Detection of some virulence genes in Aspergillus fumigatus isolated from the nasal cavity of cattle in Al-Qadisiyah Province

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Abstract
Our study aim to investigate in detection Aspergillus fumigatus isolated from the upper respiratory tract of cattle (nasal cavity) by culture and confirmed by (PCR) after designing the specific primer, and detection some virulence genes such as sidA and gpaB gene in Aspergillus fumigatus by PCR. Our findings are one hundred twenty (120) samples were collected randomly from the upper respiratory tract of cowsin different regions of Al-Qadisiyah province. Wherever the samples cultured on specific media Sabouraud agar for enrichment as the first step then confirmed by PCR by using specific primers for detection some virulence genes by PCR (second step). Our results found the percentage of suspected Aspergillus fumigatus by culture was 19/120 (15.8) %, and the percentage of confirmed Aspergillus fumigatus by PCR was 10/19 (52.6) %, and the rate of sidA gene and gpaB gene was7/10 (70) % and 5/10 (50) % respectively. Generally, pathogenicity depends on the quality and quantity of virulent genes. sidA gene and gpaA gene is the most common virulence genes in our isolates, which leads to the virulent genes carry Fungi are life-threatening more than non-gene carrying fungi.

Keywords: the virulence gene, sidA, gpaB, Aspergillus fumigatus, cattle


Introduction:
The fungi cause many diseases in animals and humans. The fungal species reach more than (500) species, but (100) species causing fungal diseases in humans and animals. Filamentous fungi such as Aspergillus fumigatus cause many diseased cases. Aspergillus spp is spread in the soil, the environment and the plant's tissues (1, 2). Aspergillosis is a disease that causes high death rates in immunocompromised patients, and causes several diseases in animals especially in small animals, diseased animals and inhibited immune animals (3). A. fumigatus is one of the major causative agents of Aspergillosis. Indeed, their conidia help it to get in the alveolar (4-6).

Usually, immunocompromised individuals suffer from many diseases such as tuberculosis, and fungal infection including Aspergillosis. Pathogenicity of Aspergillosis is varying among hosts depended on age, animal species, and fungal virulence. The pathogenicity of fungal depends on the type and number of the virulence genes. The virulence proteins are having a great role in the aspergillosis pathogenesis such as stains formation, adhesion antigens, toxins, and enzymes (7).

Many gene-encoded virulence proteins in Aspergillus spp (8, 9). The deletion of G-protein receptors caused growth defects, decreasing of hyphal extension (10). Protein G is encoded by the gpaB gene. The sidA genes encoded siderophore. It has important roles in the fungi pathogenesis such as iron storage, stress resistance and germination (11, 12). Protein G and siderophore proteins have a great role in the pathogenesis of the fungi (13). It showed that they are related to pathogenesis. Some are known mechanisms and some other not known.
mechanisms in *Aspergillus* (14). This study focused on the detection of some virulence genes such as *gpaB* and *sidA* in *Aspergillus fumigatus*.

**Material and methods:**

**Study design**

Swap samples (120) are collected randomly from the upper respiratory tract of cows. All the samples are keeping at 4°C until reach to the lab. When it reaches to the lab immediately cultured on savoried agar. The isolates that cultured on sabouraud agar considered positive for culture. Then positive isolates submitted to PCR examination for detection of *Aspergillus fumigatus* by specific primer, then the positive isolated is examined by PCR for detection some virulence genes including *sidA* gene, *gpaB* gene.

**Culture**

The samples were transferred to the lab, then treated by KOH 10% then cultured on Sabouraud Dextrose Agar media (Merck, Germany) for seven days at room temperature. The Aspergillus growth was identified by using its colors, shape and slide cultures after staining.

**DNA extraction**

Promega protocol was applied for extraction *Aspergillus* spp DNA. The extracted DNA was analyzed and tested by biophotometer. Standard PCR Protocol of KAPA2G Fast ReadyMix PCR Kit for b-tubulin amplification was used. The total PCR mix was (25) μL, it formed from of PCR water was (8) μL, KAPA2G Fast ReadyMix was (12.5) μL, F-Primer was (1.25) μL, R-Primer was (1.25) μL and extract DNA (2) μL.

**Primers:**

The used primers are depended on several previous studies (HAILU W. H. 2017).

**Table (1):** showed primer sequences and its size.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Molecular size</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-tubulin</td>
<td>F</td>
<td>GGAAGGGRTGTATTTATTAG</td>
<td>200 bp (HAILU W. H. 2017)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TCCTCTAAATGACCAAGTTTG</td>
<td></td>
</tr>
<tr>
<td><em>sidA</em></td>
<td>F</td>
<td>GCGACGATAGCCCATTTGTC</td>
<td>155 bp (Nayereh Ghodsa, 2017)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ATTAGCAGGATAGGATCAAGG</td>
<td></td>
</tr>
<tr>
<td><em>gpaB</em></td>
<td>F</td>
<td>CCCGACTTCTATGACGGAGC</td>
<td>140 bp (Nayereh Ghodsa, 2017)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CAACGCCTATGCATTCAGCC</td>
<td></td>
</tr>
</tbody>
</table>

**Thermo-cycler:**

Table (2): Thermocycler setting of used primers.

<table>
<thead>
<tr>
<th>The primers</th>
<th>Thermocycler setting</th>
</tr>
</thead>
</table>
| b-tubulin   | 1- 95°C for two minute  
2- 95°C for 30 second  
3- 61.8°C for 30 second  
4- 72°C for 20 second  
5- Repeat steps (2-4) for 29 times  
6- 72°C for five minute  
7- 4°C forever |
| sidA        | 1- 95°C for two minute  
2- 95°C for 30 second  
3- 61.8°C decrease 0.5°C per cycle for (30) second  
4- 72°C for 20 second  
5- Repeat steps 2-4 (14) times  
6- 95°C for 30 second  
7- 54.8°C for 30 second  
8- 72°C for 20 second  
9- Repeat steps (6-8) for 19 times  
10- 72°C for 5 minute  
11- 4°C forever |
| gpaB        | 1- 95°C for two minute  
2- 95°C for 30 seconds  
3- 65.4°C decrease 0.5°C per cycle, for 30 seconds.  
4- 72°C for 20 second  
5- Repeat steps (2-4) 14 times  
6- 95°C for 30 second  
7- 58.4°C for 30 second  
Step 8- 72°C, 20.0 sec.  
Step 9- Repeat steps 6-8 19 more times  
Step 10- 72°C, 5 min.  
Step 11- 4°C, forever |

Electrophoresis
PCR products have emigrated on agarose gel electrophoresis (1) % (Bio-Rad) with the current was (400) mA, the time was half hours and the voltage was 85V. The staining of the gel was done by ethidium bromide by adding several drops then washing by water for fifteen min. then DNA bands visualized under UV light.

Results
Our results found the percentage of suspected *Aspergillus fumigatus* by culture was 19/120 (15.8) % and the percentage of confirmed *Aspergillus fumigatus* by PCR was 10/19 (52.6) % by using b-tubulin gene primer as shown the table(1) and figure (1). Percentage of sidA gene and gpaB genes were 7/10 (70) % and 5/10 (50) % respectively as table (2) and figure (2) and (3).

Table(1): showed number and percentage of isolates of *Aspergillus fumigatus* by culture and PCR.

<table>
<thead>
<tr>
<th>The isolates</th>
<th>The culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>120</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>
Fahad et al. (2020): Detection of some virulence genes in *Aspergillus fumigatus* Nov 2020 Vol. 23 Issue 19

**Figure (1):** Showed band of the b-tubulin gene under UV light at 200 bp after using electrophoresis on agarose gel 1.5%

**Table (2):** Showed number and percentage of virulence genes that detected in *Aspergillus fumigatus*.

<table>
<thead>
<tr>
<th>The gene</th>
<th>The Number</th>
<th>The Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>sidA</td>
<td>7/10</td>
<td>70%</td>
</tr>
<tr>
<td>gpaB</td>
<td>5/10</td>
<td>50%</td>
</tr>
<tr>
<td>sidAandgpaB</td>
<td>3/10</td>
<td>30%</td>
</tr>
</tbody>
</table>

All positive isolates by culture techniques submitted to PCR methods for detection virulence genes (*sidA* gene and *gpaB* gene) by using specific primers then immigrated by electrophoresis by using agarose gel 1.5% and witched under UV lighter as figure(2) and (3).

**Figure (2):** Showed band of *sidA* gene under UV light at 155 bp after using electrophoresis on agarose gel 1.5%
Discussion

Aspergillus fumigatus is the most common fungus spread in soil, dust and the environment. It can produce spores called conidia (15). Although Aspergillus fumigatus is uncommon, the patient with aspergillosis showed high mortality (16). Our results found the percentage of suspected Aspergillus fumigatus by culture was 19/120 (15.8) %. This fungus was isolated at (3.2%) and (6.6) % by culture and that less than our percentage (17,18). The incidence is assessed to range between (0-15) percent depending on organs, wherever it varies among the organs such as heart, small bowel, liver, pancreas and kidney, and that agree with our results (19). Aspergillus fumigatus was (92%) by culture (20) and that considered more than our results.

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The percentage of confirmed Aspergillus fumigatus by PCR was 10/19 (52.6) % similar to the results of (21) wherever found A. fumigatus 50% by PCR. (22) found the percentage of A. fumigatus by PCR was (27.3%) and that represents less than our rates. While (23) found that A. fumigatus was 80% by using polymerase chain reaction (PCR).

Aspergillus species are common, invasive aspergillosis is common only in the immunocompromised population, composed of patients with AIDS, neutropenic patients, those on long-term corticosteroids, and recipients of transplants on anti-rejection medications. Invasive aspergillosis is also being seen in critically ill intensive care patient with an underlying pulmonary disease. Overall, the incidence of invasive aspergillosis has risen four-fold in the last 13 years. Patients with underlying lung diseases such as chronic obstructive lung disease, tuberculosis, asthma, lung cancer, and sarcoidosis are also at higher risk for developing the chronic form of aspergillosis. Bronchial aspergillosis is almost exclusively found in asthma and cystic fibrosis patients. Those working in the construction and farming industries may be at increased risk of Aspergillus infection due to chronic exposure in their work environments. Smoking marijuana contaminated with the fungus may also place an individual at risk for infection. Nosocomial Aspergillus infections have been reported from hospital showers and healthcare facilities undergoing construction (24). Aspergillus spp is spread in all regions of the world. It found in tobacco, building materials, food, flower, and water. Type od the sounding conditions are determined of fungi spreading (25).

The percentage of the sidA gene and gpaB gene was 7/10 (70) % and 5/10 (50) % respectively according to our results. sidA gene and gpaA gene are genes encoding to important proteins that have a great role in the pathogenesis of the A. fumigatus.

Generally, the similarity and difference in the prevalence of virulence genes are very normal because there are many factors related to host and factor related to an accusative agent that determines their spread, such as the ability of fungi to exchange of virulence genes. The host’s weak or strong immunity allows or prevents freedom of reproduction and genetic exchange between microorganisms; also, it depends on the ability of the fungus to create a genetic mutation that enables it to develop a necessary protein that plays a role in pathology (26). In spite of that, there are no recent studies dealing with the epidemiological issue of virulence genes in Aspergillus fumigatus, but the variation of results is very natural depending on the conditions surrounding the causative agent that compel it to mutate in order to be more virulent (27,28).
It is necessary to make new studies related to all aspects of virulence genes such as detection and the expressions in all hosts, healthy and patient especially in hepatitis and tuberculosis. The sidA and gpaB genes have a role in the pathogenesis of Aspergillus spp.

References


