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"التحري الجزيئي عن *Aspergillus niger* و *Candida tropicalis* المعزولة من مرضى التهاب الاذن الوسطى"

رسالة مقدمة الى

مجلس كلية العلوم – جامعة ديالى وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

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Summary

This study was aimed to identify of otomycotic species that isolated from immunocompromised and immunocompetent patients with otitis media at the molecular level. One hundred swaps were collected from patients with otitis media who attended the ENT consulting clinic at Baqubah Teaching Hospital for a period extending from the 1st of November 2022 to the 28th of February 2023. The specimens were collected from immunocompromised (patients whom underwent malignancies, diabetes and whom treated with steroids for a long periods), and immunocompetent patients (50 each of). Fifty eight were males and forty two were females, with a percentage (58% and 42%, respectively) ranging between 1 to 80 years old. Clinical diagnosis was done by a consultant otolaryngologist.

Otomycotic species were isolated using Sabouraud′s dextrose agar medium, and the identification of *Aspergillus niger* and *Candida tropicalis* was done using both of conventional cultural methods such culture on (Hi-CHROM Candida agar and CZapic Dox agar media) and molecular methods (PCR for the ITS region and ITS region sequencing) for resistant isolates of *A. niger* and *C. tropicalis* toward fluconazole and miconazole together. Molecular detection of virulence genes was done using (PCR and gene sequencing) methods for the (*NIG1* and *β-tub*) genes for *A. niger* isolates and the (*Als2*) gene for *C. tropicalis* isolates. The DNA was extracted from those isolates by commercial kit, then the studied virulence genes were detected by the single-plex polymerase chain reaction specific primers for those genes. Subsequently, the PCR products of all studied genes were subjected for gene sequencing.

The results of the present study showed that (*Aspergillus flavus*, *A. fumigates*, *A. niger*, *Candida albicans*, *C. tropicalis*, *C. glbrata* and *C.*

krusei) were identified as causative agents of otomycosis among immunocompromised patients. *Aspegillus niger* and *Candida tropicalis* reported high percentages among immunocompromised patients with otitis media which were (26.0%) and (14.0%), respectively. In gender terms, males were more percentage infected with otitis media among immunocompromised patients than females (58.0%). According to the age, among immunocompromised patients with otitis media, the highest rate was in the (>61) age group, which was (34.0%), whereas among immunocompetent patients in the (21-40) age group, the highest rate was (36.0%).

According to the results of the susceptibility test of otomycosis toward the studied antibiotics, only 7 isolates of *A. niger* and 3 isolates of *C. tropicalis* (33.3% and 25.0%, respectively) were resistant to both antibiotics (fluconazole and muconazole). According to the single-plex PCR analysis of the ITS gene for *A. niger* and *C. tropicalis* isolates, the molecular weight of the PCR products was (599 bp and 520 bp, respectively). The results of substitution mutations in the studied genes indicated that four isolates of *A. niger* have substitution mutations in the *NIG1* gene. whereas two isolates of *A. niger* have substitution mutations for *β-tub* gene. For *C. tropicalis*, only one isolate has two substitution mutations in the *Als2* gene.

Chapter One

(Introduction)

1. Introduction

Otomycosis is a fungal infection that affects the external ear canal and can spread to the middle ear when the tympanic membrane is perforated (Vennewald and Klemm, 2010). Yeasts and molds especially, *Candida spp*. and *Aspergillus spp*. are the mainly fungal causes (Morinaka, 2005). 7–15% of otitis media which caused by fungi, and the cure is frequently drawn-out and difficult (Anwar and Gohar, 2014). Warm, muggy weather, frequent swimming, eczema, overuse of cotton swabs, a small ear canal, allergies, chronic drainage, etc. are all risk factors (Viswanatha *et al*., 2012). Patients with impaired immune systems are more likely to develop fungal necrotizing otomycosis, the clinical signs and symptoms, such as swelling, skin redness, itching, debris, wetness, pain, and discharge, are frequently all that are used to make the diagnosis. (Tarazi *et al*., 2012).

Aspergillus genus is made up of widespread molds that can be found in soil, water, on plants, in excrement, on dead materials, suspended in the air, one of the most significant industrial filamentous fungi used in biotechnology is *Aspergillus niger* (Andersen *et al*., 2011). It is widely utilized to produce organic acids and extracellular enzymes (Pariza and Cook, 2010). To infect the host, pathogenic *Aspergillus* strains need virulence factors which aids in the establishment of infection, which later on may result in invasive or widespread infections (Sales *et al*., 2013).

Candida tropicalis is characterized with a round or oval vegetative cell with a diameter of 2 to 10 um, they produce single-celled blastoconidia and are dimorphic, they reproduce by budding unipolar or bipolar (Ellis *et al*., 2007). Regarding epidemiology and virulence, *Candida tropicalis* emerges as one of the most significant *Candida* species in this situation because it has been regarded as a powerful biofilm builder, capable of producing

genuine hyphae, and extremely adherent to epithelial and endothelial cells (Marcos-Zambrano *et al*., 2014).

Traditional methods used to identify otomycosis, such as microscopic examination, isolation and culture are based on the morphological characteristics of fungus, the characterization and identification of various fungal isolates up to a species level is made possible by molecular identification techniques based on ITS region fungal using DNA sequencing methods (Alsohaili and Bani-Hasan, 2018). Also, The sequencing of virulence genes of otomycoses is used to detect their role in the pathogenicity of otitis media (Monod *et al*., 2006).

This study was planned to achieve the following aim:

To detect the virulent genes of otomycosis isolated from immunocompromised in comparison with immunocompetent patients with otitis media.

To reach this aim, the steps of this study were:

1- Isolation and Identification of two genus *Aspergillus* and *Candida* from immunocompromised and immunocompetent patients with otitis media using cultural methods.
2- Molecular identification of ITS region for two genus *Aspergillus and Candida* isolated from immunocompromised and immunocompetent patients with otitis media using single-plex PCR and gene sequencing methods.
3- Detection of the substitution mutations in sequences of some virulence genes for isolating otomycosis from immunocompromised and immunocompetent patients with otitis media.