



Isolation and Molecular Characterization of *Aspergillus fumigatus* and *Aspergillus flavus* Isolated from Poultry Birds in Ado- Ekiti, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author EDF designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KJA and AJTF managed the analyses of the study. Authors KJA and ATJF managed the literature searches. Authors EDF and KJA read and approved the final manuscript.

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ABSTRACT

Aim: The study was carried out to isolate and identify *Aspergillus* species from commercial birds with suspected aspergillosis in the poultry farms within Ado Ekiti metropolis Nigeria.

Place and Period of Study: The study was carried out in the Department of Microbiology, Faculty of Science, Ekiti State University Ado Ekiti, Nigeria in August 2016.

Methodology: A total of 35 sick/suspected birds were collected randomly from three poultry farms. At Ago-Aduloju poultry farms, 15 samples were randomly collected from 1000 birds while at Ekiti State University poultry farms, 10 samples were randomly collected from 500 birds. At Federal Polytechnic Ado Ekiti poultry farms, 10 samples were randomly collected from 700 birds. The bird's selection was on the basis of clinical signs and symptoms such as difficulty in breathing, weight loss, drooping of wings and exercise intolerance. Swab samples were collected from each suspected/sick bird for mycological culture and molecular characterization of the isolates from each

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bird was carried out. The isolates were identified based on the color of the culture on Potato Dextrose Agar and microscopic examination. Molecular identification was done using 23S Ribosomal RNA Gene and Partial Sequence.

Results: Six fungal strains that showed similar morphological and cultural characteristics of *Aspergillus* species were isolated. The isolates were coded ASP 1, ASP 2, ASP 3, ASP 4, ASP 5, and ASP 6. The identified organisms were; *Aspergillus fumigatus* qH 107 (ASP 1), *Aspergillus fumigatus* qH 107 (ASP 2), *Aspergillus flavus* M09 (ASP 3), *Aspergillus flavus* UOMS6 (ASP 4), *Aspergillus fumigatus* qH 107 (ASP 5), *Aspergillus flavus* qH 107 (ASP 6).

Conclusion: It is evident that *Aspergillus* species were predominant in poultry farms selected in this study. Necessary precaution should be put in place to prevent the spread of aspergillosis. Poultry farmers are advised to avoid damp environments, moldy feeds, dry and dusty litters. Adequate ventilation should always be provided in poultry farms to prevent Aspergillosis.

Keywords: Avian Aspergillosis; poultry birds; molecular identification; *Aspergillus fumigatus*; *Aspergillus flavus*; Aspergillosis; molecular characterization.

1. INTRODUCTION

Poultry is one of the fastest growing segments of the agricultural sector in Nigeria that serves as a source of income to both skilled and unskilled labours [1]. Over the past three decades, the poultry sector has been growing at more than 5 percent *per annum* (compared to 3 percent for pig meat and 1.5 percent for bovine meat) and its share in world meat production increased from 15 percent three decades ago to 30 percent currently [2]. *Poultry* can be defined as domestic birds, including turkeys, geese, chickens and ducks, raised for the production of meat or eggs and the word is also used for the flesh of these birds used as food. Birds belong to the members of the superorder Galloanserae (fowl), in the order Galliformes (which includes chickens, quails and turkeys) and the family Anatidae, in order Anseriformes, commonly known as "Waterfowl" and including domestic ducks and domestic geese [3].

In poultry industry there are different methods of production such as village flocks, small scale commercial flocks and large-scale commercial farms. Challenges in poultry production are; lack of knowledge and skills, lack of enough capital at all levels and marketing [4]. Birds in poultry are often exposed to adverse climatic conditions and often not vaccinated; while some may lack proper nutrition and bio-security leading to frequent fungal, viral, bacterial, parasitic and nutritional diseases [5]. Poultry production is affected by many fungal diseases which include Aspergillosis, Candidiasis, Dactylariosis, Favus, Mucormycosis, Histoplasmosis and Cryptococcosis. Among these fungal diseases, Aspergillosis and Candidiasis are prominent

while Cryptococcosis and Histoplasmosis have some zoonotic importance [6].

Aspergillosis is a non-contagious, opportunistic infection caused by members of the fungal genus *Aspergillus*. *Aspergillus* species are saprophytic moulds. Most live in the environment without causing diseases. However, *Aspergillus* can cause both acute and chronic disease varying in spectrum from local involvement to systemic dissemination. Acute aspergillosis occurs in chicks with high morbidity and mortality. The chronic form is usually sporadic with lesser mortality and affecting older birds, with a compromised immune system [7]. Aspergillosis has emerged as a significant poultry health concern for poultry producers and human's health officials. Moreover, *Aspergillus* species have been isolated from many pulmonary infections in human. Aspergillosis is the most important fungal infection being caused by *Aspergillus fumigatus* and the most important air borne saprophytic fungus [8]. Clinical signs of Aspergillosis include Dyspnea, hyperpnea, somnolence and other signs of nervous system, anorexia, emaciation, and increased thirst may be seen. However, a definitive diagnosis requires confirmation of the organism by cytology, culture, histopathology or DNA testing [9]. Lungs are most frequently involved in chicks or poults up to 6 weeks old. Pulmonary lesions are characterized by white to yellow plaques and nodules which are few mm to several mm in diameter; occasionally, mycelial masses may be seen within the air passages [10]. Thus this study aim to isolate the common agent of Aspergillosis in the poultry farms within Ado Ekiti metropolis Nigeria using cultural and molecular techniques.

2. MATERIALS AND METHODS

2.1 Clinical Examination of Affected Birds

The general health condition and age of the chickens were recorded. The clinical signs were observed from the visual examination in case of diseased birds. The clinical signs such as emaciation, difficulty in breathing, appear unthrifty and weak, with drooping wings and may fail to attempt to escape if pursued [11]. These signs were recorded during the physical visit to the affected flocks. Farmer's complaints about the affected birds were considered in some cases.

2.2 Collection of Samples from Birds

A total 35 sick and suspected chickens were collected from poultry farms in Ado Ekiti Nigeria; Ekiti State University farm (10), Ago-Aduloju's poultry farms (15) and Federal Polytechnic Ado-Ekiti poultry farms (10). The poultry birds were diagnosed for avian Aspergillosis on the basis of clinical signs such as emaciation, difficulty in breathing, appeared unthrifty and weak, with drooping wings and may fail to attempt to escape if pursued [11]. Samples were collected from the trachea of infected/ suspected birds using sterile swab stick as described by Sultana et al. [12] for mycological culture.

Samples collected were carefully transported to the laboratory in sterile ice packs for further processing.

2.3 Isolation of *Aspergillus* Species

An inoculum was prepared from each swab samples, and streaked on Potato Dextrose Agar media in each petri dish. The petri dishes were incubated at 25±2°C temperature for 7 days. After period of incubations at room temperature, the cultural characteristics such as color of colony, growth pattern and colonial morphology were observed and recorded [12]. Subculture of each isolates was carried out until pure culture of each isolate was obtained, and a culture of each isolate was transferred to a PDA slant and stored at 4°C in a refrigerator.

2.4 Identification of Fungal Isolates

Macro- and microscopic morphological features such as surface color of the colony, morphology and color of conidiophore, growth rate and

microscopic structures which include sclerotia, cleistothecia, aleuriconidia and hulle cells were used to identify the different fungi isolated from the poultry bird's samples [13].

The pure cultures of each isolates were examined using standard mycological techniques such as slide culture techniques and needle mount preparation as described below.

2.4.1 Needle mounts preparation method

The procedure was carried out according to the methods of Fagbohun et al. [14] whereby fragments of the sporing surface of the initial culture was taken midway or between the centre and the edge of the colony. This was teased out in drop of alcohol on a sterilized glass slide using a botany needle. The teased out portions were stained by adding a drop of lactophenol blue, covered with cover slip and the preparation was examined under ×10 and ×40 objective lens of the microscope.

2.4.2 Slide culture technique

From a plate approximately 2mm deep, 1 cm² PDA was cut and placed on a sterile glass slide. Fungus was inoculated into the four vertical sides using a sterile needle and covered with sterile coverslip. The preparation was placed on a suitable support medium in a petri dish containing blotting paper soaked in 20% glycerol in water. The preparation was kept moist at 28°C until adequate growth was observed. After removing the medium with scalpel, the fungus adhering to both coverslip and slide was examined [15]. A drop of alcohol was added followed by a drop of lacto-phenol blue and the preparation was covered and examined under the low power objective of microscope.

2.5 Molecular Characterization

The Pure culture of each isolate was inoculated on malt extract broth for molecular characterization and identification.

2.5.1 Extraction of fungal DNA

Genomic DNA was prepared from a loopful of cells grown in Nutrient Broth for 24 h. The cell pellet was re-suspended in 250 µl of solution I (50 mM glucose, 25 mM Tris-HCl pH 8.0, and 10 mM EDTA). The cells were lysed by adding 25 µl of solution II [200 mM NaOH and 1% (w/v) SDS], and mixed for 5 min. Then, 500 µl of solution I

and 2.5 µl of RNase A (10 mg/ml) was added and incubated for 2 h at 37°C. This methodology was adapted from alkaline lysis first described by Vuong et al. [16]. DNA was then purified with phenol-chloroform using a standard laboratory protocol and after precipitation; DNA was re-suspended in 30 µl of TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA).

2.5.2 Polymerase chain reaction (PCR)

About 2.5 g of fungal genomic DNA was added to a 50 µl PCR mix which contained 1 X Hot start reaction buffer, 0.25 mM dNTPs, 0.01 M (each), and 2.5 U Hot start polymerase (Jenabioscience). Thermal cycling was done in a veriti thermal cycler (Applied Biosystems, USA) and cycling conditions were 95°C for 3 min followed 45°C cycles of 95°C for 1 min, by 45°C for 1 min, 72°C for 1 min 45 secs with ramp from 45°C to 72°C set at 40%. Subsequently, the reaction was held at 72°C for 10 min after which it was held at 4°C till terminated. PCR products were resolved on 1% (w/v) agarose gel stained with ethidium bromide and viewed on a transilluminator [17].

2.5.3 Sequencing of amplified 23s rRNA gene

The PCR products were purified using Montage PCR Clean up kit (Millipore). The purified PCR products of approximately 1,500 bp and the fungal sequencing and identification were performed as described by Lachance et al. [18]

3.1 RNA Sequence of the Isolates

3.1.1 Isolate ASP 1, ASP 2 and ASP 5

Transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: gb|CW198110.1|Length: 761Number of Matches:

Related Information

Range 1: 7 to 708GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand	Frame
1166 bits(631)	0.0()	680/702(97%)	10/702(1%)	Plus/Plus	

Features:

sequencing sequenced using two primers ITS4 (TCCTCCGCTTATTATTGACATG) and ITS 1 (TCCGTAGGTGAACCTGCGG). The sequences of PCR products were analyzed using standard protocols with a dideoxy nucleotide dye terminator (Big Dye vs. 3.1—Applied Biosystems, CA, USA) and Genetic Analyzer 3130 (Applied Biosystems, CA, USA). All 23S rRNA gene sequences were checked for quality, aligned, and analyzed with Codon-Code Aligner v.3.7.1 (CodonCode Corp., Centerville, MA, USA).

2.5.4 Bioinformatics analysis

All sequences were compared with reference sequences in the Ribosomal Data base Project (RDP) using sequence Match and the sequence were analyzed in GenBank using the BLAST (Basic Local Alignment Search Tool) bioinformatics program on the NCBI (National Center for Biotechnology Information) website. BLAST was done to identify 23S rRNA sequences in Genbank most similar to the query sequence sent.

3. RESULTS

Six fungal strains were isolated. The isolates were coded ASP 1, ASP 2, ASP 3, ASP 4, ASP 5 and ASP 6. The growth characteristics of each isolates are showed in Table 1. The genomic DNA of the isolates was extracted and 8S rRNA gene was amplified and sequenced. The PCR results of the isolated fungi are displayed on plate 1 revealing different bands of DNA.

Query 70 CCTCCC-CCCGAGTTT-
 CTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCC 127
 ||||| ||| ||| ||||||||||||||||||||||||||||||||||
 Sbjct 7
 CCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCC 66

Query 128
 GGGGGCTCTCAGCCCCGGGCCCGCGCCCGCGGAGACACCACGAACTCTGTCTGATCTAG
 187
 ||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct 67
 GGGGGCTCTCAGCCCCGGGCCCGCGCCCGCGGAGACACCACGAACTCTGTCTGATCTAG
 126

Query 188
 TGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAACTTTCAACAATGGATCTCTTGGTT 247
 ||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct 127
 TGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAACTTTCAACAATGGATCTCTTGGTT 186

Query 248
 CCGGCATCGATGAAGAACGCATAAAAGTGCATAACTAGTGTGAATTGCATAATTCCGTG 307
 |||||||||||| || |||||||||||||| ||||||
 Sbjct 187
 CCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTG 246

Query 308
 AATCATCTAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCC 367
 ||||| ||||||||||||||||||||||||||||||||||
 Sbjct 247
 AATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCC 306

Query 368
 GAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCGCCCTCTCCggg 427
 ||||||||||||||||||||||||||||||||||||||||||
 Sbjct 307
 GAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCGCCCTCTCCGGG 366

Query 428
 ACGGGCCCCAAAGGGAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTT 487
 ||||||||| ||||||||||||||||||||||||||||||
 Sbjct 367
 GGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTT
 426

Query 488
 GTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGT 547
 ||||||||||||||||||||||||||||||||||||||
 Sbjct 427
 GTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGT 486

Query 548 TGACCTCGGATCAAGTAGGGATACCCGCTGAACTTAAGCATATCTTAA--CCGG-AG-
 GA 603
 ||||||||| |||||||||||||||||| || ||||| ||
 Sbjct 487
 TGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAAAACCGGGAGAGA 546

Query 604 ATC-TTA-CGAGTGTAGGGTT-
 CTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCT 660

```

||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
Sbjct 547
ATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCT 606
    
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Query 661
TAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCG
720
    
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||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
Sbjct 607
TAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCG
666
    
```

Query 721 CCCGCCGAT-CACCACGAACTCTGTCTGATCTAGTGAAGTC 761

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||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
Sbjct 667 CCCGCCGAGACACCACGAACTCTGTCTGATCTAGTGAAGTC 708
    
```

Identification: *Aspergillus fumigatus* internal qH107

3.1.2 Isolate ASP 3

Transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

Sequence ID: gb|CW198110.1|Length: 761Number of Matches:
 Related Information

Range 1: 7 to 708GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand	Frame
1166 bits(631)	0.0()	680/702(97%)	10/702(1%)	Plus/Plus	

Features:

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Query 433
CCCTTYMAACCGGCTGGATTATTTTCTTCACCCTTGCTTTTGCGCACTTGTTGTTTCC 492
||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
Sbjct 5 CCCTTCAAACCGGCTGGATTATTTTCTTCACCCTTGCTTTTGCGCACTTGTTGTTTCC
64
    
```

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Query 493
TGGGCGGGTTCGCTCGCCACCAGGACCACACCATAAACCTTTTTGTTAATGCAATCAGCG 552
||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
Sbjct 65
TGGGCGGGTTCGCTCGCCACCAGGACCACACCATAAACCTTTTTGTTAATGCAATCAGCG 124
    
```

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Query 553
TCAGTAAAAGTAATAATTATTTTACAACCTTCAACAACGGATCTCTTGGTTCTGGCATC 612
||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
Sbjct 125
TCAGTAAAAGTAATAATTATTTTACAACCTTCAACAACGGATCTCTTGGTTCTGGCATC 184
    
```

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Query 613
GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCG 672
||||| ||| ||||||||||| |||||||||||||||||||||||||||||||
    
```


Sbjct 59
 TCGCGAATCTTACCCGTACGGTTGCCTCGGCGCTGGCGGTCCGGAAGGCCCTCGGGTCC 118

Query 461
 TCCCGGATCCTCGGGTCTCCCGCTCGCGGGAGGCTGCCCGCCGGAGTGCCGAACTAAAC 520
 |||

Sbjct 119
 TCCCGGATCCTCGGGTCTCCCGCTCGCGGGAGGCTGCCCGCCGGAGTGCCGAACTAAAC 178

Query 521
 TCTTGATATTTTATGTCTCTCTGAGTAACTTTTAAATAAGTCAAACTTTCAACAACGG 580
 |||

Sbjct 179 TCTTGATATTTTATGTCTCTCTGAGTAACTTTTAAATAAGTCAAACTTTCAACAACGG 238

Query 581
 ATCTCTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC 640
 |||

Sbjct 239
 ATCTCTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC 298

Query 641
 AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGTATTCTGGCGAG 700
 |||

Sbjct 299
 AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGTATTCTGGCGAG 358

Query 701
 CATGCCTGTTGAGCGTCATTTCAACCATCAAGCTCTGCTTGCGTTGGGGATCCGCGGCT 760
 |||

Sbjct 359
 CATGCCTGTTGAGCGTCATTTCAACCATCAAGCTCTGCTTGCGTTGGGGATCCGCGGCT 418

Query 761
 GCCCGCGGTCCCTCAAATCAGTGGCGGGCTCGCTAGTCACACCGAGCGTAGTAACTCTA 820
 |||

Sbjct 419
 GCCCGCGGTCCCTCAAATCAGTGGCGGGCTCGCTAGTCACACCGAGCGTAGTAACTCTA 478

Query 821
 CATCGCTATGGTCGTGCGGCGGGTTCTTGCCGTAAAACCCCCATTTCTAAGGTTGACCT 880
 |||

Sbjct 479
 CATCGCTATGGTCGTGCGGCGGGTTCTTGCCGTAAAACCCCCATTTCTAAGGTTGACCT 538

Query 881 CGGATCRGGWAGGAATACCCAGCYGAACTTAAGCATATCAATA 923
 ||| || ||| |||

Sbjct 539 CGGATCAGGTAGGAATACCC-GCTGAACTTAAGCATATCAATA 580

Identification: *Aspergillus flavus* internal UOMS6

3.1.4 Isolate ASP 6

Transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: gb|CW198110.1|Length: 761Number of Matches:

Related Information

Range 1: 7 to 708GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand	Frame
1166 bits(631)	0.0()	680/702(97%)	10/702(1%)	Plus/Plus	

Features:

```

Query                               70                               CCTCCC-CCCGAGTTT-
CTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCC 127
||||| ||| |||||||||||||||||||||||||||||||
Sbjct                                                                7
CCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCC 66

Query                               128
GGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAG
187
|||||||||||||||||||||||||||||||||
Sbjct                                                                67
GGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAG
126

Query                               188
TGAAGTCTGAGTTGATTGTATCGCAATCAGTAAAACCTTTCAACAATGGATCTCTTGGTT 247
|||||||||||||||||||||||||||||||||
Sbjct                                                                127
TGAAGTCTGAGTTGATTGTATCGCAATCAGTAAAACCTTTCAACAATGGATCTCTTGGTT 186

Query                               248
CCGGCATCGATGAAGAACGCATAAAAAGTGCATAACTAGTGTGAATTGCATAATCCGTG 307
||||||||||| || ||||||||||||||| |||||||
Sbjct                                                                187
CCGGCATCGATGAAGAACGCAGCGAAATGCATAACTAGTGTGAATTGCAGAATCCGTG 246

Query                               308
AATCATCTAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCC 367
||||| |||||||||||||||||||||||||||||||
Sbjct                                                                247
AATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCC 306

Query                               368
GAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCTCTCCggg 427
|||||||||||||||||||||||||||||||||
Sbjct                                                                307
GAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCTCTCCGGG 366

Query                               428
ggggACGGGCCCCAAAGGGAGCGGCGGCACCGGTCCGATCCTCGAGCGTATGGGGCTTT 487
||||||||||| |||||||||||||||||||
Sbjct                                                                367
GGGGACGGGCCCCAAAGGCAGCGGCGGCACCGGTCCGATCCTCGAGCGTATGGGGCTTT
426
    
```

```

Query 488
GTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGT 547
|||||
Sbjct 427
GTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGT 486

Query 548 TGACCTCGGATCAAGTAGGGATACCCGCTGAACTTAAGCATATCTTAA--CCGG-AG-
GA 603
||||| ||||| || ||| ||
Sbjct 487
TGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAAAACCGGGAGAGA 546

Query 604 ATCTTACGAGTGTAGGGTT-
CTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCT 660
||| ||||| ||||| ||||| |||||
Sbjct 547
ATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCT 606

Query 661
TAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCG
720
|||||
Sbjct 607
TAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCG
666

Query 721 CCCGCCGAT-CACCACGAACTCTGTCTGATCTAGTGAAGTC 761
|||||
Sbjct 667 CCCGCCGAGACACCACGAACTCTGTCTGATCTAGTGAAGTC 708
    
```

Identification: *Aspergillus flavus* qH107

3.1.5 Result of DNA and PCR

The extracted and amplified DNA revealed different band width. The isolates that revealed clear bands with large amplicon value were ASP 1, ASP 2, ASP 3, ASP 4 and ASP 5 while isolate 6 did not have a clear band. The amplicon size showed that isolates were from the same species.

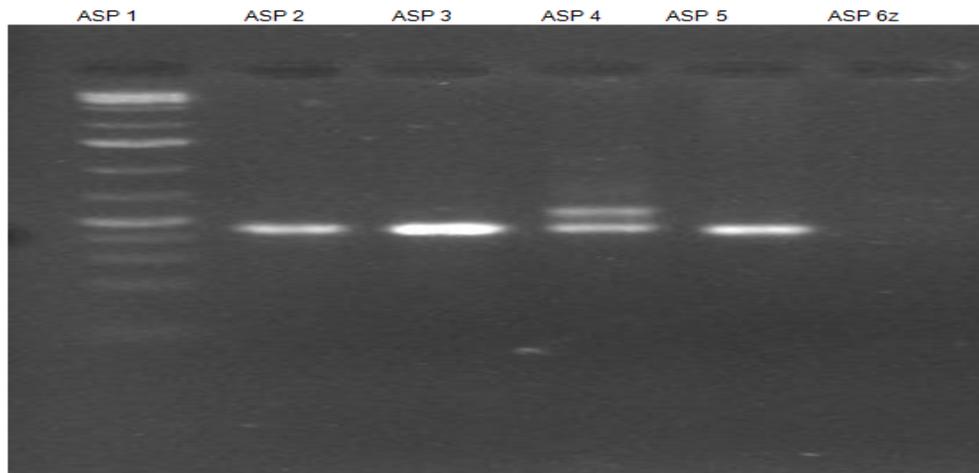


Plate 1. PCR result of the amplified DNA of isolated fungi
Isolates Name Key; ASP = Aspergillus

Table 1. Growth characteristics of isolated *aspergillus* species

Isolates	Temperature	Cultural and morphology description	Identification
ASP 1	25±2°C	It grew well on PDA at the temperature range of 25±2 ⁰ C. it has black colour with white margin; reverse was cream. Conidiophores were smooth and uncolored. Conidia were smooth to finely roughened, subglubose. Hyphae were septate and hyaline	<i>Aspergillus fumigatus</i> qH 107.
ASP 2	25±2°C	Grew well on PDA. The colony had grayish with white margin colour on Potato Dextrose Agar. Exudate was absent. Mycelium was white. Conidiophore was smooth to finely roughened and uncolored	<i>Aspergillus fumigatus</i> qH107
ASP 3	25±2°C	The growth was rapid to moderately rapid with bright yellow- green colour on PDA. Conidia were round to elliptical. Conidiophores were roughened. Hyphae were septate and hyaline.	<i>Aspergillus flavus</i> M09.
ASP4	25±2°C	Grew rapidly and had bright yellow- green colour on PDA. Conidia were round to elliptical. Conidiophores were roughened. Hyphae were septate and hyaline. Mycelium was Fluffy. Surface texture was smooth to finely roughen	<i>Aspergillus flavus</i> UOMS6
ASP5	25±2°C	Grew well on PDA. It possesses black colour with white margin. Reverse had wrinkled mycelium growth. Phialides were uniseriate. Conidiophore was smooth and colorless.	<i>Aspergillus fumigatus</i> qH107.
ASP 6	25±2°C	Isolate ASP 6 had parrot green colony colour. Mycelium was Parrot green fluffy. Reverse was pale yellow to uncoloured and wrinkled mycelia growth. Conidiophore was smooth to finely roughen.	<i>Aspergillus flavus</i> qH107.

3.1.6 Sequencing result

Out of the eight organisms sequenced for molecular characterization, only six showed sequenced identity that revealed their genetic information from Gen bank. The revealed organisms were; *Aspergillus fumigatus* strain qH 107 (ASP 1), *Aspergillus fumigatus* strain qH 107 (ASP 2), *Aspergillus flavus* strain M09 (ASP 3), *Aspergillus flavus* strain UOMS6 (ASP 4), *Aspergillus fumigatus* strain qH 107 (ASP 5), *Aspergillus flavus* strain qH 107 (ASP 6).

4. DISCUSSION

The genus *Aspergillus* includes over 185 species. About 20 have so far been reported as causative agents of opportunistic infections such as Aspergillosis. Aspergillosis is a non-contagious, opportunistic infection referring to any disease condition caused by members of the fungal genus *Aspergillus* [19]. Immunosuppression is the major factor predisposing birds to the development of opportunistic *Aspergillus* infections. Warris et al. [20].

In this work, *Aspergillus* species associated with poultry birds in farms in Ado- Ekiti metropolis Nigeria were investigated. *Aspergillus fumigatus* and *A. flavus* implicated. However, *A. fumigatus* was predominant organism in the studied farms based on our findings. Previous studies by Ustimenko [21] showed that *Aspergillus* species causing aspergillosis in fowls and in unsanitary condition of incubators in U.S.S.R were isolated. In this study Preliminary Identification was based on cell and colony morphology characteristics (morphological and microscopic features). Among the characteristics used were colonial characteristics such as colour of the colonies, surface appearance, reverse colouration, texture and optimum temperature [15]. In addition, microscopy revealed vegetative mycelium including presence or absence of cross walls, diameter of hyphae and types of sexual and asexual reproductive structures (Table1). The final identification was based on molecular characteristic of the isolates using 23S Ribosomal RNA Gene and Partial Sequence (Plate 1). This is similar to the findings of Ustimenko, [21] who isolated *Aspergillus fumigatus* from lung tissue of dead chicken. *A. fumigatus* can grow in litter of poor quality, feed stored, poor conditions and in air from infected broiler farms [22]. Furthermore, inadequate ventilation increases the risk of exposure of susceptible host to inhalation of spores causing high morbidity and mortality [22].

Aspergillus fumigatus is widespread in nature. Birds are exposed to fungal spores on a regular basis, and many carry them in their lungs and air sacs without ill effects. The nature of disease that occurs is depend on the resistance of the avian host and the number and distribution of fungal spores [23] Isolation of *Aspergillus fumigatus* in this work is similar to that of Ustimenko, [21] who isolated *Aspergillus fumigatus* from the lung tissue of dead chicken tissue in U.S.S.R. Similarly, Harold and John [24] also reported that *A. fumigatus* were responsible for a disseminated Aspergillosis in 5-week old broiler in Iowa State University USA. *Aspergillus* spp is one the most common and pathogenic fungi that constitutes serious threat to the poultry industry in Nigeria. Most prevention in many poultry industry is focus on bacterial infection which allowed fungal infection such as aspergillosis to thrive.

The result of this study is similar to that of Sultana et al. [25] who isolated *Aspergillus flavus* from the tracheal and lung of commercial broiler chicken in Bangladesh. It is concluded that *Aspergillus flavus* is predominant in poultry birds suffering from Aspergillosis. The prevalent of *A. fumigatus* and *A. flavus* in the study area may be linked to the climatic conditions of Ado Ekiti metropolis in Nigeria. In related study carried out by Shivaprakash et al. [26] it was reported that Infection due to *A. flavus* is predominant in Asia, Middle East and Africa possibly due to its better ability to survive in hot and arid climatic conditions compared to other *Aspergillus* spp. The fungal pathogens (*Aspergillus* sp.) mainly target the respiratory and nervous system of poultry and cause sickness leading to death. Avian Aspergillosis is responsible for death of chickens which can lead to economic loss to the farmers.

5. CONCLUSION

Conclusively, *Aspergillus* species associated with poultry birds in Ekiti State University poultry farms, Ago Aduloju poultry farms and Federal Polytechnic Ado-Ekiti, Ekiti State were predominantly *Aspergillus fumigatus* and *Aspergillus flavus*. In general, poultry is constantly exposed to these fungi in its environment, long exposition, highly contaminated environment, litters, high humidity in poultry houses, poor ventilation, malnutrition and stress.

Protective immunity following vaccination is useful in treating and preventing avian Aspergillosis. To prevent Aspergillosis, factors

such as stress and exposure to spores are to be minimized along with adopting strict hygiene and sanitation measures in the poultry. Affected birds should be removed and culled/ destroyed to avoid further spread of pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oladunmoye MK, Ayantola KJ, Ogunleye AG. Antibacterial and phytochemical activity of *Gliricidia sepium* against poultry pathogens. Microbiology Research Journal International. 2018;24(4):1-10.
2. FAO. World agriculture: Towards 2030/2050 interim report. Rome; 2006.
3. Pond WG, Bell AW. (Poultry: Behavior, Management and Well-Being. Marcell Dekker, Inc., New York. 2010;847–849.
4. Van Pelt W, Van Der See H, Wannet WJB, Van De Giessen AW, Mavius DJ, Bolder NM, Komijn RE, Van Duyenhoven YTHP. Explosive increase in salmonella java in poultry in the Netherlands. Consequences for Public Health. Eurosurveillance. 2003; 8:31-50.
5. Fagbohun ED, Ayantola KJ, Dada AT. Isolation and molecular characterization of Candida SPP from poultry with symptoms of candidiasis in Ado Ekiti, Nigeria. 2019; 3(3):1-9. DOI: 10.9734/IJPR/2019/v3i330097
6. Jand SK, Kaur P, Sharma NS. Mycoses and Mycotoxicosis in poultry. Indian J. Anim. Sci. 2005;75:465-475.
7. Jordan F, Pattisson M, Alexander D, et al. Poultry Disease. 5th ed. UK: Elsevier Limited. 2002;387–390.
8. Lobna MAS, Abdel FA. Epidemiological study of Aspergillosis in chickens and human contacts in chicken farms at Kalyoubia Governorate. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS). 2014;7(7):20-24.
9. Bauck L. Mycoses. In: Ritchie BW, Harrison GJ, Harrison LR. Editors. Avian Medicine: Principles and Application. Lake Worth: Wingers Publishing. 1994;165- 175.
10. Shoukat S, Wani H, Jeelani R, et al. An overview of avian aspergillosis. Int J Avian & Wildlife Biol. 2018;3(3):215–216. DOI: 10.15406/ijawb.2018.03.00088
11. Douglas F. Mycotic Infection. 2007;1-4.
12. Sultana S, Rashid SMH, Islam MN, Ali MH, Islam MM, Azam MG. Pathological investigation of avian Aspergillosis in commercial broiler chicken at Chittagong District. International Journal of Innovation and Applied Studies. 2015;10(1):366-376.
13. Pitt JJ, Hocking AD. Fungi and food spoilage. 3rd Edn, Springer, USA. 2009; 519.
14. Fagbohun ED, Anibijuwon I, Egbebi AO, Lawal O. Fungi associated with spoilage of dried cocoa beans during storage in Ekiti State of Nigeria. J. Microbiol. Biotech. and Food Sci. 2011;1(2):204-214.
15. Crowley N, Bradley JM, Darrell JH. Practical bacteriology. Butterworth and Co., Ltd. London. 1969;164-168.
16. Vuong C, Gotz F, Otto M. Construction and characterization of an agr deletion mutant of *Staphylococcus epidermidis*. Infection and Immunity. 2000;68:1048-1053.
17. Pontes DS, Pinheiro FA, Lima-Bittencourt CI, Gue-des RL, Cursino L, Barbosa F, Santos FR, Chartone-Souza E, Nascimento AM. Multiple antimicrobial resistance of gram negative bacteria from natural oligotrophic lakes under distinct anthropogenic influence in a tropical region. Can. J. Microbiol. 2009;58(4):762-772.
18. Lachance MA, Bowles JM, Starmer WT, Barker JSF. Kodamaea kakaduensis and Candida tolerans, two new ascomycetous yeast species from Australian hibiscus flowers. Canadian Journal of Microbiology. 1999;45:172-177.
19. Shabu S, Henna W, Rizwan J, Umer A, Mudasir. An overview of avian Aspergillosis. International Journal of Avian & Wildlife Biology. 2018;3(3):215-216.
20. Warris A, Bjorneklett A, Gaustad P. Invasive pulmonary Aspergillosis associated with infliximab therapy. New England Journal of Medicine. 2001;344: 1099– 1100.
21. Ustimenko AN. Aspergillosis of fowls and sanitary condition of incubators in U.S.S.R. Poultry Abstract. 1982;8(4):138.
22. Lobna MAS, Abdel FA. Epidemiological study of Aspergillosis in chickens and human contacts in chicken farms at Kalyoubia Governorate. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS). 2014;7(7):20-24.
23. Bauck L. Mycoses. In: Ritchie BW, Harrison GJ, Harrison LR. Editors. Avian Medicine: Principles and Application.

- Lake Worth: Wingers Publishing. 1994; 165-175.
24. Harold LC, John LR. Fungal infections. In: Diseases of poultry. Calnek BW, John BH, Charles WB, Larry RM, Saif YM (Eds), 10th Edition, Iowa State University Press, Ames, Iowa. 1997;251-359.
25. Sultana S, Rashid SMH, Islam MN, Ali MH, Islam MM, Azam MG. Pathological investigation of avian Aspergillosis in commercial broiler chicken at Chittagong District. International Journal of Innovation and Applied Studies. 2015;10(1):366-376.
26. Shivaprakash MR, Raees AP, Arunaloke C, Johan WM, Jacques FM. Invasive Aspergillosis by *Aspergillus flavus*: Epidemiology, diagnosis, antifungal resistance, and management. Journal of Fungi. 2019;5(55):1-23. DOI: 10.3390/jof5030055

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