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## JOURNAL OF BIOMEDICAL RESEARCH AND CLINICAL PRACTICE

## **Original Article**

2018 Journal Impact Factor: 1.10 Print ISSN: 2636-7378 | Online ISSN: 2651-5865

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# Molecular identification of Fusarium species recovered from Hospital Environment in Jos, Nigeria

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doi) : https://doi.org/10.46912/jbrcp.151

## ABSTRACT

Fusarium species are opportunistic fungi that play an important role in nosocomial infection. The reservoir of Fusarium species in the hospital is not well understood in our environment. Therefore, the present study sought to identify the reservoir of Fusarium species in hospital environment. Three hundred and sixty (360) samples were collected from the environment of two tertiary health care facilities A and B. The sample consists of water (120), soil (120) and plants (120) which were sourced from hospital environments. Cultures of these samples were performed and polymerase chain reaction was used to confirm Fusarium species. The most predominant specie was Fusarium oxysporum Hospital A: 47(57.3%) and Hospital B: 65(64.4%). Most of the Fusarium isolates 92(76.7%) were recovered from soil samples, followed by water 54(45.0%) and the least were from plants 37(30.8%). In conclusion the present study has demonstrated that hospital environment is a reservoir for Fusarium species. However, identification of such reservoir would further enhance effective infection control measures.

Keywords: Fusarium species, Hospital environment, Opportunistic fungi, Reservoir

## **INTRODUCTION**

usarium species are opportunistic fungi associated with nosocomial infections, especially in immunocompromised patients.<sup>1,2</sup> Fusarium species are considered the third most common fungal genus after Candida and Aspergillus.<sup>34</sup> Both the pathogenic and nonpathogenic strains of Fusarium form chlamydospores

which enhance their survival in adverse conditions.<sup>57</sup> Fusarium species are widely distributed in soil, plants water and air, this may be attributed to their ability to grow on a wide range of substrates.<sup>8,94,10-12</sup> More than 50 species of Fusarium have been identified, but few of these species are known to cause human infections. The clinical form of fusarial infection depends largely on the immune status of the host and the portal of entry of the

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infection.<sup>13,15</sup>*Fusarium* species cause a broad spectrum of infections in humans, including superficial, (such as keratitis and onychomycosis), locally invasive or disseminated infections, with the latter occurring mostly in immunocompromised patients. It may also cause allergic diseases (sinusitis) in immunocompetent individuals,<sup>12,14,16,17</sup> and mycotoxicosis in humans and animals following ingestion of food contaminated by toxin-producing *Fusarium spp*.<sup>18,19</sup>

In a review of over 200 cases of fusariosis, 12 species were associated with infection; Fusarium solani was the most frequent with about 50% of cases, followed by Fusarium oxysporum 20% while Fusarium verticillioidis and Fusarium moniliforme account for 10% each. In addition, Fusarium solani is the most frequent pathogen in fusarial keratitis while F. oxysporum accounts for most cases of onychomycosis caused by Fusarium species.<sup>14,19</sup> Several studies have established the genetic relatedness between Fusarium species isolated from patients and hospital environment. 5,9,11,15,20 However, there are few or no documented studies on Fusarium species recovered from hospital environment and its association with nosocomial infection in Nigeria, particularly in Jos, Plateau State. We hereby report the results of a molecular study of Fusarium species in the environment of two tertiary healthcare facility in Jos, Nigeria.

## **MATERIALS AND METHODS**

#### Sample collection

This study was conducted within the environment of two tertiary healthcare facilities A and B. Environmental sampling was focused on water, soil and plant samples. Twenty milliliters of water samples (n=60) each was collected into sterile screw-capped universal containers from different stagnant water of hospital environments. Twenty grams of soil samples (n=60) each was collected into sterile polythene bags from different point, and diseased part of 60 different plants were also collected. These samples were transported to the Laboratory for analysis.

## Sample processing Water

Water sample were processed by a method described by Anaissie *et al.*, (2001).<sup>11</sup> Briefly, 10mls of each water sample was transferred into a sterile centrifuge tube and

spurn at 3500G for 15minutes. The resulting pellet was resuspended in 10mls of sterile normal saline, then 0.2ml of the suspension was inoculated onto Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) supplemented with biphenyl agent. All the culture plates were incubated for minimum of 7days at  $25^{\circ}$ C.

#### Soil

The soil samples were analyzed by a method described by Zhang *et al.*, (2012).<sup>3</sup> The samples were washed thrice in universal containers with 10ml distilled water after which 10ml distilled water was added and the sample vortexed. From the supernatant, 0.5ml was collected and inoculated onto agar medium (PDA and SDA plus biphenyl agent), and incubated at  $25^{\circ}$ C for a minimum of seven (7) days.

#### Plant

The plants were washed twice with sterile water, once with 0.1% Sodium hypochlorite (Household bleach) and rinsed with sterile water. The stem of the plants were cut using sterile surgical blades and the stem cuttings placed aseptically onto agar medium (PDA and SDA plus biphenyl agent) and incubated at 25°C for a minimum of seven (7) days.<sup>3</sup>

#### Identification of Fusarium spp.

*Fusarium species* were initially identified based on macroscopic characteristics carried out, pigment production and microscopic examination. Tease-mount preparation was carried out using portion of the cultures on clean grease-free glass slides with a drop of lactophenol cotton blue stain. A coverslip was placed over the preparation and examined using x10 and x40 objectives. Identification of *Fusarium species* microscopically was by the characteristic appearance of the macro- and micro-conidia, and conidiophores.

### Molecular identification Growth condition and DNA extraction

All *Fusarium species* isolated were grown in 20 ml of potato dextrose broth for 48 h at 28 °C. The cultures were vortex for 10min and total genomic DNA extraction from the isolates was performed using Zymo Quick-DNA kits reagents (Zymo Research Corporation, U.S.A) in accordance with the manufacturer's instructions.

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#### PCR condition

Polymerase chain reaction (PCR) assay optimization was performed using specific primer sequences for the internal transcribed spacer 1 and 4 (ITS 1 and ITS 4) regions; ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as forward primer and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as reverse primer.13,17,21,22 Amplification was performed in 100µl of reaction mixture containing 50 pmol of primers, 2.5 units of Taq DNA polymerase, 200 mM of each dNTP, 10 ml of 10. PCR buffer and 0.2 mg of template DNA. The mixture was subjected to PCR in a Thermo cycler. The PCR cycles began with an initial denaturation for 3 min at 95 C, followed by 30 cycles of annealing for 40 s at 58 C, extension for 40 s at 72 C and denaturation for 40 s at 94 C before a final extension for 5 min at 72 C. The PCR product obtained was run on 1.4% agarose gels, stained with ethidium bromide and visualized under a UV transilluminator.

#### RESULT

In this study, a total of 183 Fusarium isolates were

 Table 1: Distribution of Fusarium spp isolated from two
 hospital environment.

Fusarium species	Hospital A n=180	Hospital B n=180	
F. oxysporum	47(57.3)	65(64.4)	
F. solani	20(24.4)	19(18.8)	
F. moniliformes	12(2.4)	6(5.9)	
F. sporotrichoide	2(2.4)	8(7.9)	
F. equiseti	1(1.2)	2(1.9)	
F. semitectum	0(0.0)	1(0.9)	
Total	82(45.6)	101(56.1)	

 Table 2: Frequency distribution of Fusarium Species in relation to sources of specimen

Fusarium species	Soil n=120	Water n=120	Plant n=120
F. oxysporum	73(60.8)	29(24.2)	10(8.3)
F. solani	10(8.3)	9(7.5)	20(16.7)
F. moniliformes	4(3.3)	13(10.8)	1(0.8)
F. sporotrichoide	4(3.3)	3(2.5)	3(2.5)
F. equiseti	1(0.8)	0(0.0)	2(1.7)
F. semitectum	0(0.0)	0(0.0)	1(0.8)
Total	92(76.7)	54(45.0)	37(30.8)

obtained from 360 specimens of plants, water and soil from two tertiary hospital environments. One hundred and eighty (180) specimens each were collected from Hospital A and Hospital B. However, Fusarium species isolated from Hospital B 101(56.1%) were more than those isolated from Hospital A 82(45.6%). The isolates were identified into six different species, the most predominant specie was *Fusarium oxysporum* 47(57.3%) from Hospital A and 65(64.4%) from Hospital B followed by *Fusarium solani* 20(24.4%) from Hospital A and 19(18.8%) from Hospital B, the least isolated specie was *Fusarium semitectum* 1(0.9%) from Hospital B and none from Hospital A as shown in table 1.

Table 2 reveals the frequency distribution of *Fusarium species* based on sources of specimen. Result shows that a greater number of *Fusarium species* 92(76.7%) were obtained from soil samples, followed by water specimens 54(45.0%) and the least *Fusarium species* 37(30.8%) came from Plant specimens.

A PCR product from two *Fusarium species* was amplified using primer pair ITS1 and ITS4. *Fusarium oxysporum* was identified at approximately 530bp band region, *F. solani* species complex 420bp as presented in figure1.



Figure 1. Conventional PCR using primers ITS1 and ITS4. Lane L, 100-bp DNA ladder. Lanes 1,2,4,8,9,10 are *F. oxysporum*. Lanes 4, 7, 9 are *F. solani*.

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Figure 2: Microscopic morphology of *Fusarium spp* (magnification X400)



Figure 3. Potato dextrose agar plate showing the morphology of Fusarium spp

#### DISCUSSION

This study was conducted to demonstrate that hospital environment can be a reservoir for opportunistic fungi. Samples of soil, water and diseased plants yielded 183 *Fusarium* isolates. Based on their morphological characteristics these isolates were identified as *F*. *oxysporum, F. solani, F. moniliformes, F. sporotrichioides, F. equiseti, F. semitectum.* The isolation of these *Fusarium species* in hospital environments is an indication of its possible reservoir for contamination of hospital,<sup>5,11,22</sup> surfaces that could result in nosocomial infections.

However, the isolation of these species of Fusarium in this study is similar to findings from several studies elsewhere that linked the possibility of nosocomial spread by molecular epidemiologic techniques.<sup>13,13,23,24</sup>

This study also reveals that the isolation of *Fusarium* oxysporum 47(57.3%) and *Fusarium solani* 20(24.4%) may be explained by the fact that their spores survive longer in water, soil and equally infect plants.<sup>3,25,26</sup> Similar findings were reported by Scheel *et al.* in which *Fusarium* oxysporum species complex (FOSC) and *Fusarium solani* species complex (FSSC) were mainly isolated from hospital environment samples.<sup>13</sup> Several other studies carried out in hospitals showed higher isolation rate for both *Fusarium solani* and *Fusarium oxysporum*.<sup>6,7,14,21</sup>

Based on water source, *F. oxysporum* was the most predominant isolates 29(24.2%) followed by *F. moniliformes* 13(10.8%), this finding is in tandem with the report of Anaissie *et al.*, (2001) who documented the persistence of the spores of these species in water. And the reason for this may be due to the fact that their spores are lighter and can spread easily on water.<sup>5,11</sup> Considering the isolation of Fusarium species from infected/diseased plants from hospital environment, *F. solani* was most predominant followed by *F. oxysporum*. Several studies have shown that plants can be infected by Fusarium species.<sup>3,4,6,21,27</sup>

Regarding soil samples, *F. oxysporum* was the most recovered isolates 73(60.8%) followed by *F. solani* 10(8.3%). On the contrary, few studies have documented *F. solani* species complex as the most frequently isolated specie.<sup>24,28,29</sup> This difference in the species isolated may be due to variation in geographical location of the studies.

#### CONCLUSION

In conclusion, we have demonstrated that soil, water, and diseased plants, within hospital environment are reservoirs for *Fusarium species*. However, this will go a long way to enhance infection control measures against opportunistic fungi.

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