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ORIGINAL ARTICLE

Spectrum of central nervous system mycoses and antifungal susceptibility: a two-year retrospective analysis from a tertiary care hospital in India

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ABSTRACT

Background and Objectives: The frequency of central nervous system (CNS) fungal infections is rising, leading to increased mortality. These infections pose diagnostic challenges, and therapy depends on the specific fungal pathogen identified. Only a few studies from India have examined the spectrum of fungal pathogens causing CNS infections. The objective of this study was to analyze the clinical and microbiological diversity of fungal pathogens responsible for CNS infections.
Materials and Methods: This was a retrospective study conducted at a tertiary care center in India from January 2023 to December 2024. The study included patients in whom fungi were isolated from cerebrospinal fluid, brain abscess pus, and paraspinal abscesses.
Results: Nine fungal pathogens were identified during the study period. Three isolates were yeasts and six were molds. Brain abscess was the predominant clinical presentation. The yeast isolates included *Cryptococcus neoformans* (n = 1) in meningitis and *Candida tropicalis* (n = 1) and *Candida parapsilosis* (n = 1) in VP shunt infections. The molds isolated from brain abscesses included *Cladophialophora bantiana* (n = 1), *Rhizopus arrhizus* (n = 1), *Aspergillus flavus* (n = 2), *Scedosporium apiospermum* (n = 1), and *Chaetomium lucknowensis* (n = 1). Mortality was observed in 4 of 9 cases (44.4%).
Conclusion: In the present study, nine fungal pathogens were isolated over a two-year period from varied clinical presentations. This highlights the rarity of the condition, which should not be overlooked.

Keywords: Brain abscesses; Meningitis; Ventriculoperitoneal shunts; *Cladophialophora bantiana*; *Scedosporium apiospermum*; *Chaetomium lucknowensis*

INTRODUCTION

Fungal infections of the central nervous system have increased in recent decades, especially in immunocompromised patients and patients in intensive care units (1, 2). These infections exhibit many clinical symptoms, predominantly meningitis, hydrocephalus, and space-occupying lesions (1).

The mode of infection is via inhalation or direct transmission from the sinuses and orbit. Upon inhalation, the pathogen disseminates hematogenously into the systemic circulation or to the brain (2). The

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fungi that most frequently lead to infections include *Cryptococcus* spp., *Aspergillus* spp., *Mucorales*, *Histoplasma* spp., and *Candida* spp. A few emerging fungi, such as *Scedosporium* spp. and *Lomentospora* spp., cause infections in immunocompromised individuals (3).

The diagnosis of fungal infections remains challenging (4, 5). Molecular techniques such as sequencing can diagnose rare fungi, which are difficult to identify using phenotypic approaches. A delay in diagnosis may result in complications in therapy.

CNS fungal infections are challenging to manage due to the varying treatment protocols for each type of fungus (4). Antifungal drugs, administered individually or in combination, will assist in the treatment of central nervous system fungal infections. Identifying the pathogen responsible for the infection and administering appropriate treatment is crucial to mitigating mortality associated with the disease. Limited research exists in India concerning the range of microorganisms responsible for CNS fungal infections (6, 7).

The objective of this study is to analyze the clinical and microbiological range of fungal pathogens responsible for CNS infections at our institution.

MATERIALS AND METHODS

This is a retrospective study conducted from January 2023 to December 2024 (2 years) by the Department of Microbiology at a tertiary care center in India. Patients with growth of fungi from cerebrospinal fluid, pus from brain abscess, and paraspinal abscess were included in the study.

The data regarding demography, clinical diagnosis, risk factors, site of infections, organisms isolated, management, and outcomes were collected from medical records. The pus and CSF samples received were subjected to direct microscopy by KOH-Calcofluor white stain and inoculated into Sabouraud's Dextrose Agar, followed by incubation at 30°C & 37°C for 5-7 days. Identification and antifungal susceptibility testing of the yeast isolates were done by the VITEK-2C system (bioMérieux, Marcy-l'Étoile, France) using the YST and YS08 panels. The results of the yeast were interpreted as per CLSI guidelines (8).

Identification of the molds was performed by slide culture. Confirmation of identification was carried out using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany), and rare molds were confirmed by DNA sequencing of the internal transcribed spacer (ITS) regions. The primer sets used for sequencing are listed in Table 1. Sequencing was performed using a Sanger sequencer, and the resulting sequences were BLASTed against the NCBI and Westerdijk (CBS) databases.

Table 1. Primer sets used for the sequencing of rare molds

Primer name	Sequence (5′–3′)
ITS4	TCCTCCGCTTATTGATATGC
ITS5	GGAAGTAAAAGTCGTAACAAGG

AFST for molds was performed using the microbroth dilution method as per CLSI guidelines (9). The antifungals (Sigma-Aldrich, Bengaluru, India) tested were voriconazole, itraconazole, amphotericin B, posaconazole, and isavuconazole. Terbinafine was additionally tested for *S. apiospermum*.

The results were interpreted as per CLSI guidelines for *Aspergillus flavus* (10). No interpretative breakpoints are available for other molds.

Statistical analysis. Descriptive statistics were used for analysis, and categorical data were described as frequencies with percentages. The data were entered into a Microsoft Excel sheet and analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0.

Ethical approval. Ethical approval has been obtained with the letter number EC/NIMS/3038.

RESULTS

Nine fungal pathogens were identified during the study period. Three (33.3%) isolates were yeast, and six (66.6%) were molds.

The median age of the patients was 38 years, and the male: female ratio was 8:1.

Molds caused six (66.6%) cases of brain abscess, which was the predominant clinical presentation. The clinical features were hemiparesis (n=4), headache (n=3), seizures (n=2), and fever (n=1).

Ventriculoperitoneal (VP) shunt infections were observed in two patients (22.2%) and meningitis in one case (11.1%). The clinical features were fever (n=2),

headache (n=2), seizures (n=1), and vomiting with abdominal distension (n=1).

The patients' risk factors are listed in Table 2. Risk factors included diabetes (n=4, 33.3%), renal transplant (n=1, 11.1%), steroid therapy (n=1, 11.1%), retroviral disease (n=1, 11.1%), trauma (n=1, 11.1%), and congenital aqueduct stenosis (n=1, 11.1%).

The yeast isolates included *Cryptococcus neoformans* (n=1) in meningitis, and *Candida tropicalis* (n=1) and *Candida parapsilosis* (n=1) in VP shunt infection. *C. neoformans* was susceptible to fluconazole, amphotericin B, and flucytosine. Both *Candida* spp. were susceptible to fluconazole, voriconazole, caspofungin, micafungin, and amphotericin B.

The molds isolated from brain abscesses included *Cladophialophora bantiana* (n=1) (Figs. 1A and B), *Rhizopus arrhizus* (n=1) (Figs. 2A and B), *Aspergillus flavus* (n=2) (Figs. 3A and B), *Scedosporium apiospermum* (n=1) (Figs. 4A and B), and *Chaetomium lucknowensis* (n=1) (Fig. 5). Confirmation of identification was performed using MALDI-TOF MS for *A. flavus* and *R. arrhizus.* Rare molds such as *S. apiospermum*, *C. bantiana*, and *C. lucknowensis* were confirmed by DNA sequencing of the internal transcribed spacer (ITS) regions.

The MICs of the mold isolates are given in Table 3. *A. flavus* belonged to the wild type based on the ECVs for the antifungals tested.

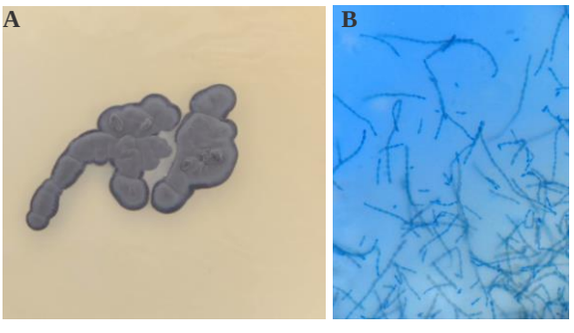

Fig. 1. A) Culture of *Cladophialophora bantiana* on Sabouraud Dextrose Agar showing olivaceous-grey, suede-like colonies. B) Lactophenol cotton blue mount of *Cladophialophora bantiana* with long, sparsely branched one-celled conidia on undifferentiated pigmented conidiophores.

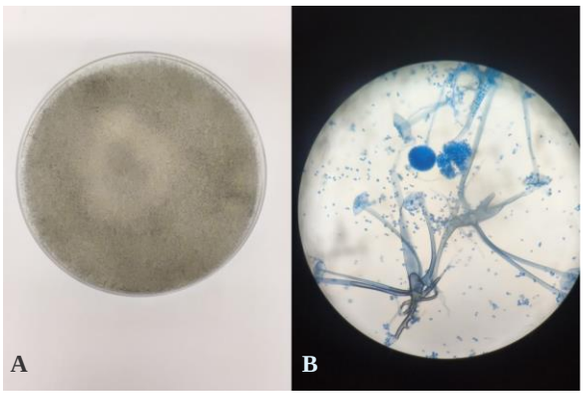

Fig. 2. A) Culture of *Rhizopus arrhizus* on Sabouraud Dextrose Agar showing blackish-grey colonies. B) Lactophenol cotton blue mount of *Rhizopus arrhizus* showing rhizoids, long aseptate sporangiophores bearing globose sporangia and ellipsoidal sporangiospores.

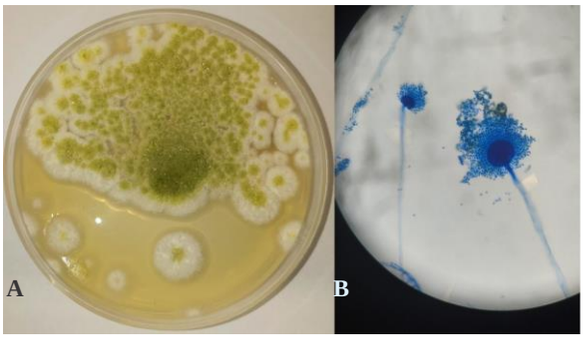

Fig. 3. A) Culture of *Aspergillus flavus* on Sabouraud Dextrose Agar showing granular, flat yellow-green colonies. B) Lactophenol cotton blue mount of *Aspergillus flavus* showing hyaline conidiophore with globose-subglobose conidia.

Table 2. Risk factors of patients with CNS fungal infections

Host status	Risk factor	No. of patients	Percentage
Immunocompromised	Diabetes mellitus	2	22.2
(n=5, 55.5%)	Diabetes mellitus, renal transplant	1	11.1
	Diabetes mellitus on steroid therapy	1	11.1
	Retroviral disease	1	11.1
Immunocompetent	Trauma	1	11.1
(n=4, 44.4%)	Congenital aqueduct stenosis	1	11.1
	No identifiable risk factor	2	22.2

The clinical features of the yeast cases are given in Table 4, and those of the mold cases in Table 5. Mortality was observed in 4 of 9 patients (44.4%). In 5 of 9 patients (55.5%), the clinical symptoms improved, and they were discharged in stable condition with advice to follow up after seven days.

DISCUSSION

There is an increasing incidence of fungal infections in immunocompromised patients. In tropical countries like India, the environment is conducive to fungal infections (11). The use of immunosuppressive drugs, transplant recipients, malignancy, and autoimmune diseases are the risk factors of fungal infections (12). In a cohort study of CNS mold infections from India, the patients were young adults. The risk factors were steroid intake in 18.1%, renal transplant in 5.5%, diabetes in 11%, and trauma in 1.6% of the cases (11). In the present study, the median age was 38 years, and the risk factors were similar, as observed in other studies.

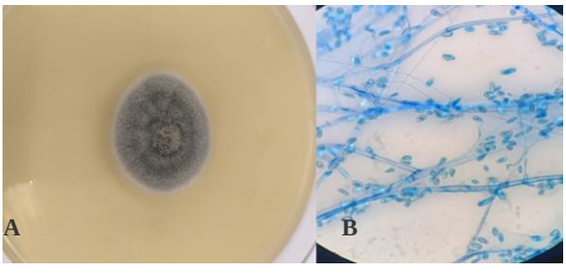

Fig. 4. A) Culture of *Scedosporium apiospermum* on Sabouraud Dextrose Agar showing greyish cottony colonies. B) Lactophenol cotton blue mount of *Scedosporium apiospermum* showing hyaline conidiophore bearing single ovoid unicellular conidia.

Fig. 5. Culture of *Chaetomium lucknowensis* on Sabouraud Dextrose Agar showing a greyish colony.

Cryptococcus is the predominant etiological agent of fungal meningitis worldwide (2), resulting in significant mortality, especially among patients with AIDS (13). The case-fatality rate in the United States is approximately 12% (14). The mortality rate remains high despite antifungal treatment. Diagnosis may be established using microscopy, culture, and serology (14). Treatment comprises induction therapy utilizing amphotericin B and flucytosine/fluconazole, followed by consolidation therapy with fluconazole (15). In the present study, *C. neoformans* was isolated from a patient with de novo retroviral disease who presented with meningitis. His CD4 level was 22 cells/µL. The patient received amphotericin B and fluconazole for seven days but succumbed during treatment.

Fungal infections of ventriculoperitoneal (VP) shunts are infrequent, with only a limited number of cases documented in the literature. *Candida* spp. are the most common cause of fungal VP-shunt infections (16), which may result in significant mortality. Infections of VP shunts are more prevalent in children, with fungi responsible for 17% of these infections (17). Research conducted in Mumbai revealed that 25% of shunt infections in children were attributed to *Candida* spp. (16). Only two instances of *C. parapsilosis* VP shunt infections in adults have been documented; one case resulted from a severe fall, while the other was associated with sinus surgery and brain abscess drainage (18, 19). Treatment encompasses shunt replacement and antifungal ther-

Table 3. The minimum inhibitory concentration (MIC) of molds to antifungal agents according to the CLSI protocol

Pathogen	MIC (µg/ml)					
	Voriconazole	**Itraconazole**	**Posaconazole**	**Amphotericin B**	**Isavuconazole**	**Terbinafine**
Apergillus flavus, n=2	0.12, 0.12	0.06, 0.06	0.06, 0.03	0.25, 0.5	0.12	-
Rhizopus arrhizus, n=1	-	0.5	0.12	0.25	0.12	-
Scedosporium apiospermum , n=1	0.5	0.5	0.5	0.25	1	0.25
Chaetomium lucknowensis, n=1	0.12	0.25	0.12	0.06	0.25	-

Table 5. Molds isolated from central nervous system fungal infections

S. No.	Age	Sex	Comorbidities	Clinical Features	Site of Abscess	Microscopy KOH-Calcofluor	Culture	Type of Surgery	Antifungal Therapy	Outcome
1.	37	M	Nil	Seizures, hemiparesis	Parietal	septate hyphae	*Cladophialophora bantiana*	Burr Hole evacuation of the abscess	Intravenous Voriconazole 400mg twice daily for 18 days.	Symptoms subsided, and they were discharged in stable condition. Death
2.	52	M	Uncontrolled Diabetes	Hemiparesis	Fronto-parietal	Aseptate hyphae	*Rhizopus arrhizus*	Burr hole evacuation of the abscess Ultrasound-guided aspiration of paraspinal collection	Intravenous Amphotericin B 250mg once daily for 2 days. Intravenous Voriconazole 400mg twice daily for 2 days, followed by 200mg twice daily for 12 days	Death
3.	39	M	Nil	Hemiparesis	Paraspinal	septate hyphae	*Aspergillus flavus*	Left frontal craniotomy with abscess excision	Intravenous voriconazole 200mg twice daily with intravenous liposomal amphotericin B 250mg once daily for 11 days	Symptoms subsided, and they were discharged in stable condition.
4.	37	M	Diabetes & Renal transplant recipient	Headache, seizures	Frontal	septate hyphae	*Scedosporium apiospermum*	minicraniotomy and excision of the abscess	Intravenous liposomal amphotericin B 400mg and intravenous voriconazole 400mg twice daily for 19 days	Symptoms subsided, and they were discharged in stable condition
5.	39	M	Diabetes, steroid therapy	Fever, headache	Fronto-parietal	septate hyphae	*Aspergillus flavus*	Minicraniotomy with evacuation of the abscess and excision of the abscess cavity	Intravenous Amphotericin B 250mg once daily for 4 days	Death
6.	44	M	Diabetes mellitus	Hemiparesis, Headache	Frontal	septate hyphae	*Chaetomium lucknowensis*			

Table 4. Yeasts isolated from central nervous system fungal infections

S. No.	Age	Sex	Comorbidities	Clinical Features	Type of Infection	Microscopy Gram stain/India Ink	Culture	Antifungal Therapy	Outcome
1.	36	M	Retroviral disease CD4 count 22 cells/µL	Headache, seizures	Meningitis	India Ink showed encapsulated budding yeast cells	*Cryptococcus neoformans*	Injection Amphotericin B 250mg once daily and fluconazole 800mg once daily orally for 7 days	Death
2.	7 months	F	Aqueduct stenosis, Hydrocephalus	Fever, vomiting, and distension of the abdomen	Ventriculoperitoneal shunt infection, meningitis	Gram stain showed budding yeast cells	*Candida parapsilosis*	VP shunt removal with extraventricular drain placement. Syrup fluconazole 12mg/kg/day for two weeks	Symptoms subsided, and discharged in stable condition
3.	34	M	Post-traumatic hydrocephalus	Fever, headache	Ventriculoperitoneal shunt infection, meningitis	Gram stain showed budding yeast cells	*Candida tropicalis*	VP shunt removal with extraventricular drain placement and intravenous fluconazole 400mg once daily	Symptoms subsided, and they were discharged in stable condition.

apy according to the etiological agent. Amphotericin B has demonstrated efficacy in treating shunt infections (17). In the present study, there were two cases of VP shunt infections; one from a seven-month-old baby with congenital hydrocephalus. VP shunt was inserted; however, due to shunt obstruction and infection, the infant developed meningitis and received antibiotic treatment. An extraventricular drain (EVD) was inserted, and the cerebrospinal fluid (CSF) culture revealed the presence of *C. parapsilosis.* The infant received treatment with fluconazole syrup and was discharged in stable condition. The second case involved a 33-year-old male with post-traumatic hydrocephalus. The patient sustained a subdural hematoma following a road traffic accident. Craniotomy was performed, after which the patient developed communicating hydrocephalus, for which a VP shunt was inserted. The patient underwent effective treatment involving shunt removal and intravenous fluconazole administration.

CNS abscess is an uncommon yet potentially fatal condition. It is a localized infection of the brain parenchyma resulting from inflammation and the accumulation of infected material (6). Fungal abscesses by molds may occur due to environmental exposure to fungi (11). In the present study, all patients were male and aged 30-50 years, and the infection likely occurred due to outdoor exposure to fungi.

Fungal brain abscess is a serious complication associated with immunosuppression (20). The mortality rate ranges from 85% to 100%, as the diagnosis is often missed antemortem (6). The location of the abscess is contingent upon the initial source of infection. Direct extension from the sinuses results in frontal and temporal abscesses, whereas dissemination from other regions of the body leads to parietal abscesses (1). The frontal lobe is the most common site of infection in studies from India (11). In the present study, there were six cases of CNS abscess. The abscess locations included two frontoparietal, two frontal, one parietal, and one paraspinal site.

The clinical features of the infection include fever, headache, hemiparesis, and seizures (11), similar to the findings in the present study. These infections are caused by both hyaline fungi, such as *Aspergillus* spp. and Mucorales, as well as dematiaceous fungi, including *Cladophialophora bantiana*.

C. bantiana is an uncommon neurotropic fungus that causes severe infections in both immunocompetent and immunocompromised individuals, with higher mortality rates in the latter group. In a review of 124 cases, more than 50% of the infections originated from India (7). According to studies from India, *C. bantiana* was the pathogen responsible for CNS fungal infections in 28-44% of cases (6, 11). Treatment comprises surgical excision in conjunction with antifungal therapy (7). In the present study, there was one case of *C. bantiana* in an immunocompetent patient. He had no comorbidities or identifiable risk factors. The abscess was successfully managed with surgical evacuation followed by voriconazole therapy.

CNS mucormycosis is an invasive infection predominantly affecting diabetics and is associated with high mortality. Rhino-orbital cerebral mucormycosis is the predominant manifestation, with isolated mucormycosis being uncommon. *R. arrhizus* is the predominant pathogen responsible for CNS mucormycosis. Treatment comprises surgical debridement and antifungal therapy to prevent severe complications and improve patient outcomes (21, 22). In the present study, *R. arrhizus* was isolated from a patient with uncontrolled diabetes and an HbA1c of 14.7. This was a case of isolated CNS mucormycosis without any rhino-orbital involvement. The patient underwent surgical treatment and received amphotericin B, but unfortunately could not be saved.

Neuroaspergillosis constitutes approximately 5% of central nervous system infections (23). In immunocompetent individuals, it manifests either by direct extension from the sinuses or as a result of trauma. In immunocompromised patients, it arises from hematogenous dissemination originating in the lungs (24). *Aspergillus fumigatus* is the predominant species responsible for CNS infections in immunosuppressed individuals, while *A. flavus* more commonly affects immunocompetent individuals (25). In a study from India, *Aspergillus* spp. were the predominant molds, isolated in 56.3% of cases, among which *A. flavus* was the most common (11). Surgical excision combined with voriconazole therapy constitutes the primary treatment for CNS aspergillosis (26). In the present study, *A. flavus* was isolated from two cases. The first case was a paraspinal abscess accompanied by a cerebral infarct and pulmonary consolidation. This may represent a case of disseminated aspergillosis, though *Aspergillus* was not isolated from other sites. The patient succumbed despite antifungal treatment. In the second case, the patient was a de novo diabetic and had received steroid therapy and

intravenous immunoglobulin for one month at an external hospital for post-dengue acute disseminated encephalomyelitis. The patient underwent effective treatment with abscess excision and combined therapy using amphotericin B and voriconazole.

S. apiospermum is a saprophytic fungus that causes invasive infections in immunocompromised patients, often resulting in high mortality. Brain infections typically arise from pulmonary transmission (27). There have been reports of *S. apiospermum* brain abscess in leukemia (27), renal transplant (28, 29), and in a case of near-drowning (30). Surgical drainage and voriconazole therapy constitute the primary therapeutic modalities (30). In the present study, *S. apiospermum* was isolated from a renal transplant recipient, and the patient was successfully treated with surgery and voriconazole. The isolate was identified by slide culture and confirmed by sequencing of the internal transcribed spacer (ITS) region.

Chaetomium spp. are saprophytic fungi that rarely cause human infections. There have been reports of brain abscesses caused by *Chaetomium* spp. in intravenous drug users (31) and in a renal transplant recipient (32). The fungus poses diagnostic challenges, and there are currently no established management methods for treating these infections (33). In the present study, *Chaetomium lucknowensis* was identified by sequencing the internal transcribed spacer (ITS) region. The patient received treatment with amphotericin B and underwent surgery but succumbed within four days post-operation.

The efficacy of antifungal therapy for CNS fungal infections depends upon the drug's capacity to permeate the CNS. Liposomal amphotericin B, fluconazole, and voriconazole have good CNS penetration and can be used for the treatment of these infections. The contemporary azoles isavuconazole and posaconazole have insufficient central nervous system penetration and are not commonly employed for treating CNS fungal infections (34). In the present study, the antifungals evaluated against molds demonstrated low minimum inhibitory concentrations (MICs), ranging from 0.03 to 0.5 µg/mL. Despite the low in vitro MICs of posaconazole and isavuconazole, these agents were not used for treatment because of their inadequate penetration, and most patients instead received azoles and liposomal amphotericin B.

Effective antifungal therapy combined with surgery, when necessary, constitutes the primary treatment for CNS fungal infections; yet, due to the invasive characteristics of these fungi, mortality rates remain high despite intervention (1). Timely diagnosis and intervention may mitigate the mortality associated with these infections. In the present study, four patients succumbed despite intervention.

Limitations. Only nine fungal pathogens were identified during the study period. A longer study duration will help us better understand the varied presentations associated with a larger number of fungal pathogens.

CONCLUSION

In the present study, conducted over two years, we isolated nine fungal pathogens from diverse clinical presentations associated with significant mortality rates. This underscores the rarity and severity of the condition. The clinical features may be non-specific, which can delay diagnosis, particularly when the pathogens are rare and not easily identified, potentially leading to severe outcomes. Advanced molecular diagnostic tests facilitate the identification of rare fungi and enable targeted antifungal therapy. Therefore, a high index of clinical suspicion is crucial for the early identification and appropriate management of these infections to prevent mortality.

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