Diagnostic allele-specific PCR for the identification of Candida auris clades and common resistance mutations

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Candida auris (C. auris) is an opportunistic pathogenic yeast that emerged worldwide during the past decade. This fungal pathogen poses a significant public health threat due to common multidrug resistance (MDR), alarming hospital outbreaks, and frequent misidentification. Genomic analyses have identified five distinct clades that are linked to five geographic areas of origin and characterized by differences in several phenotypic traits such as virulence and drug resistance.

Typing of C. auris strains and the identification of clades can be a powerful tool in molecular epidemiology and might be of clinical importance by estimating outbreak and MDR potential. As C. auris has caused global outbreaks, including in low-income countries, typing C. auris strains quickly and inexpensively is highly valuable. We report five allele-specific multiplex polymerase chain reaction (AS-multiplex PCR) assays for the identification of C. auris and each of the five described clades of C. auris based on conserved mutations in the internal transcribed spacer (ITS) rDNA region and a clade-specific gene cluster. Additionally, we developed AS-PCR assays for the identification of SNPs in FKS1 and ERG11 that are commonly linked to echinocandin andazole resistance, respectively.

This PCR method provides a fast, cheap, sequencing-free diagnostic tool for the identification of C. auris, C. auris clades, and common resistance mutations.

High positive and rapid detection of clinical urine samples of fungal infection based on modified calcium fluorescence

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Successful management of urinary fungal infection depends upon the detection positive and efficient. The aim of this study was to evaluate the detection positive and efficiency of modified calcium fluorescence (m-CFP) staining to detect detection of Candida spp. in urine samples of patients with suspected fungal infection. We collected 130 clinical urine samples from different departments and analyzed the detection positive rate of the methods of culture, KOH sequence, and modified CFP. The results indicated that the positive rate of the methods was 32%, 8%, 14%, and 11%, respectively. The positive rate of modified CFP staining was significantly higher than that of ordinary microscopic examination and fungal culture (P < 0.05). Modified CFP in the detection of fungi in urine can significantly improve the positive rate of fungi in clinical urine samples and shorten the detection time. It has a certain reference value for clinical diagnosis and medication.
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Efficient and accurate diagnosis of otomycosis using an ensemble deep learning model
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Objective: Otomycosis accounts for ~15% of the cases with external otitis worldwide. And otomycosis is more frequently observed in humid regions and people enjoying the culture of ear cleaning in China. Aspergillus and Candida are the major pathogens that could cause long-term infection. Early endoscopic and microbiological examinations are important for appropriate medical treatment to otomycosis. However, accurate diagnosis always needs experts such as otologist and microbiologist. Deep-learning model is a novel efficient method to provide quick diagnosis which is an automatically diagnostic program using a large database of images acquired in the clinic. This paper put forward a mechanic learning model to address the diagnosis of otomycosis caused by Aspergillus and Candida accurately and quickly.

Method: We proposed a computer-aided diagnosis system that is based on a deep learning model consisting of two subnetworks, a meta-based web application, and picture classification. The web application subsystem mainly provides a user-friendly page for collecting consulted pictures as well as displaying the calculation results. The picture classification subsystem mainly uses trained neural network models for end-to-end data inference. The end-user only needs to upload a few pictures of the ear endoscope, and the system will return the classification results to the user in the form of category probability value.

In order to accurately diagnose otomycosis, we generally kept endoscopic images and took the occurrence for fungal culture for further identification. Positive fluorescence fungal staining, culture, and further DNA sequencing were taken to confirm the pathogens, Aspergillus or Candida. In addition, imprinted cerumen, external otitis, and normal external auditory canal endoscopic images are retained for reference. We merged these four types of images into an endoscopic image gallery.

Results: In order to achieve better accuracy and generalization ability after model training, we selected 2750 samples from nearly 4000 ear endoscopic images as training samples and 474 as validation samples. On the selection of deep neural network models, we tested the resnet, seer, and efficientnet neural network models with different numbers of layers. Considering the accuracy and operation speed, we finally chose the efficientnet-b6 model and output the probability values of the four categories of otomycosis, external otitis, impacted cerumen, and normal canal. After multiple iterative sample training, the overall validation sample accuracy reached 94.71%, and the average cross-validation accuracy of the 4 classifications reached 94.3%.

Conclusions: The results suggest that the system can be used as a reference for general practitioners to make better decisions in the diagnosis of otomycosis.

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Evaluation of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)-Bruker Biotyper Silus for identification of invasive molds
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Objective: Susceptibility to various antifungal drugs varies between different species and subspecies within the same genus. Phenotypic identification of fungi has limitations for species-level identification. Correct identification of species and subspecies in invasive mold infections is important to initiate the appropriate antifungal therapy. Matrix Assisted Laser Desorption Ionization Time-Of-Flight mass spectrometry (MALDI-TOF MS) with its proteomic analysis overcomes this limitation and helps in administering the correct antifungal therapy. A total of seven mold isolates from invasive fungal infections were evaluated for identification by MALDI-TOF MS and conventional morphological methods.

Method: Total of seven isolates from invasive mold infections were identified by the conventional method of culturing specimens on Sabouraud’s dextrose agar and Potato Dextrose agar with incubation at room temperature and 37°C in Biological oxygen demand (BOD) incubator. Micro-morphological identification of the fungus was done by Laser Photol Cotton Blue (LPCB) mount. Some isolates were processed on MALDI-TOF MS Bruker Biotyper Silus (Bruker Daltonik, Bremen-Germany) following recommended extraction protocol using ethyl alcohol, acetonitrile, and 90% formic acid.

Results: As per the below figure.

Conclusions: In four out of seven isolates phenotypic identification up to species level based on LPCB morphomycology was confirmed on MALDI-TOF MS. In the remaining three isolates we could only give a genus level identification based on LPCB mount. These three isolates were further identified up to the level of species after processing on MALDI-TOF as Aspergillus niger, Phaeoacremonium cinerum, and Alternaria sp. All mold isolates were identified with good quality mass spectra. In our experience, mold identification by MALDI-TOF MS using the Bruker Biotyper Silus platform definitely has an edge over conventional phenotypic methods in species-level differentiation of various molds, impacting targeted antifungal management.