Research Article

Detection of Intestinal Fungi in Chickens Naturally Infected with Infectious Bursal Disease

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Abstract | A little is known about intestinal fungal population and its role during health and disease particularly in chickens. Thus, this preliminary study was performed to identify intestinal fungal population in immunosuppressed chickens. Cloacal swabs were collected from freshly dead chickens suffering from infectious bursal disease (IBD) for isolation of fungal species on specific medium. A total of 19 purified fungal isolates have been identified morphologically. *Aspergillus* isolates were the most identified (42%), followed by *Trichosporon* (10.5%), *Penicillium* (10.5%), *Fusarium* (5%), *Candida* (1%) and non-identified isolates (26%). Furthermore, the obtained 8 *Aspergillus* isolates were further morphologically identified into *A. fumigatus* which was most frequent species identified (4/19 of total) and (4/8 of *Aspergillus* species); *A. flavus* (2), *A. niger* (1) and *A. terreus* (1). Molecular identification of 5 representative *Aspergillus* isolates indicated *A. fumigatus* (2), *A. flavus* (2), and *A. niger* (1) were also performed. We recommended further studies to identify the intestinal fungi of chickens in large scale and different ages which help to understand their role.

Keywords | Aspergillus, Chickens, fungi, immunosuppression, molecular identification, Histopathology.

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INTRODUCTION

The gastrointestinal tract (GIT) is occupied by a diverse microbial population (microbiota) consisting of bacteria, fungi, protozoa, and viruses (Roto et al., 2015). The microbiota which is essential for gut homeostasis has been extensively characterized. GIT microbiota plays an important role in carbohydrate and lipid metabolism, immunity development, synthesis of amino acids and vitamins, and strengthen the gut barrier by producing short-chain fatty acids (Cisek and Binek, 2014).

Dysbiosis, the microbial imbalance, is mostly associated with intestinal inflammation (Frank et al., 2011; Chang and Lin, 2016; Hughes et al., 2017). Intestinal microbial changes after infection with a wide range of pathogens have been reported. These changes characterized by reduction in the abundance and diversity of the intestinal flora (Day et al., 2015; Liu et al., 2018; Ma et al., 2017; Macdonald et al., 2017). Dysbiosis has been reported in chickens after infection with Newcastle disease virus (Cui et al., 2018), Marek's disease virus (Perumbakkam et al., 2014), avian leucosis viruses (Ma et al., 2017), or infectious bursal disease virus (IBDV) (Li et al., 2018). IBDV is a non-enveloped virus belongs to the genus *Avibirnavirus* and the family *Birnaviridae* (Müller et al., 2003; Delmas et al., 2005). It is an immunosuppressive virus which replicates in gut associated lymphoid tissue causing histological lesions, changes in immune cells, and alteration of microbial population (Hoerr, 2010; Jackwood, 2017; Li et al., 2018).

Although extensive knowledge is available about GIT microbiota, little is known about intestinal fungal population and its role during health and disease particularly

in chickens. The term mycobiome used to differentiate the mycology aspect of the microbiome (Gillevet et al., 2009). A total of 88 different fungal and yeast species were identified from cecal samples taken from commercial broiler and layer flocks, including *Aspergillus, Penicillium,* and *Sporidiobolus* species, and 18 unknown genera by using automated repetitive sequence-based PCR (Byrd et al., 2017). Furthermore, fifty fungal isolates belonged to seven species (*Aspergillus fumigatus, Aspergillus niger, Chrysonilia crassa, Mucor circinelloides, Mucor sp, Rhizopus oligosporus and Rhizopus oryzae*) have been isolated from different parts of GIT of chickens (Yudiarti et al., 2012). Recently, Next-generation sequencing of chicken intestinal mycobiota has shown the presence of four phyla and 125 genera in which 3 genera (*Microascus, Trichosporon*, and *Aspergillus*) representing over 80% of the total fungal population (Robinson et al., 2020). Interestingly, many species belong to these genera (*Microascus, Trichosporon, and Aspergillus*) are considered opportunistic pathogens particularly in immunocompromised humans (Colombo et al., 2011; Iwen et al., 2012; Sandoval-Denis et al., 2013; Sugui et al., 2014). Therefore, it is important to continue monitoring these intestinal fungi which could be a source of contamination in poultry meat in processing plant and increase fungal load inside poultry house to ensure food safety and low personal risk.

The objective of this study was to isolate and identify fungal populations that passed through the GIT of the immunosuppressed chickens during IBDV natural infection.

MATERIALS AND METHODS

Flocks history

Two native chicken flocks suffering from infectious bursal disease were sampled from Menoufia governorate. The first flock was 35 day old chickens (n=5000). While the second flock was 28 day old chickens (n=1200). They only vaccinated with intermediate IBD vaccine at 12 day of age. Chicken flocks showed signs including whitish diarrhea, ruffled feathers, anorexia, depression and mortalities (5%-10%). At postmortem examination enlarged bursa with gelatinous transudate covering its serosal surface was observed. Furthermore, hemorrhagic bursae, hemorrhage on thigh muscle and between gizzard and proventriculus was also observed in dead chickens.

Samples

Cloacal swabs and intestinal specimens

Five cloacal swabs and intestinal specimens per flock were collected from freshly dead birds for isolation and identification of fungi and histopathological study respectively.

Isolation and identification of fungi

Culture media

Sabouraud Dextrose Agar (Oxoid) was used for isolation and primary morphologicl identification of the obtained isolates. Preliminary cultures were established with Sabouraud, and glucose-potato (PDA) agars with the addition of antibiotics, and incubated at 25 and 37 for 48 h up to 7 days. After 72 where fungi mostly showing especial growth.

Morphological identification

Species of fungi including *Aspergillus* were identified by observing the characteristic conidial head and colony (Girma et al., 2016). Taxonomic identification was carried out with the method (Kurtzman et al., 2011; Raper and Fennel, 1965).

Molecular identification of fungi

Four representative isolates from identified were subjected to molecular identification as follows:

DNA extraction

DNA extraction from samples was performed using QIAamp DNeasy Plant Mini kit (Qiagen, Germany, GmbH). Briefly, 100 mg of the sample was added to 400 µl Buffer AP1 and 4 µl RNase. A stock solution (100 mg/ml), tungsten carbide bead were added to the previous mixture in a 2 ml safe-lock tube. Tubes were placed into the adaptor sets, which are fixed into the clamps of the Tissue Lyser. Disruption was performed in two 1–2 minute high-speed (20–30 Hz) shaking steps. The mixture was incubated for 10 min at 65°C and mixed 2 or 3 times during incubation by inverting tube. Then, 130 µl Buffer P3 was added to the lysate, mixed, and incubated for 5 min on ice. The lysate was centrifuged for 5 min at 14,000 rpm, and then pipetted into the QIA shredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifuged for 2 min at 14,000 rpm. The flow-through fraction from was transferred into a new tube without disturbing the cell-debris pellet and then applied to silica column. The lysate was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 50 µl of elution buffer provided in the kit.

Oligonucleotide primers

Primers used were supplied from Biobasic Canada and were listed in Table 1 and cycling conditions in Table 2.

PCR amplification

DNA samples: Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied bio system 2720 thermal cycler.

Table 1: Primers sequences, target gene, and amplification size.

Target gene	Primers sequences	Amplified segment (bp)	Reference
Fungi ITS	ITS1: TCCGTAGGTGAACCTGCGG	Variable	Tarini *et al.*, 2010
	ITS4: TCC TCC GCT TAT TGA TAT GC		

Table 2: Cycling conditions for conventional PCR.

Target	Primary denaturation	Amplification cycles			Final extension
		Secondary denaturation	Annealing	Extension	
Fungi ITS	94°C 5 min.	94°C 30 sec.	56°C 40 sec.	72°C 45 sec.	72°C 10 min.

Analysis of the PCR Products

Conventional PCR products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A generuler 100 bp DNA Ladder (Fermentas, thermofisher, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software. *A. flavus* (ATCC® 9643™) was used as positive control (Figure 2).

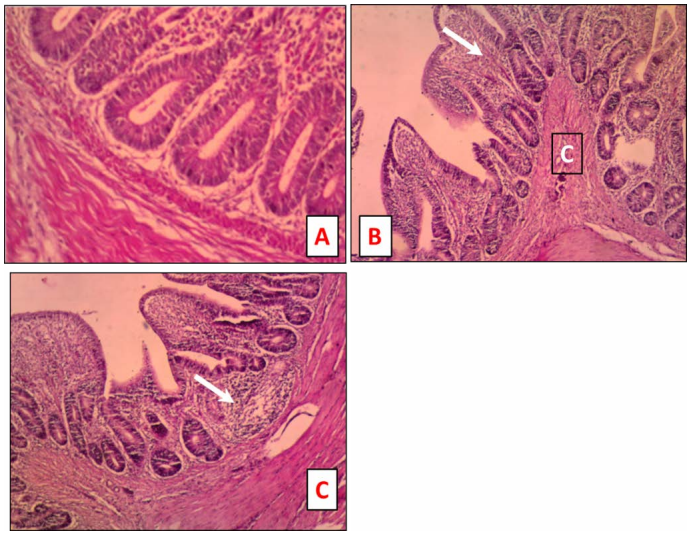

Figure 1: Intestine tissue sections of naturally IBDV infected chicken stained by H and E. A. Normal intestine tissue sections stained (x 200) B: Chicken intestine showing inflammatory cells infiltration of the mucosa (white arrow) and congestion (C) of the submucosa (x 100). C: Chicken intestine showing inflammatory cells infiltration of the mucosa (white arrow) (x 100).

Gene sequence

PCR products were purified using QIAquick PCR Product Extraction Kit (Qiagen, Valencia, CA, USA). Big Dye Terminator V3.1 Cycle Sequencing Kit (PerkinElmer, Foster city, CA, USA) was used for the sequence reaction, and then, the product was purified using Centri-Sep™ spin columns. DNA sequences were obtained from Applied Biosystems 3130 genetic: analyzer (Hitachi, Japan). A BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequence identity to GenBank accessions (Altschul et al., 1990). Amino acid sequence of the TSI gene of the isolated fungal strains in comparison to published strains were shown (Figure 4). A phylogenetic tree was created using the CLUSTAL W multiple sequence alignment program, MegAlign module of Lasergene DNASTAR version 12.1 (Thompson, et al. 1994) (Figure 3).

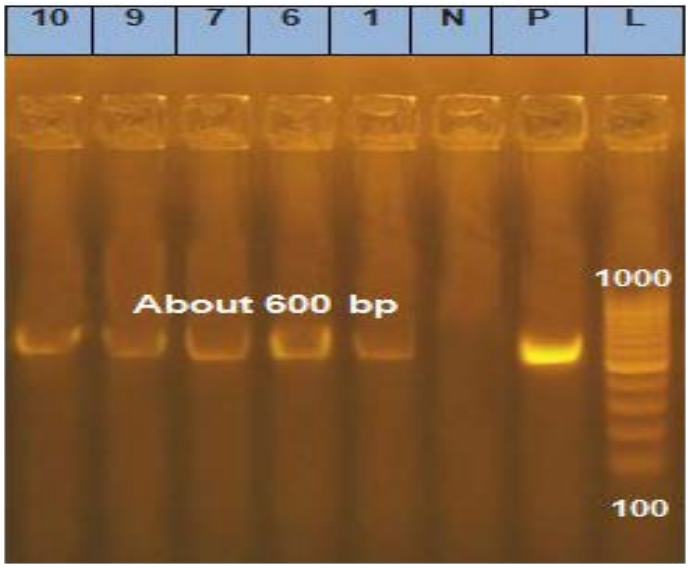

Figure 2: Amplified ITS gene of isolated fungi. Lane L = 100 bp marker; Lane P = Positive control. Lanes 1 (*A. niger*), 6 (*A. fumigatus*), 7 (*A. flavus*), 9 (*A. fumigatus*) and 10 (*A. flavus*); Lane N = Negative control.

Phylogenetic analyses

Phylogenetic analyses were performed with published Fungi sequences of ITS gene (Table 3) using the maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6 (Figure 3) (Tamura et al., 2013).

Histopathological examination

Intestine specimens were collected and fixed in 10% neutral buffered formalin for preparing paraffin tissue sections at 4-6 µm thickness. These sections were stained with hematoxylin and eosin (Bancfort and Stevens 1996).

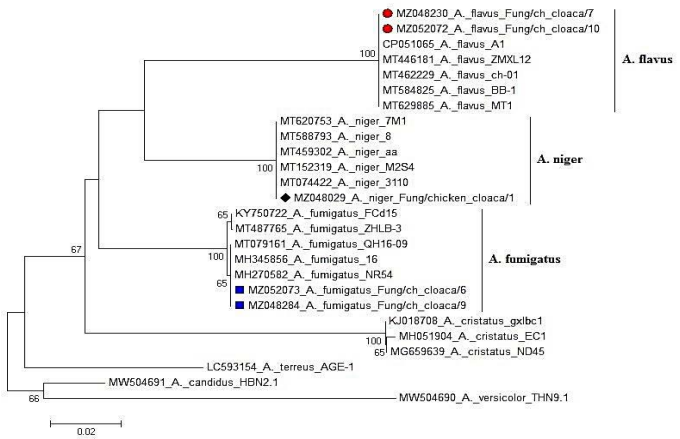

Figure 3: Phylogenetic tree 5 Aspergillus strains named MZ052072, MZ052073, MZ048029, MZ048030 and MZ052073 are compared with 21 sequences of ITS gen published in GenBank. Branched distances correspond to sequence divergence.

Table 3: Percentage of nucleotide identities for the ITS genes of 5 Aspergillus strains named MZ052072, MZ052073, MZ048029, MZ048030 and MZ052073as compared with 21 sequences published in GenBank.

Percent identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
1		100.0	100.0	100.0	100.0	100.0	100.0	78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	1
2	0.0		100.0	100.0	100.0	100.0	100.0	78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	2
3	0.0	0.0		100.0	100.0	100.0	100.0	78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	3
4	0.0	0.0	0.0		100.0	100.0	100.0	78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	4
5	0.0	0.0	0.0	0.0		100.0	100.0	78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	5
6	0.0	0.0	0.0	0.0	0.0		100.0	78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	6
7	0.0	0.0	0.0	0.0	0.0	0.0		78.1	78.3	78.3	86.9	86.9	86.7	86.9	86.9	86.9	88.5	88.5	88.5	88.5	88.5	88.5	88.5	84.2	84.6	77.3	7
8	13.6	13.6	13.6	13.6	13.6	13.6	13.6		99.5	99.6	79.1	79.1	79.5	79.1	79.1	79.1	77.0	77.0	77.0	77.0	77.0	77.0	77.0	76.5	77.7	75.2	8
9	13.6	13.6	13.6	13.6	13.6	13.6	13.6	0.2		99.6	79.5	79.5	79.5	79.5	79.5	79.5	77.5	77.5	77.5	77.5	77.5	77.5	77.5	76.4	77.5	75.2	9
10	13.6	13.6	13.6	13.6	13.6	13.6	13.6	0.2	0.0		79.1	79.1	79.5	79.3	79.1	79.1	77.3	77.3	77.3	77.3	77.3	77.3	77.3	76.6	77.7	75.2	10
11	10.8	10.8	10.8	10.8	10.8	10.8	10.8	11.7	11.4	11.4		100.0	100.0	99.6	99.6	100.0	100.0	90.8	90.8	90.8	90.8	90.8	90.8	86.3	87.1	77.5	11
12	10.8	10.8	10.8	10.8	10.8	10.8	10.8	11.7	11.4	11.4	0.0		100.0	99.6	99.6	100.0	100.0	90.8	90.8	90.8	90.8	90.8	90.8	86.3	87.1	77.5	12
13	10.8	10.8	10.8	10.8	10.8	10.8	10.8	11.7	11.4	11.4	0.0	0.0		99.6	99.6	100.0	100.0	90.8	90.8	90.8	90.8	90.8	90.8	86.3	87.1	77.5	13
14	10.8	10.8	10.8	10.8	10.8	10.8	10.8	11.4	11.1	11.1	0.2	0.2	0.2		99.6	99.6	90.4	90.4	90.4	90.4	90.4	90.4	90.4	86.7	87.1	77.3	14
15	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.4	11.1	11.3	0.2	0.2	0.2	0.0		99.6	99.6	90.8	90.8	90.8	90.8	90.8	90.8	86.7	87.1	77.5	15
16	10.8	10.8	10.8	10.8	10.8	10.8	10.8	11.7	11.4	11.4	0.0	0.0	0.0	0.2	0.2		100.0	90.8	90.8	90.8	90.8	90.8	90.8	86.3	87.1	77.5	16
17	10.8	10.8	10.8	10.8	10.8	10.8	10.8	11.7	11.4	11.4	0.0	0.0	0.0	0.2	0.2	0.0		90.8	90.8	90.8	90.8	90.8	90.8	86.3	87.1	77.5	17
18	9.7	9.7	9.7	9.7	9.7	9.7	9.7	14.4	14.1	14.4	7.9	7.9	7.9	8.2	8.2	7.9	7.9		100.0	100.0	100.0	100.0	100.0	86.3	85.2	77.0	18
19	9.7	9.7	9.7	9.7	9.7	9.7	9.7	14.4	14.1	14.4	7.9	7.9	7.9	8.2	8.2	7.9	7.9	0.0		100.0	100.0	100.0	100.0	86.3	85.2	77.0	19
20	9.7	9.7	9.7	9.7	9.7	9.7	9.7	14.4	14.1	14.4	7.9	7.9	7.9	8.2	8.2	7.9	7.9	0.0	0.0		100.0	100.0	100.0	86.3	85.2	77.0	20
21	9.7	9.7	9.7	9.7	9.7	9.7	9.7	14.4	14.1	14.4	7.9	7.9	7.9	8.2	8.2	7.9	7.9	0.0	0.0	0.0		100.0	100.0	86.3	85.2	77.0	21
22	9.7	9.7	9.7	9.7	9.7	9.7	9.7	14.4	14.1	14.4	7.9	7.9	7.9	8.2	8.2	7.9	7.9	0.0	0.0	0.0	0.0		100.0	86.3	85.2	77.0	22
23	9.7	9.7	9.7	9.7	9.7	9.7	9.7	14.4	14.1	14.4	7.9	7.9	7.9	8.2	8.2	7.9	7.9	0.0	0.0	0.0	0.0	0.0		86.3	85.2	77.0	23
24	12.7	12.7	12.7	12.7	12.7	12.7	12.7	14.7	14.7	14.9	11.2	11.2	11.2	10.9	10.9	11.2	11.1	11.1	11.1	11.1	11.1	11.1	11.1		89.1	78.5	24
25	11.1	11.1	11.1	11.1	11.1	11.1	11.1	14.2	14.2	14.5	8.6	8.6	8.6	8.9	8.9	8.6	8.6	10.8	10.8	10.8	10.8	10.8	10.8	9.8		91.8	25
26	17.6	17.6	17.6	17.6	17.6	17.6	17.6	16.4	16.6	16.6	14.7	14.7	14.7	14.7	14.9	14.7	14.7	15.7	15.7	15.7	15.7	15.7	15.7	14.5	11.0		26
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	

Divergence

1 MT629885 A. flavus MT1
2 MT584825 A. flavus BB-1
3 MT462229 A. flavus ch-01
4 MT446181 A. flavus ZMXL12
5 CP051065 A. flavus A1
6 MZ048230 A. flavus Fung/ch cloaca/7
7 MZ052072 A. flavus Fung/ch cloaca/10
8 MH051904 A. cristatus EC1
9 MG659639 A. cristatus ND45
10 KJ018708 A. cristatus gxlbc1
11 MT079161 A. fumigatus QH16-09
12 MH345856 A. fumigatus 16
13 MH270582 A. fumigatus NR54
14 KY750722 A. fumigatus FCd15
15 MT487765 A. fumigatus ZHLB-3
16 MZ052073 A. fumigatus Fung/ch cloaca/6
17 MZ048284 A. fumigatus Fung/ch cloaca/9
18 MT620753 A. niger 7M1
19 MT588793 A. niger 8
20 MT459302 A. niger aa
21 MT152319 A. niger M2S4
22 MT074422 A. niger 3110
23 MZ048029 A. niger Fung/ch cloaca/1
24 LC593154 A. terreus AGE-1
25 MW504691 A. candidus HBN2.1
26 MW504690 A. versicolor THN9.1

RESULTS AND DISCUSSION

Pathological findings

Intestine of chickens naturally infected with IBDV showed inflammatory cells infiltration in the mucosa and congestion of the submucosa (Figure 1).

Isolation and identification of fungi

Morphological examination of purified 19 fungi isolates on Sabouraud agar revealed the identification of 8 *Aspergillus* species, 2 *trichosporon* , 2 *pencillium*, 1 *Fusarium*, 1 *candida* and 5 non-identified species. The obtained 8 *Aspergillus* isolates were further morphologically identified into *A. fumigatus* which was most frequent species identified (4/19 of total) and (4/8 of *Aspergillus* species); *A. flavus* (2), *A. niger* (1) and *A. terreus* (1). A representative 5 *Aspergillus* species those are invasive fungi associated with excessive morbidity and mortality, also of toxigenic importance to chickens including (*A. fumigatus* (2), *A. flavus* (2) and *A. niger* (1)) were taken for further molecular identification.

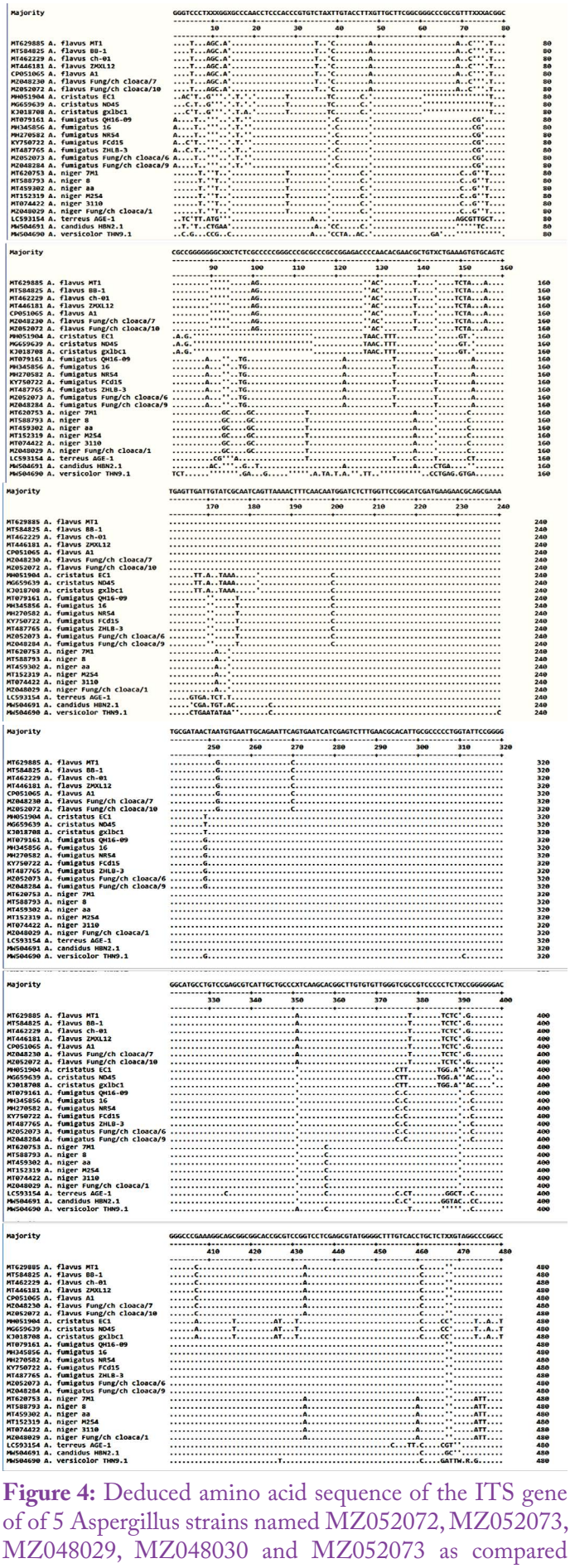

Figure 4: Deduced amino acid sequence of the ITS gene of of 5 Aspergillus strains named MZ052072, MZ052073, MZ048029, MZ048030 and MZ052073 as compared with 21 sequences published in GenBank.

The PCR product of ITS gens were sequenced and submitted to genebank: *A. fumigatus*, *A. flavus*, and *A. niger* with accession numbers MZ052073 and MZ048284; MZ048230 and MZ052072 as well as MZ048029, respectively.

The intestinal tract of humans and animals has a diverse populations of bacteria, fungi, archaea, protozoa, and viruses (Fan and Pedersen, 2021; Peixoto et al., 2021). Despite large diversity of the fungal community in humans, it is approximately estimated 0.02% of the intestinal mucosa-associated microbiota and 0.03% of the fecal microbiota (Ott et al., 2008). A little is known about intestinal fungal population in chickens particularly during immunosuppression. Thus, this study was carried out to identify fungal population passed through intestine of chickens during IBDV infection. IBDV replicates in gut associated lymphoid tissue causing immunosuppression, histological lesions, changes in immune cells, and alteration of microbial population (Hoerr, 2010; Jackwood, 2017; Li et al., 2018).

Samples for fungi isolation were collected as cloacal swabs. Small intestine and caecum are the place where the more existences of microbe (Romach et al., 2004). Furthermore, Chicken ileum was dominated by lactobacilli, whereas in caecum was found more diverse microbial community (Bjerrum et al., 2006). However, the largest number of fungi was found in ileum, and then followed by caecum, jejenum and duodenum (Yudiarti et al., 2012).

The morphological characterization was performed as a preliminary step to identify fungi followed by further identification using molecular methods (Zulkifli and Zakaria, 2017). Based on fungal morphology on sabouraud agar, we identified 19 purified fungal isolates. A total number of 8 out of 19 isolates (42%) were *Aspergillus* species. *Aspergillus* species have been reported to be one of the most identified fungal population from cecal swabs obtained from broiler and layer flocks (Byrd et al., 2017). Furthermore, it was one of the most 3 identified chicken intestinal mycobiota by next-generation sequencing (Robinson et al., 2020). In this study, the obtained 8 *Aspergillus* isolates were further morphologically identified into *A. fumigatus* which was the most identified species (4/19 of total) and (4/8 of *Aspergillus* species); *A. flavus (2)*, *A. niger* (1) and *A. terreus (1)*. Noticeably, *A. fumigatus* is a ubiquitous pathogen in poultry farms causing aspergillosis which reported in different avian species and production types (Arné et al., 2011). Additionally, *Aspergillus* species produce mycotoxins which cause high economic losses in poultry industry represented by mortalities, immunosuppression, and main cause of vaccination failure (Girma, 2016).

Histological characteristics of fungi in various organs including GIT were associated to systemic form of Aspergillosis in which fungal hyphae from lungs spread by hematogenous route (Beytut et al., 2004). Besides, pathological lesions in lungs were developed gradually after infection of specific pathogen free chickens with high dose of *A. fumigatus* conidia. Inflammatory cell infiltration in parabronchi was observed at 1 day post infection (dpi) followed by emergence of typical granulomatous lesions at 3-5 dpi (Cheng et al., 2020). In our study, the histopathological findings in intestinal sections were inflammatory cells infiltration of the mucosa and congestion of the submucosa (Figure 1). Since fungal populations are a part of GIT ecosystem (Fan and Pedersen, 2021; Peixoto et al., 2021), further experimental studies are required to illustrate and quantify different fungal species during IBDV infection comparing to non-infected chickens which help to further understanding of its role.

Molecular identification of fungi at the genus or species level by amplification of ITS gene have been used (Gaskell et al., 1997). In this study, a representative 5 *Aspergillus* species (*A. fumigatus* (2), *A. flavus* (2), and *A. niger* (1)) were taken for further molecular identification. Phylogenetic analysis and nucleotide identity based on ITS sequence (Figure 3 and Table 3) confirmed the morphological identification of these *Aspergillus* species.

Finally, the opportunistic fungi in intestine may be a source of contamination of poultry meat in processing plant and increasing fungal load inside poultry house. Thus, continuous monitoring of fungal population in chickens during health and disease conditions is required for deep understanding of its role, food safety, and low personal risk.

CONCLUSIONS AND RECOMMENDATIONS

This preliminary study was performed to identify intestinal fungal population in immunosuppressed chickens. A total of 19 purified fungal isolates have been identified morphologically. *Aspergillus* isolates were the most identified (42%) fungal isolates from cloacal swabs of IBD infected chickens, followed by *Trichosporon* (10.5%), *Penicillium* (10.5%), *Fusarium* (5%), *Candida* (1%) and non-identified isolates (26%). Furthermore, the obtained 8 *Aspergillus* isolates were further morphologically identified into *A. fumigatus* which was most frequent species identified (4/19 of total) and (4/8 of *Aspergillus* species); 2 *A. flavus*, 1 *A. niger* and 1 *A. terreus*. Furthermore, molecular identification of 5 representative *Aspergillus* isolates indicated *A. fumigatus* (2). *A. flavus* (2), and *A. niger* (1) were performed.

NOVELTY STATEMENT

The study of intestinal fungal population and its role during health and disease is essential particularly in chickens. Thus, our study was accomplished to isolate and identify fungal populations that passed through the GIT of the immunosuppressed chickens during IBDV natural infection. Different purified fungal isolates have been identified morphologically in our study. Furthermore, molecular identification of 5 representative Aspergillus isolates were also performed. Our results help to further understand of intestinal fungal population in immunosuppressed chickens.

AUTHOR'S CONTRIBUTION

Mohamed M. Amer and Kh. M. Elbayoumi carried out the research design and revised the manuscript. Aly M. Ghetas, Dalia M. Sedeek, Hanaa S. Fedawy and Hoda M. Mekky isolated and identified fungal isolates. M. A. Bosila performed histopathology for intestinal samples. Aly M Ghetas wrote the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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