



Prevalence of Nocardiosis in Sputum of HIV Positive/AIDS Patients in a Tertiary Health Institution in North Central Nigeria

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Authors' contributions

Author MBAS was involved in conceptualizing the study, carrying out of the field works, data collection processes and literature search. Author AAAI was involved in study design and review of the draft of this manuscript while author KAS was involved in the statistical analysis and writing of the first draft of this manuscript. All authors contributed financially to the successful completion of this work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to determine the prevalence of *Nocardia* species in the sputum of HIV/AIDS patients attending Highly Active Antiretroviral Therapy (HAART) clinic at a tertiary health institution in the North central part of Nigeria.

Study Design: This study was an analytical cross-sectional laboratory based research.

Place and Duration of Study: University of Ilorin teaching hospital (UITH), Ilorin, Nigeria between August 2012 and February 2013.

Method: Two hundred and forty eight (248) patients were recruited for this study and had their sputum collected in duplicates for investigation. The samples were cultured on Buffered Charcoal Yeast Extract (BCYE) medium. Gram and Auramine Rhodamine staining were carried out on the isolates respectively for microscopy.

Result: Only one (1) patient tested positive to *Nocardia* species giving a prevalence rate of 0.4%.

Conclusion: This study gives an indication that Pulmonary Nocardiosis may be found within UITH among HIV patients.

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1. INTRODUCTION

Bacterial infection is one of the causes of severe complications in HIV-infected patients [1]. AIDS patients are infected by pathogenic bacteria that infect immunocompetent individuals and these patients may as well be infected by opportunistic bacteria such as *Mycobacterium tuberculosis* and *Salmonella species* [2]. However, an emerging bacterium worthy of note is *Nocardia species* [2].

Nocardia are obligate aerobes, partially acid-fast, beaded, branching, gram-positive bacilli. Several *Nocardia species*, in the order Actinomycetales cause human disease like pulmonary and disseminated infection and skin infections [3]. Pulmonary disease is one of the most common manifestations of *Nocardia* infection; Nocardiosis, an uncommon but potentially fatal complication of advanced AIDS [2]. In the lung, the organism usually provokes a suppurative response [1].

Nocardia bacteria are found in soil around the world [4]. Nocardiosis occurs worldwide in all age groups, but prevalence is higher in adults, especially men [5]. While individuals with normal immune systems could be infected, the main risk factor for Nocardiosis is immunosuppression, particularly those with defects in cell-mediated immunity [5]. Although it is not written in the WHO clinical guidelines/management algorithm, a study suggested that in areas where sputum culture for tuberculosis is not practicable, patients with apparent pulmonary tuberculosis who are sputum-negative for AFB should have further samples stained with Gram stain to search for *Nocardia*. Moreover, partial acid-fastness is a unique characteristic of *Nocardia* that is not exhibited by other actinomycetes [6]. *Nocardia* are not commensal in humans or animals and there is no evidence of animal to human or person to person transmission [4]. The opportunistic nature of the infection is now emphasized and there are approximately 1, 000 cases each year in the United States. Unfortunately data for this infection in Nigeria is unavailable as studies done in this area are still very scanty and there is no report from the northern part of the country. Hence, this study was designed to determine the prevalence of *Nocardia* species in the sputum of HIV/AIDS patients attending HAART clinic at University of Ilorin Teaching Hospital (UITH), a tertiary health institution found in the North central part of Nigeria.

2. METHODS

2.1 Setting

The study was conducted in the HAART clinic and microbiology laboratory of the University of Ilorin Teaching Hospital, Ilorin, Nigeria, a tertiary health care facility with about 450 beds and an average admissions rate of 10,000 patients annually. The hospital attends to patients from Kwara and the neighboring states. The HAART clinic is an Institute of Human Virology of Nigeria (IHVN) supported facility and it is conducted by the Medicine, Microbiology, Haematology and Family Medicine departments of the hospital.

2.2 Study Design

This study was an analytical cross-sectional laboratory based research.

2.3 Study Population

The study population included all HIV positive patients registered at the HAART clinic of University of Ilorin Teaching Hospital which was estimated at 4,521 patients assessing care at the clinic.

2.4 Inclusion and Exclusion Criteria

The study included Adult patients who were HIV positive registered with the UITH HAART clinic, were presenting with cough and sputum production and were not on any antibiotics within the last four weeks before recruitment including prophylactic cotrimoxazole. This study excluded all adult patients who did not fit into the above categories listed above.

2.5 Sample Size

The sample size for this study was Two hundred and forty eight (248) patients derived from using the Fisher formula for proportion with population less than 10,000 [7].

2.6 Sampling Technique

Purposive non-probability sampling technique was employed in this study where the selection of subjects was based on assumption/specific criteria typical of the population to be studied [7].

2.7 Specimen Collection

Early morning Sputum specimen was collected in duplicates by the researcher from all HIV-positive patients recruited for the study using appropriate and well labeled universal bottles.

2.8 Laboratory Handling of Specimen

2.8.1 Culture

Collected sputum samples were taken to the microbiology laboratory for culture within one hour of collection. The collected samples were streaked on Buffered charcoal-yeast extract (BCYE) medium and was incubated aerobically at 35°C for 14 days and examined every 24 hours for possible growth.

2.8.2 Microscopy

The positive culture was Gram stained; which included making and fixing a smear of the culture on a glass slide and passing it over Bunsen burner flame about 3-4 times, the fixed smear was stained with crystal violet for 30-60 seconds and was rapidly washed off with clean water, then the smear was covered with Lugol's iodine for 30-60 seconds after which it was washed off with clean water. Acetone alcohol was used to decolorize rapidly for a few seconds and washed immediately with clean water followed by the staining with neutral red for two minutes then the stain was washed off with clean water. The organism appeared under the microscope as a dark purple Gram positive bacilli or coccobacillary cells with branching filaments.

Also the positive culture was stained using Auramine Rhodamine staining procedure. This procedure required making a thin smear on glass slide which was allowed to air dry and then it was heat fixed. The slides are flooded with Auramine Rhodamine for 15 minutes, washed with distilled water and decolorized with 1% aqueous hydrochloric acid for 2 minutes 30 seconds, it was washed with water, excess water were drained off and potassium permanganate was added for five minutes. It was air dried then viewed with fluorescence microscope (Carl Zeiss Microimaging GmbH, Primo Star iLED). *Nocardia species* was seen as thin, branching, filamentous bacilli showing yellow-green fluorescence.

2.8.3 Biochemical test

The identity of the positive culture obtained was biochemically confirmed using catalase and urea hydrolysis test to which the isolate tested positive.

2.9 Data Collection

Biodata and important information about each patient recruited for the study was entered into a designed proforma and these was retrieved from the patient and patients' case file through the HAART clinic records department.

2.10 Data Analysis

Data generated in this study was entered into the computer and analyzed using SPSS software version 15.

3. RESULTS AND DISCUSSION

3.1 Results

Two hundred and forty eight (248) patients were recruited for the study and had their sputum collected in duplicates for investigation. About 44.8% of them were males while 55.2% were females. Mean age of patients was 39.96 ± 12.97 years with the ages ranging from 22 years to 80 years. The mean CD4 count was 362.4 cells/ μ l with a minimum of 14 cells/ μ l and a maximum of 1184 cells/ μ l. About 65.7% of patients on recruited for the study were on HAART drugs while 34.3% were yet to commence HAART drugs. Mean Body Mass Index (BMI) of patients was 23.15 ± 4.57 kg/m² with the BMI ranging from 14 to 34.6kg/m². The recruited patients cut across Oyo state (5.2%), Niger state (1.2%), Lagos State (2.8%), Kwara State (86.7%), Kogi State (3.3%) and Kaduna State (0.8%).

Only one (1) patient tested positive to *Nocardia species* giving a prevalence rate of 0.4%. The species isolated grew when cultured on Buffered charcoal-yeast extract (BCYE) medium appearing as whitish, waxy and raised. It was Gram positive cocco-bacillary filamentous branched organism viewed with light microscope and appeared as thin, branching, filamentous bacilli showing yellow-green fluorescence with Auramine Rhodamine stain viewed with fluorescence microscope (Carl Zeiss Microimaging GmbH, Primo Star iLED) (Figs. 1 to 3). Figure 4 compares the *Nocardia* spp as seen by Kumar R. et al and that seen in the present study (Fig. 3) when viewed under Fluorescence microscope positive to Auramin Rhodamine stain. The isolate obtained from the present study was positive to catalase and urease test.



Fig. 1. Isolated *Nocardia* species on Buffered Charcoal Yeast Extract (BCYE) medium in University of Ilorin Teaching Hospital

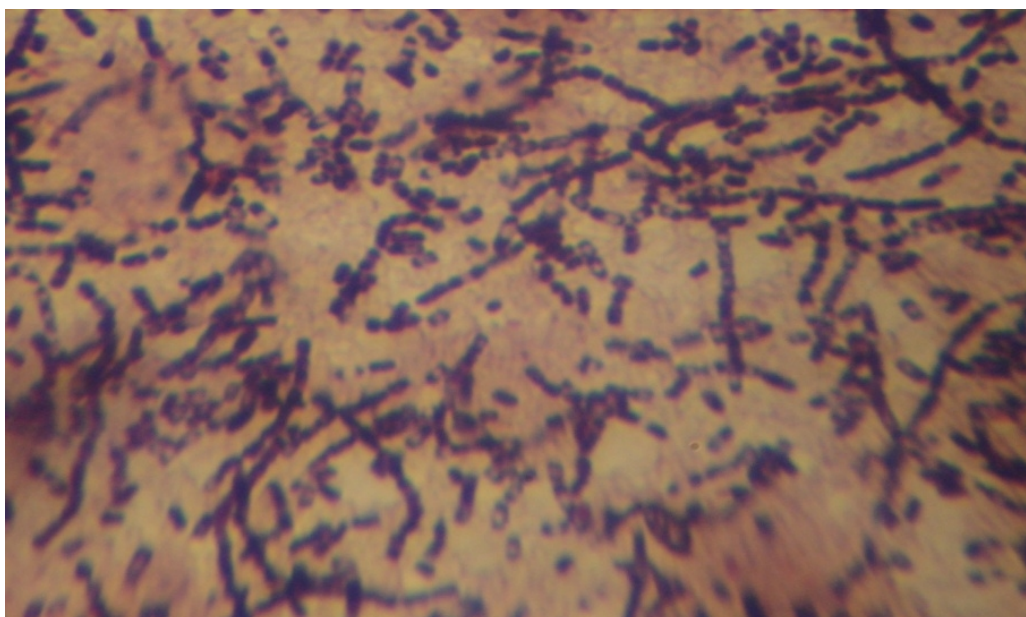


Fig. 2. *Nocardia* spp- Gram positive cocco-bacillary filamentous branched organism viewed with light microscope under x 100 objective with oil immersion in University of Ilorin Teaching Hospital

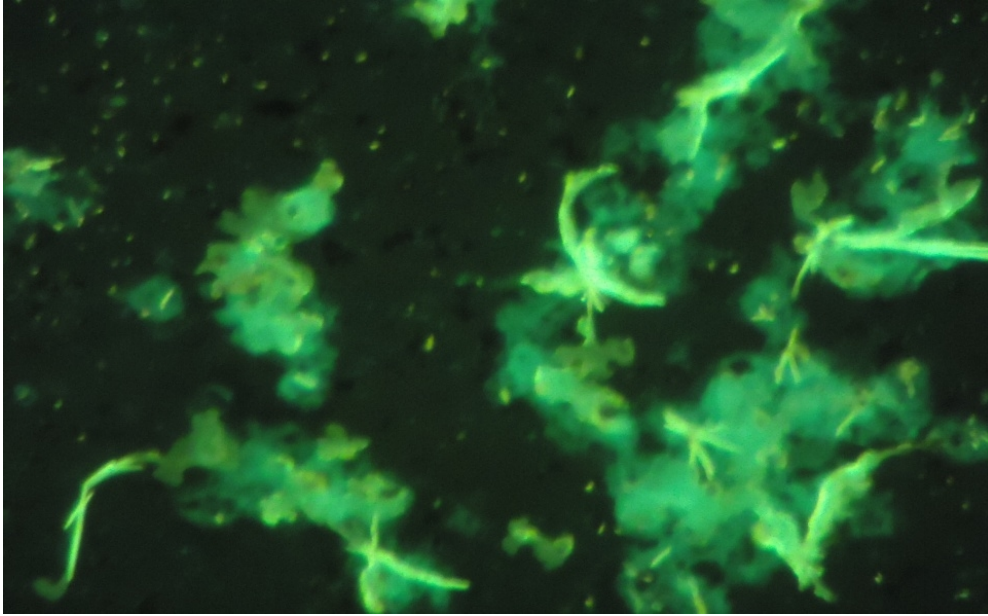


Fig. 3. *Nocardia* spp viewed under Fluorescence microscope positive to Auramine Rhodamine stain in University of Ilorin Teaching Hospital



Fig. 4. Auramine Rhodamine staining of *Nocardia* spp as seen by Kumar R et al. [8]

3.2 Discussion

The prevalence rate of *Nocardia species* in sputum of HIV patient in this study is 0.4%. This represent one patient out of the Two hundred and forty eight (248) patients recruited for the

study group. Eight cases in 20 years were reported in Madrid, Spain giving a prevalence rate of 0.4% which may be similar with that reported in this study [9]. The result from this study represents the experience of a single tertiary health care center over a period that accommodates referral from several other states and other health centres. However, other studies were in variance with the prevalence reported in this study such as 3.4% reported by Nwuba et al. [10,11] and 4.1% reported by Gughani et al where prevalence were higher.

The *Nocardia* positive patient in this study was a 25 years old male who was a sawmill operator. An HIV positive patient on first line HAART drugs with a BMI of 20.2kg/m² and a CD4 count of 419cells/μL. He complained of low grade fever and chronic cough for up to a month and associated weight loss. These symptoms are consistent with those reported in literatures by Martinez et al., Muñoz et al. and Mari et al. [12,13,14] where low grade fever, chronic cough and weight loss are common factors. Immunocompromised individuals with underlying illness including organ transplant, HIV infection and long-term steroid therapy have high risk of developing *Nocardia* infection [15].

The *Nocardia* species isolated in this study grew on culture on BCYE medium (Fig. 1) as documented in earlier studies [16-18] though it was recovered in mixed culture but the sub culture grew within 24 hours to give a pure culture, hence, giving an indication that BCYE may be used as a selective media for *Nocardia* species. This study was able to demonstrate the importance of special stains in confirming the diagnosis of *Nocardia* species in clinical specimen. The isolated *Nocardia* species was catalase, urease and Gram positive coccobacillary under light microscope and also positive to Auramine Rhodamine stain viewed by fluorescence microscope. It was the first time that fluorescent filamentous bacteria morphologically resembling *Nocardia* were observed in the laboratory at UITH Ilorin. According to a case report of *Nocardia* abscess of spinal cord, Auramine Rhodamine staining and fluorescent microscopy of pus showed weak acid fast filamentous forms [19]. Another report highlights the utility of special stains and fluorescent microscopy when applied to clinical specimen as it allowed quick and accurate diagnosis of opportunistic infectious organisms, specifically Nocardiosis and Actinomycosis [8]. The ability to identify *Nocardia* in acid fast and Auramine Rhodamine stained smears varies considerably between different species. Identification by standard methods is a lengthy process that can delay the start of appropriate antibiotic therapy and such a delay can have serious consequences. Quick identification of this uncommon pathogen using special stains like Auramine Rhodamine stain helped in timely diagnosis and successful treatment of the patient. Thus, a high index of suspicion for *Nocardia* species in infection must be maintained while assessing immunosuppressed individuals as it may be masked by the intense inflammatory exudates associated with this infection [20].

4. CONCLUSION

This study has shown that, although rare, *Nocardia* infection may occur within UITH in form of pulmonary infection among HIV patients. The study also indicates that *Nocardia* species may be cultured using BCYE medium. This study also show that the isolate was from a young person hence equal suspicion should also be given to aged individual who are likely to be more susceptible especially if immunosuppressed.

Based on the findings of this study, it is recommended that a higher index of suspicion to *Nocardia* infection should be demonstrated in the assessment of immunosuppressed patients, epidemiological and molecular studies should be carried to establish the extent and spectrum of *Nocardia* infection within the community and *Nocardia* infection should be

included in the list of possible opportunistic infection in respect of HIV/AIDS patients and appropriate management protocol developed and disseminated.

CONSENT AND ETHICAL APPROVAL

Written informed consent was obtained from the participants. Ethical approval was also sought and obtained from the Ethical review committee of the University of Ilorin Teaching Hospital, Ilorin, Nigeria (Approval number ERC1132).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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