyspnea. Both were found to have pulmonary disease with collapsed thoracic vertebrae and patient 8 had a purulent discharge from a sinus tract at T5 vertebral level (Figure 1). Both patients were treated empirically for tuberculosis (TB). Patient 8 died on February 19, 2021.
Human blastomycosis in South Africa

soon after starting amphotericin B, while patient 9 recovered after receiving a cumulative
total of 1.8 g of amphotericin B (Table 1). B. emzantsi was isolated in Limpopo (case 8),
North West (case 9) and Northern Cape provinces (case 10) of South Africa (Figure 4D).

Genome assembly sizes

The genomic sizes of B. percursus (32.1-34.2 Mb) and B. emzantsi (27.4 Mb -32.5 Mb) were
similar to other closely-related dimorphic fungi; however, they differed from those reported
for B. dermatitidis (66.6 Mb) and B. gilchristii (75.4 Mb) [5].

Nucleotide diversity

The estimated genome-wide nucleotide diversity (\(\pi\)) for B. emzantsi is 0.00029, and is almost
twice as large in the case of B. percursus 0.00052. Relative to the Blastomyces genus (\(\pi =
0.00905\), this represents a ~31-fold and ~17-fold lower diversity for B. emzantsi and B.
percursus populations than observed at the genus level, respectively. Comparing to other
dimorphic fungal pathogens from Ajecellomycetaceae causing endemic mycoses, the level of
diversity in B. emzantsi is lower than that reported in Paracoccidioides brasiliensis S1a and
PS2 (\(\pi = 0.00053\) and 0.00066, respectively; but higher than the clonal diversity reported for
P. brasiliensis PS3 that is mainly found in regions of endemicity in Colombia (\(\pi = 0.00008\))
[33]. The degree of genetic variation supports that B. emzantsi is a single species, well
separated within the Blastomyces genus.

Mating types analysis

Of the 18 Blastomyces strains analysed for mating type, only the three recently-diagnosed
cases of B. percursus contained MAT1-2 gene. Furthermore, all B. emzantsi were MAT1-1.
The population ratio for each mating type is 1:1 in other dimorphic pathogens from the
Human blastomycosis in South Africa

Ajellomycetaceae such as Paracoccidioides. Either MAT1-1 or MAT1-2 have been reported in other dimorphic fungal pathogens demonstrating that these strains are heterothallic [7, 29].

BAD-1 gene identification from WGS

The full orthologue of the BAD-1 gene was absent in all 18 Blastomyces strains; only a partial paralogue region was found.

Taxonomy

Blastomyces emzantsi sp. nov.: Maphanga, Govender, Birkhead, Frean; MycoBank MB828102.

Etymology: Referring to South Africa, the country of origin of the type species. Emzantsi means south in the isiXhosa language.

Morphologically, B. emzantsi can be differentiated on the combination of saprobic phase helical hyphae, abundant conidiogenesis, and clavate, complanate cells terminally or extending laterally from hyphal cells, together with incomplete transition to the yeast phase at 37°C (hyphae, adiaspore-like/giant cells and broad-based budding yeasts are all present in the thermotolerant phase).

Holotype: South Africa, National Institute for Communicable Diseases, dried herbarium specimen culture from subcutaneous abscess of an HIV-seronegative black African man with blastomycosis, described by Frean et al. in 1993; living strain NICD (SA-NICD-15; case 9)
Human blastomycosis in South Africa

Physiology: Minimum growth temperature 8°C, optimum 25°C reaching 55 mm on Sabouraud agar in 29 days, maximum 37°C; urease positive.

Differential molecular diagnosis: *B. emzantsi* can be diagnosed by the following nucleotide characters, which are fixed in *B. percurus, B. dermatitidis, B. gilchristi, B. parvus, B. helicus* and *B. silverae*: internal transcribed spacer 2 of rDNA at position 140 (T:C); 169 (A:T)
We re-examined clinical isolates from 20 human cases of blastomycosis from South Africa from 1967 to 2014, and found that these were not *B. dermatitidis*, as initially believed [3-5, 17-21], but rather different species: twelve isolates belonged to the recently-described *B. percursus* [5] and eight isolates to a novel species, described here as *B. emzantsi*. None of the 20 isolates clustered with *B. dermatitidis*, *B. gilchristii* or *B. parvus*.

Blastomycosis is an infrequently-diagnosed endemic mycosis in South Africa compared to emergomycosis and histoplasmosis [22, 34]. This is more likely to reflect a diagnostic bias rather than a true difference in prevalence owing to a lack of awareness among clinicians and a lack of access to simple non-culture-based diagnostic methods. The very closely-related fungi, *B. dermatitidis* and *B. gilchristii* cause infections with varying clinical phenotypes [35, 36, 37, 38]. 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 [3, 4, 35]. Similar to *Emergomyces* and *Histoplasma*, the lungs are the primary site of infection and acquisition of infection occurs following inhalation of conidia. Dissemination of the fungus from the lungs via the bloodstream is presumed to lead to skin and bone involvement. Our study supports the latter hypothesis, mainly because we found that South African cases of blastomycosis were caused by species other than *B. dermatitidis*. However, our sample size was too small to draw any firm conclusions about any differences in the clinical presentation of South African versus North American cases of blastomycosis. Nevertheless, four of the ten cases with detailed clinical information in our study had pulmonary infiltrates on chest X-ray, while all ten cases had extra-pulmonary disease. This is consistent with previous reports on cutaneous and bone disease being more common than pulmonary disease in African blastomycosis in...
Human blastomycosis in South Africa

contradistinction to the pattern in North America [3, 39]. Six of ten cases had cutaneous lesions. It is possible that extrapulmonary disease could have resulted because of delayed or inaccurate diagnosis and/or lack of pulmonary clinical assessment [35]. Both B. percursus and B. emzantsi caused vertebral and bone collapse, which is a classical feature noted in patients with African blastomycosis [3, 4, 35]. The clinical picture of vertebral blastomycosis mimics that of vertebral TB, thus making clinical diagnosis challenging, particularly in a high-prevalence setting for TB [40].

The treatment of choice for North American blastomycosis is itraconazole for mild infections and amphotericin B followed by itraconazole for severe infections [40, 41]. Four people with B. percursus infection, for whom treatment and outcome data were available, recovered after receiving azole or amphotericin B treatment. Although there are no clinical trials to compare the efficacy of these regimens for treatment of blastomycosis, in vitro MIC data for B. dermatitidis, B. percursus, and B. helicus suggest that the azoles (excluding fluconazole), and amphotericin B are active [9, 42-45]. There were some important differences between the susceptibility profile of B. percursus and B. emzantsi. For instance, the amphotericin B Etest GM MIC for the mould phase was 0.01 mg/L for B. percursus versus 0.26 mg/L for B. emzantsi. Also, both B. percursus and B. emzantsi isolates had high anidulafungin BMD GM MICs. According to previous reports, anidulafungin is weakly active against the mould-phase of B. percursus, B. parvus and B. silverae [42]. Zhanel et al. (1997) reported an MIC range of 4.0 µg/ml to 64.0 µg/ml for the mould-phase for B. dermatitidis with this antifungal agent [46]. Similarly, we observed a high micafungin activity against the two phases of B. percursus, B. emzantsi including the ATCC 10225 strain of B. dermatitidis in our study.
Macroscopically, there was incomplete transition from the mould to the yeast phase for all 20 isolates, with most of the strains producing hyphal elements at 37°C. Similar results were reported by Kaufman et al (1983) and Lombardi et al (1988) [11,12]. The observation of hyphal elements in tissue warrants special mention, as histopathologists should be aware of these findings. Microscopically, the maximum size of the yeast cells of *B. percurcus* (9 µm x 14 µm) were larger than those previously reported (6.5 µm x 12.2 µm) [5,8]. *B. emzantsi* produced larger broad-based budding yeast cells (9.0–10.2 x 13.8-20.9 µm) that were more similar to those of *B. parvus*, which until recently were thought to produce adiaspores [8]. Helical hyphal gyres bearing conidia were present in the mycelial phase, similar to those previously described for *B. parvus* and *Emmosia sola* (at 21°C) [8, 47]. Urease was positive within 24 h of incubation for *B. emzantsi*, however, similar results has been reported for other dimorphic pathogens such as *E. africanus, E. europaeus, E. pasteurianus, Emmmosia sola* and *Emmosia crescens* [8].

The ITS region is a formal fungal barcode, which cannot always distinguish closely-related cryptic species [48]. In this study, all eight *B. emzantsi* isolates were erroneously identified as *B. helicus* or *B. parvus* based on ITS sequencing and a BLAST search; however, phylogenetic analysis of these ITS sequences separated the species into different clades. Of the 20 presumptive *B. dermatitidis* South African strains analysed, none clustered with *B. dermatitidis, B. gilchristii* or *B. parvus*. In 2013, Brown *et al.* reported the existence of a clade distinct from North American *B. dermatitidis* strains; this cryptic species was named *B. gilchristii* [7]. Dukik *et al.* (2017) recently reported a novel species, *B. percurcus* which were isolated from cases in South Africa and Israel [5]. Thereafter, using MLST, other *Blastomyces* species were identified which cause disease in mammalian hosts [8]. Our concatenated sequences of the ITS-LSU-PRP8-β-tubulin-actin genes could not separate *B.*
Human blastomycosis in South Africa

dermatitidis from B. gilchristii but clearly differentiated B. percursus, B. parvus, and B. emzantsi from the former two species, forming four clades and confirming the existence of

Blastomyces species different to B. dermatitidis/ B. gilchristii. Furthermore, based on whole genome sequence analysis, the degree of genetic variation supported that B. emzantsi is a single species well separated within the Blastomyces genus. Although none of the 20 Blastomyces isolates currently in the NICD’s collection belong to B. dermatitidis/B. gilchristii, other studies have reported B. gilchristii [CDC B1566 (UAMH10245)] from South Africa [7].

BAD-1 is an important conserved adhesion-promoting protein and virulence factor in B. dermatitidis strains [49]. We could not identify the entire BAD-1 gene in our strains. Previous studies have also reported the absence of the BAD-1 gene from African Blastomyces strains [14, 50]. A study of African Blastomyces strains which lacked the BAD-1 gene exhibited an attenuated disease profile [35]. Furthermore, in a mouse model, mice infected with B. dermatitidis strain containing the BAD-1 gene died within 4 weeks while those which lacked the BAD-1 gene survived [51]. The BAD-1 gene seems to be an important virulence factor in North American Blastomyces strains; however, the impact of the absence of this gene in African strains remains unclear. More studies are required to determine virulence genes which may contribute to differences in clinical manifestations noted between cases of African and North American blastomycosis.

Sexual reproduction greatly increases genetic diversity within Blastomyces and may contribute to the emergence of new genotypes with different virulence profiles [29]. Of the 18 Blastomyces strains analyzed for mating type distribution, all B. emzantsi strains contained MAT1-1, while the B. percursus isolates contained both MAT1-1 and the MAT1-2 genes. The
Human blastomycosis in South Africa

fact that two alleles are not evenly distributed in *B. emzantsi* and *B. percursus* suggests clonal expansion of *MAT1-1* and selection favouring *MAT1-1*; however, wider sampling of clinical and environmental strains and examination of additional loci are needed to confirm these trends [29].

Our study has several limitations. Only cases reported to a reference laboratory were included, which precludes estimation of the prevalence of blastomycosis in South Africa. Our sample size was small (20 isolates over almost 5 decades), which could be due to cases being missed because of limited resources and the lack of clinical awareness in our setting. Furthermore, if *B. percursus* and/or *B. emzantsi* are more likely to cause cutaneous disease than other species, then given that skin lesions (or draining pus) may be more accessible for culture, there may be a bias towards these species being represented in NICD’s culture collection. This is plausible because we suspect that cases of pulmonary blastomycosis are misdiagnosed as smear- or Xpert MTB/RIF-negative pulmonary TB in South Africa. Our analysis included only clinical isolates from humans. In fact, neither veterinary blastomycosis nor environmental isolation of *Blastomyces* species has been reported from South Africa. Small numbers and incomplete clinical details limited our comparison of the diseases caused by these species. With this in mind, the two persons infected with *B. emzantsi* for whom clinical data were available had systemic pyogranulomatous pulmonary and vertebral blastomycosis consistent with disease observed for *B. percursus* and not unusual for North American blastomycosis [14]. Whether *B. emzantsi* is more likely to be associated with this syndrome will require characterization of additional cases.

**Conclusion**
Human blastomycosis in South Africa

South African cases of blastomycosis reported to a national mycology reference laboratory over five decades were caused by two species, *B. percurus* and *B. emzantsi*, and not *B. dermatitidis* as had been presumed. Both taxa caused disease with overlapping clinical presentations, with high proportion of extra-pulmonary disease. Although histology and culture are useful methods in the diagnosis of blastomycosis, our analysis highlights the importance of molecular methods to identify emerging species correctly as causes of old diseases. Increasing awareness of African blastomycosis among clinicians and clinical microbiologists and access to simple rapid non-culture-based diagnostics in resource-limited settings may improve early diagnosis of this illness.
Human blastomycosis in South Africa

**Funding:** This work was supported by the National Institute for Communicable Diseases, a division of the National Health Laboratory Service, South Africa.

**Acknowledgements**

We acknowledge Sydney Mogokotleng (National Institute for Communicable Diseases) for his assistance with retrieving case records from NICD archives and Rudzani Mathebula for constructing the map [Figure 4(d)].

**Author contributions**

Electron microscopy: M.B.
Antifungal susceptibility testing: T.G.M., T.G.Z., R.S.M.
Whole genome sequencing: T.G.M., J.F.M., M.A., A.I.
Data analysis and manuscript writing: T.G.M., I.S.S., M.B., J.F.M., N.P.G.
Human blastomycosis in South Africa

References

Human blastomycosis in South Africa


Human blastomycosis in South Africa


Mycol Appl 50(1):1-84.


26
Human blastomycosis in South Africa


Human blastomycosis in South Africa


Human blastomycosis in South Africa


Human blastomycosis in South Africa

Cryptococcus neoformans, Blastomyces dermatitidis, and Aspergillus species.


<table>
<thead>
<tr>
<th>Case #</th>
<th>Year of isolation/Publication (Accession #)</th>
<th>Demographic information</th>
<th>Clinical presentation, &amp; radiology findings</th>
<th>Laboratory findings</th>
<th>Treatment, outcome (duration of follow-up)</th>
</tr>
</thead>
</table>
| 1      | 1986/[5] (QKW00000000; NCPF4691)          | Age: Unknown
Race & sex: Unknown
Province: Gauteng
Comorbidities: Unknown | History: Ulcerative skin lesions
Examination: Unknown
Radiology: Unknown | Specimen type: Skin
Histopathology: Yeasts morphologically resembling B. dermatitidis
Culture identification: B. dermatitidis
ITS amplification & sequencing: B. percursus (99%–100%) | Unknown |
| 2      | 2006/[18,17] (LGTZ00000000;BP222)         | Age: 52-year-old
Race & sex: White, Male
Province: Western Cape
Comorbidities: HIV seronegative | History: 7-month history of headache with features suggestive of raised intracranial pressure, and 2-month history of ataxia
Examination: Ataxic and bilateral papilloedema. Tone, power, reflexes, and sensation of extremities were normal. Respiratory examination findings were not noted
Radiology: Computed tomography brain scan. Solid contrast-enhancing cerebellar lesion with multiple small abscesses; Chest X-ray: bilateral perihilar disease and pulmonary infiltrates | Specimen type: Brain tissue
Histopathology: Yeasts with broad-based budding and double refractile walls, suggestive of B. dermatitidis
Culture identification: B. dermatitidis
ITS amplification & sequencing: B. percursus (99%–100%) | Amphotericin B followed by itraconazole Recovered (3 years) |
|        |                                             | Age: 52-year-old
Race & sex: Black, Male | History: 6-month history of cough with haemoptysis, weight loss, which had progressed in spite of empiric therapy for tuberculosis; and 3-month history of fleshy skin lesions on right lower lip and right nasolabial fold | Specimen type: Skin & endobronchial tissue
Histopathology: Periodic acid Schiff (PAS) and Grocott stains; broad-based budding yeast and double refractile walls suggestive of B. dermatitidis | |
### Human blastomycosis in South Africa

<table>
<thead>
<tr>
<th>Case</th>
<th>Year</th>
<th>Province</th>
<th>Age</th>
<th>Race &amp; sex</th>
<th>Comorbidities</th>
<th>History</th>
<th>Examination</th>
<th>Radiology</th>
<th>Pathology</th>
<th>Culture identification</th>
<th>Antifungal Therapy</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2014</td>
<td>Western Cape</td>
<td>34-year-old</td>
<td>White, Male</td>
<td>HIV seronegative</td>
<td>5-year history of cutaneous lesions of the groin that resolved without intervention, followed by a 5-month history of skin lesions in the perineum and groin that began as pustules</td>
<td>Partially healed cutaneous ulcer with undermined edges and cribiform scarring in the perineum</td>
<td>Not done</td>
<td>Dermatitidis</td>
<td>B. dermatitidis</td>
<td>None</td>
<td>Recovered (11 months)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITS amplification &amp; sequencing: B. dermatitidis (99%-100%)</td>
<td>Amphotericin B for 14 days followed by itraconazole</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2014/This study</td>
<td>Gauteng</td>
<td>63-year-old</td>
<td>White, Male</td>
<td>Unknown</td>
<td>7-month of an ulcerative growth on the tongue.</td>
<td>Lesion measured 3 cm x 4 cm and involved the right edge and lower surface about 2 cm from the tip of the tongue. Enlarged, mobile submandibular lymph nodes were present in the right side. The rolled edge of the ulcer and its site suggested a malignant lesion.</td>
<td>Not done</td>
<td>Dermatitidis</td>
<td>B. dermatitidis</td>
<td>Itraconazole</td>
<td>Recovered (unknown)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITS amplification &amp; sequencing: B. dermatitidis (99%-100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The raw data extracted from the image is presented in a structured table format with relevant information about each case, including age, race, sex, province, comorbidities, history, examination results, radiology findings, pathology, culture identification, antifungal therapy, and outcomes.
### Human blastomycosis in South Africa

<table>
<thead>
<tr>
<th>Case</th>
<th>Year/Reference</th>
<th>Age</th>
<th>Race &amp; Sex</th>
<th>Province</th>
<th>Comorbidities</th>
<th>History</th>
<th>Examination</th>
<th>Radiology</th>
<th>Specimen Type</th>
<th>Histopathology</th>
<th>Culture Identification</th>
<th>ITS Amplification &amp; Sequencing</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1983/[3,4]</td>
<td>34</td>
<td>Black, Male</td>
<td>Free State</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Post mortem identified disease of lung, skin, kidney, liver, spleen and thyroid</td>
<td>Not done</td>
<td>Skin biopsy</td>
<td>Yeasts morphologically resembling <em>B. dermatitidis</em></td>
<td>Unknown</td>
<td>Died</td>
<td><em>B. dermatitidis</em> (99%-100%)</td>
</tr>
<tr>
<td>7</td>
<td>1987/[19]</td>
<td>27</td>
<td>Black, Male</td>
<td>Gauteng</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Swelling of scalp for 4 weeks prior to admission. Subsequently developed similar lumps over the right iliac crest, the left clavicle and the chest wall over the left axilla. Burning on micturition, pain in both thighs, a feeling of lameness and low back pain for 2 weeks. Good appetite but lost weight for the past four weeks.</td>
<td>New lesion appeared in the fibula and there was collapse of the first lumbar vertebra</td>
<td>Unknown</td>
<td>Culture identification: <em>B. dermatitidis</em></td>
<td>Unknown</td>
<td>Died</td>
<td><em>B. dermatitidis</em> (99%-100%)</td>
</tr>
<tr>
<td>8</td>
<td>1989/[20]</td>
<td>40</td>
<td>Black, Male</td>
<td>Limpopo</td>
<td>HIV seronegative</td>
<td>Cough, weight loss, night sweats and dyspnoea; Readmitted 3 months later, with chest pain and dyspnoea and paravertebral abscess at the level of T4-5</td>
<td>Emaciated. Clinical signs of hepatomegaly. Paraplegic, with no sensation in the legs and in moderate respiratory distress. Discharging sinus at T5 level.</td>
<td>Unknown</td>
<td>Paravertebral abscess</td>
<td>Chromoblastomycosis; broad-based budding yeasts resembling <em>B. dermatitidis</em> on potassium hydroxide stain</td>
<td>Unknown</td>
<td>Died</td>
<td><em>B. dermatitidis</em> (99%-100%)</td>
</tr>
</tbody>
</table>
# Human blastomycosis in South Africa

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Race &amp; sex</th>
<th>Province</th>
<th>Comorbidities</th>
<th>History</th>
<th>Examination</th>
<th>Radiology</th>
<th>Specimen type</th>
<th>Histopathology</th>
<th>Culture identification</th>
<th>ITS amplification &amp; sequencing</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>31-year-old</td>
<td>Black, Male</td>
<td>North West</td>
<td>HIV seronegative</td>
<td>3-month history of productive cough, weight loss and dyspnoea</td>
<td>Raised erythematous patches on the skin of the face, forehead, ears and anterior chest developed 4 months admission; the skin was biopsied and blastomycosis was diagnosed</td>
<td>Chest x-ray showed a fluffy infiltrate in the left lung and bilateral nodular infiltrates, hilar lymphadenopathy and erosion of several ribs.</td>
<td>Subcutaneous abscesses</td>
<td>Yeasts resembling <em>B. dermatitidis</em></td>
<td><em>B. dermatitidis</em></td>
<td><em>B. helicus</em> or <em>B. parvus</em> 97%</td>
<td>Recovered (3 months)</td>
</tr>
<tr>
<td>10</td>
<td>Adult, unknown</td>
<td>Black, Male</td>
<td>Northern Cape</td>
<td>Severe combined immune deficiency</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Subcutaneous abscesses</td>
<td>Unknown</td>
<td><em>B. dermatitidis</em></td>
<td><em>B. helicus</em> or <em>B. parvus</em> 97%</td>
<td>Died</td>
</tr>
</tbody>
</table>

654
Table 2: Yeast-phase MIC distribution of *Blastomyces* isolates (9 *B. percursus* isolates, 8 *B. emzantsi* isolates, 1 *B. dermatitidis* ATCC 10225)

<table>
<thead>
<tr>
<th>Case 6</th>
<th>Assession #</th>
<th>Reference</th>
<th>E-test MIC (mg/L)</th>
<th>BMD MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AMB   VRC   POS   TFC</td>
<td>FLG   VRC   POS   TFC   SFC   CAS   MSF   AMG</td>
</tr>
<tr>
<td>Group 1 (<em>Blastomyces percursus</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(QKQK00000000; SSCP09991)</td>
<td>2</td>
<td>[5]</td>
<td>0.004 0.002 0.002 0.002</td>
<td>4</td>
</tr>
<tr>
<td>(LZT20000000; BPFT22)</td>
<td>2</td>
<td>[17, 13]</td>
<td>0.004 0.002 0.002 0.002</td>
<td>8</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-06)</td>
<td>4</td>
<td>[17]</td>
<td>0.002 0.002 0.002 0.002</td>
<td>1</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-00)</td>
<td>4</td>
<td>This study</td>
<td>0.047 0.002 0.002 0.002</td>
<td>4</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-05)</td>
<td>6</td>
<td>[21]</td>
<td>0.004 0.012 0.002 0.003</td>
<td>8</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-00)</td>
<td>7</td>
<td>[3, 4]</td>
<td>0.005 0.002 0.002 0.002</td>
<td>0.25 0.008 0.008 0.008 32</td>
</tr>
<tr>
<td>(SA-NICD-06)</td>
<td>7</td>
<td>[19]</td>
<td>0.005 0.002 0.002 0.002</td>
<td>0.5 0.008 0.008 0.008 32</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-05)</td>
<td>7</td>
<td>This study</td>
<td>0.004 0.002 0.002 0.002</td>
<td>2</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-05)</td>
<td>7</td>
<td>This study</td>
<td>0.004 0.002 0.002 0.002</td>
<td>2</td>
</tr>
<tr>
<td>MBC range</td>
<td></td>
<td></td>
<td>0.005-0.004 0.002-0.012 0.002-0.002 0.002-0.003</td>
<td>0.25-8 0.008-0.03 0.008-0.25 0.008-0.25 32-256 0.06-4 0.008-0.12 0.12-4</td>
</tr>
<tr>
<td>GM4</td>
<td>0.02 0.002 0.002 0.002</td>
<td>2</td>
<td>0.01 0.02 0.02 0.02</td>
<td>59.26 0.59 0.83 0.54</td>
</tr>
</tbody>
</table>

**Group 2 (*Blastomyces emzantsi*)**

<table>
<thead>
<tr>
<th>Case 6</th>
<th>Assession #</th>
<th>Reference</th>
<th>E-test MIC (mg/L)</th>
<th>BMD MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AMB   VRC   POS   TFC</td>
<td>FLG   VRC   POS   TFC   SFC   CAS   MSF   AMG</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-13)</td>
<td>9</td>
<td>[20]</td>
<td>0.25 0.002 0.002 0.008</td>
<td>2</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-15)</td>
<td>9</td>
<td>[20]</td>
<td>0.25 0.002 0.002 0.003</td>
<td>2</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-14)</td>
<td>10</td>
<td>[3, 4]</td>
<td>0.025 0.003 0.002 0.002</td>
<td>1</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-07)</td>
<td>This study</td>
<td>0.002 0.002 0.002 0.002</td>
<td>1</td>
<td>0.008 0.008 0.008 64</td>
</tr>
<tr>
<td>(QGQK00000000; SA-NICD-07)</td>
<td>This study</td>
<td>0.012 0.002 0.002 0.002</td>
<td>2</td>
<td>0.015 0.008 0.008 128</td>
</tr>
</tbody>
</table>
Human blastomycosis in South Africa

<table>
<thead>
<tr>
<th>Study</th>
<th>MIC range</th>
<th>GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-NICD-12)</td>
<td>0.002-0.64</td>
<td>0.06</td>
</tr>
<tr>
<td>SA-NICD-09)</td>
<td>0.002-0.004</td>
<td>0.002-0.003</td>
</tr>
<tr>
<td>SA-NICD-10)</td>
<td>0.002-0.016</td>
<td>0.16</td>
</tr>
<tr>
<td>SA-NICD-11)</td>
<td>0.25-16</td>
<td>0.008-0.3</td>
</tr>
</tbody>
</table>

Note.- BMD, broth microdilution; GM, geometric mean; B. dermatitidis ATCC 10225; AMB, amphotericin B; VRC, voriconazole; POS, posaconazole; ITC, itraconazole; FLC, fluconazole; 5FC, 5-flucytosine; CAS, caspofungin; MFG, micafungin; ANG, anidulafungin

656 posaconazole; ITC, itraconazole; FLC, fluconazole; 5FC, 5-flucytosine; CAS, caspofungin; MFG, micafungin; ANG, anidulafungin

657 posaconazole; ITC, itraconazole; FLC, fluconazole; 5FC, 5-flucytosine; CAS, caspofungin; MFG, micafungin; ANG, anidulafungin
### Table 3: Mould-phase MIC distribution of Blastomyces isolates (9 B. percursor isolates, 8 B. emzantsi isolates, 1 B. dermatitidis ATCC 10225)

<table>
<thead>
<tr>
<th>Case #</th>
<th>Reference</th>
<th>E-test MIC (mg/L)</th>
<th>BMD MIC and MEC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AMB</td>
<td>VRC</td>
</tr>
<tr>
<td><strong>Group 1: Blastomyces percursor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(QW01000000001;NP4001)</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>2</td>
<td>(LL226000000001;BP222)</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>3</td>
<td>(CQ01000000000;SA-NICD-05)</td>
<td>0.008</td>
<td>0.002</td>
</tr>
<tr>
<td>4</td>
<td>(GQQR00000000;SA-NICD-08)</td>
<td>This study</td>
<td>0.012</td>
</tr>
<tr>
<td>5</td>
<td>(CQ01000000000;SA-NICD-05)</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>6</td>
<td>(GQQR00000000;SA-NICD-09)</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>7</td>
<td>(QGQR00000000;SA-NICD-09)</td>
<td>0.064</td>
<td>0.002</td>
</tr>
<tr>
<td>8</td>
<td>(GQQR00000000;SA-NICD-07)</td>
<td>This study</td>
<td>0.002</td>
</tr>
<tr>
<td>9</td>
<td>(QGQR00000000;SA-NICD-07)</td>
<td>This study</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>MBC range</strong></td>
<td></td>
<td>0.002-0.064</td>
<td>0.002-0.064</td>
</tr>
<tr>
<td><strong>GM</strong></td>
<td></td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>ATCC</strong></td>
<td></td>
<td>0.012</td>
<td>0.008</td>
</tr>
</tbody>
</table>

| **Group 2: Blastomyces emzantsi** | | | | | | | | | | | | | | | | | | | |
| 10     | (QW01000000000;SA-NICD-13) | &lt;0.002 | &lt;0.002 | &lt;0.002 | &lt;0.002 | 0.012 | 0.008 | 0.06 | 0.12 | 256 | 0.12 | 0.05 | 0.25 |
| 11     | (QW01000000000;SA-NICD-15) | 0.094 | 0.006 | 0.002 | 0.016 | 4 | 0.008 | 0.015 | 0.06 | 128 | 1 | 0.25 | 0.5 |
| 12     | (QW01000000000;SA-NICD-14) | 0.012 | 0.002 | 0.003 | 0.013 | 1 | 0.008 | 0.008 | 0.008 | 0.015 | 128 | 0.5 | 0.06 | 0.5 |
| 13     | (QW01000000000;SA-NICD-16) | This study | 0.47 | 0.002 | 0.002 | 0.003 | 1 | 0.008 | 0.008 | 0.008 | 128 | 4 | 0.25 | 1 |

Human blastomycosis in South Africa
### Human blastomycosis in South Africa

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (MIC range)</th>
<th>GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(QGQF00000000;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA-NICD125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(QGQF00000000;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA-NICD150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(QGQF00000000;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA-NICD150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(QGQF00000000;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA-NICD150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note.- BMD, broth microdilution; GM, geometric mean; *B. dermatitidis* ATCC 10225; AMB, amphotericin B; VRC, voriconazole; POS, posaconazole; ITC, itraconazole; FLC, fluconazole; CAS, caspofungin; MFG, micafungin; ANG, anidulafungin

659 Note.- BMD, broth microdilution; GM, geometric mean; *B. dermatitidis* ATCC 10225; AMB, amphotericin B; VRC, voriconazole; POS, posaconazole; ITC, itraconazole; FLC, fluconazole; CAS, caspofungin; MFG, micafungin; ANG, anidulafungin

660 posaconazole; ITC, itraconazole; FLC, fluconazole; 5FC, 5-flucytosine; CAS, caspofungin; MFG, micafungin; ANG, anidulafungin

38
Figure 1. A case of disseminated blastomycosis caused by *Blastomyces emzantsi.*

Purulent discharge from a cutaneous sinus tract at T5 vertebral level (case 8)

Figure 2. Morphology of *B. emzantsi*: saprophytic phase (25°C) on Sabouraud agar after 2 weeks (a,c,k,o-q); intermediate phase (30°C) after 3 weeks on BHI agar with 5% horse blood (b) or Sabouraud dextrose agar (l-n, r-u). (a) a floccose, white colony, with a narrow margin peripheral to the crumpled central areas; (b) at 30°C on BHI with 5% horse blood, colonies were grey (darker grey on the reverse), with numerous aerial hyphal tufts and a flattened margin; (c) two terminal conidia, a developing clavate cell (white arrow) and a clavate cell with secondary septation (black arrow); (d) hyphal septation (arrowheads) in conidiogenesis and clavate cell formation; (e) clavate, complanate cells (arrows) clearly distinguishable from developing conidia; (f) complanate terminal cells (arrows) and a developing terminal conidia (arrowhead); (g) a hyphal cell subtending single, lateral conidia and a terminal, basally septate, clavate cell; (h) the apparent clusters of conidia are usually due to the short pedicels of many of the laterally-positioned conidia; (i) multiple conidia on short secondary conidiophores can be subtended from a single basal cell; (j) conidia ornamentation is variable, from glabrous to papillate; (k) the single conidia may be lateral or clustered on vesiculate primary conidiophores; (l) clavate cell between conidiophores, with variously arranged conidia. Some inflation of subtending cells apparent at intermediate temperature; (m) increase in temperature results in conidiophores become increasingly ampulliform and vesiculate, with distended hyphal cells. Light microscopy creates the illusion that one of each of the laterally paired conidia is sessile; (n) expanded primary conidiophore with two conidia on extremely short secondary conidiophores; (o) helical hyphae, one with both developing clavate cell (black arrow) and conidia (white arrow); (p) Numerous conidia developing on
Human blastomycosis in South Africa

hyphal gyres, with bundles of older hyphae behind them; (q) abundant verrucose conidia, and
hyphae aggregated into rope-like structures; (r) development of an adiaspore-like cell
(inflated terminal conidia); (s) sectioned material provides accurate imaging of the diversity
of cell forms, including the development of giant cells (*) from enlarged, fragmented hyphal
cells, increased vacuolation of enlarging cells, numerous thin hyphal profiles, and some
thick-walled cells (arrow; tangentially sectioned); (t) a section though the hyphal bundles
seen with light and scanning microscopy, reveals numerous endo/intrahyphal profiles; (u) an
inflated intercalary cell, suggestive of a chlamydospore. Scale bars: c, d, k, l, m, n, o, q, s, u =
5 µm; e, f, g, h, i, j, p, r, t = 2 µm.

Figure 3. Morphology of B. emzantsi at 37°C: (a-e) cultured isolates on BHI agar at 4
weeks; (f) Grocott’s stain of paravertebral pus smear; (g, i-l) haematoxylin+eosin stained
section through sinus tissue of vertebral abscess; (h) potassium hydroxide stain of pus. (a)
beige, butyrous, cerebriform colony still with some white, aerial hyphal tufts; (b) incomplete
transformation in culture was typified by large, thick-walled, yeast-like cells juxtaposed to
hyphal fragments; (c) thick-walled, giant cell filled with cytoplasm (indicative of active,
viable cell). The slight disruption in the cell wall (arrow) may be the beginning of a budding
process; (d) a section through one of the large, thick-walled cells filled with cytoplasm. A
number of thin areas in the cell wall (arrows) is suggestive of the initiation of multiple
budding sites. Note the many vegetative hyphal profiles surrounding the giant cell; (e)
budding yeast-like cells with a smaller lateral cell, from cultured isolate; (f) similar
configuration of cells as in (e) though photographed from the clinical specimen; (g) section
through infected sinus tissue, again illustrating the yeast-like budding cells with a smaller
laterally-positioned cell; (h) the original image mentioned in Frean et al (1993) showing a
typical broad-based budding yeast cell; (i) a number of budding, thick walled cells, sectioned
through various planes. It would appear that one of the daughter cells (arrow) has penetrated the tissue around the phagocytic vacuole; (j) although budding cells are apparent in the section (arrow), other budding-cell profiles appear to contain hyphal cells (arrowheads), as the cytoplasm is delimited by a cell wall on the inside of the host vacuole; (k) hyphal invasion of surrounding tissue from daughter cell within phagocytic vacuole; (l) hyphal-like extrusion of thicker-walled cell into surrounding sinus tissue. Scale bars: b, d, e, f, g, h, i, k = 2 µm; c, j, l = 3 µm

Figure 4. Multi-gene phylogenies of *B. percursus* (group 1; SA-NICD-01 to SA-NICD-08, SA-NICD-17, SA-NICD-18) and *B. emzantsi* (group 2; SA-NICD-09 to SA-NICD-16). 4A and 4B: Maximum likelihood tree inferred from ITS gene and concatenated gene alignment of ITS2-LSU-PRP8-β-tubulin-actin based on 1000 replicates. 4A: Sequences of *Paracoccidioides lutzii*, *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Emergomyces africanus* were obtained from Kenyon *et al.* (2013) and *Blastomyces* *silverae*, *Blastomyces helicus*, *Blastomyces parvus* and *Emmonsia sola* were obtained from Jiang *et al.* (2018) [8,22]. 4C: Maximum likelihood tree inferred from SNP including three annotated genome assemblies of *Blastomyces* species (*B. dermatitidis* ER3, *B. parvus* UAMH 130 and *B. percursus* BP222). All nodes were supported by 100% of bootstrap replicates. The boxes indicate the mating type for each *Blastomyces* species. 4D: Geographic distribution of *B. percursus* (green) and *B. emzantsi* (blue) cases in South African provinces.