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Efficient and accurate diagnosis of otomycosis using an ensemble deep learning model

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Objectives: 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 puts forward a mechanic learning model to address the diagnosis of otomycosis caused by *Aspergillus* and *Candida* accurately and quickly.

Methods: We proposed a computer-aided decision system that is based on a deep learning model consisting of two subsystems, a java-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 secretion for fungal culture for further identification. Positive fluorescence fungal staining, culture, and further DNA sequencing were taken to confirm the pathogens, *Aspergillus* or *Candida* sp. In addition, impacted cerumen, external otitis, and normal external auditory canal endoscopic images are retained for reference. We merged these four types of images into an endoscopic images 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 454 as validation samples. On the selection of deep neural network models, we tested the resnet, senet, 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 cases. 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%.

Conclusion: 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 Sirius for identification of invasive molds

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Objectives: 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 anti-fungal therapy. A total of seven mold isolates from invasive fungal infections were evaluated for identification by MALDI-TOF MS and conventional morphological methods.

Methods: 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 Lacto Phenol Cotton Blue (LPCB) mount. Same isolates were processed on MALDI-TOF MS Bruker Biotyper Sirius (Bruker Daltonics, Bremen Germany) following recommended extraction protocol using ethanol absolute, acetonitrile, and 70% formic acid.

Results: As per the below Figure.

Conclusion: In four out of seven isolates phenotypic identification upto species level based on LPCB micromorphology 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 upto the level of species after processing on MALDI as *Aspergillus tamarii*, *Phaeoacremonium cinerum*, and *Fusarium equiseti*. All mold isolates were identified with good quality mass spectra. In our experience, mold identification by MALDI-TOF MS using the Bruker Biotyper Sirius platform definitely has an edge over conventional phenotypic methods in species-level differentiation of various molds, impacting targeted antifungal management.

SR no	Sample	Clinical details	Colony Morphology	LPCB identification	MALDI identification/(Score)	Fungal markers/Sequencing results
1	Blood	46 year old male with vertebral osteomyelitis and bilateral endophthalmitis, in the post COVID-19 setting	Velvety smoky green reverse white	Septate hyaline,uniseriate phialides covering two third vesicle s/o Aspergillus fumigatus	Aspergillus fumigatus(2.21)	Serum BDG >523 pg/ml
2	Lung biopsy	11 year old male child with refractory ALL, with pneumonia	Velvety yellow green	Septate conidiophores biserate phialides covering most of vesicle, suggestive of Aspergillus flavus complex	Aspergillus tamarii(2.05)	ITS Sequencing =Aspergillus tamarii Serum BDG 207 pg/ml, Serum Galactomannan 5.63
3	Aspirate (Gluteal abscess	57 year old male with multiple myeloma presenting with a gluteal abscess	Compact/Glabrous/pale rose /reverse colourless	Septate delicate hyphae/erect phialide/ amero conidia clustered at tip, suggestive of Acremonium species	Phaeoacremonium cinerum(1.75)	Serum BDG >523 pg/ml Serum Galactomannan 0.142
4	Bronchoalveolar lavage(BAL)	77 year old male with pneumonia and synpneumonic pleural effusion	Velvety/green/reverse tan	Septate hyaline biserate phialides,covering upper half vesicle/Hulle cells seen, suggestive of Aspergillus nidulans	Aspergillus nidulans(1.80)	BAL Galactomannan 4.64
5	PUS aspirate	22 year old female with renal abscess	Suede like/Green radial rugosities with yellow and brown spots reverse white	Septate hyphae,biserate phialides, penicillium heads suggestive of Aspergillus versicolor	Aspergillus type versicolor(1.80)	---
6	Foot tissue	42 yrs male, swelling of foot, oozing sinuses h/o of foot injury	White/cottony spreading with shades of grey and pink/reverse light	Septate hyphae with sickle shaped macroconidia and small microconidia suggestive of Fusarium species	Fusarium equiseti(1.37)	---
7	Bronchoalveolar lavage	76 year old male with CLL, presenting with pneumonia	Cinnamon brown/velvety/reverse tan	Septate hyaline ,biserate phialides short conidiophores suggestive of Aspergillus terreus	Aspergillus terreus(1.70)	BAL Galactomannan 5.280

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qPCR is a useful tool for management and surveillance of hospital contacts in the context of a *Candida auris* infection

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Objectives: *Candida auris* is an emerging fungal pathogen responsible for hospital outbreaks. It represents a serious threat due to its drug resistance profile and its potential spread within healthcare facilities. Since the global alert by the CDC in 2016, specific control measures are now available to prevent the further spread of the pathogen. These measures should be implemented immediately as soon as a case is identified to prevent patient-to-patient transmission. Until recently culture was the main technique used for the detection of *C. auris* from patient and environmental samples. Nevertheless, PCR protocols have been reported and commercial kits are now available. Our objectives were to compare culture and PCR in routine for the management of a case of *C. auris* infection in a hospital setting.

Methods: We report here the case of a patient infected by *C. auris* following injuries in a public road traffic accident in Dubai. Following the medical evacuation and transfer of the patient to our hospital in Paris, *C. auris* was isolated from several surgical specimens from the elbow. Identification of the species level was initially performed by MALDI-TOF Mass spectrometry and confirmed by ITS sequencing. Antifungal susceptibility testing was performed by Etest and EUCAST. Surveillance of the index case included bi-weekly surveillance for 2 weeks and then once a week. Contacts were also screened for *C. auris* colonization once a week by swabbing axilla and groin. Samples were analyzed by standard mycological cultures and a specific *C. auris* qPCR kit (kit Fungiplex Candida auris®, Bruker).

Results: In total 133 samples were analyzed for the patient and 52 contacts. For the index case, 14/22 samples were positive in culture for *C. auris* including elbow biopsies, urine, and axilla, groin, and rectal swabs. Other *Candida* species (*C. albicans*, *C. krusei*) were also recovered from the same samples for the patient. For the contacts, all 111 samples were negative for *C. auris* by culture, but retrieved several other yeast species (*C. albicans*, *C. glabrata*, *C. kefyr*, *C. paraspsilosis*, *C. tropicalis*, *Saccharomyces cerevisiae*, *Trichosporon inkin, and Wickerhamomyces anomalus*). By using qPCR, all culture-positive samples were positive (Ct ranged from 29.7 to 38.0, with a median at 31.4). Two culture-negative samples (one biopsy and one axillary swab) were also qPCR-positive. All samples from contacts were negative by qPCR. The strain was resistant to fluconazole (>256 μg/ml) and susceptible to all other tested antifungals (amphotericin B, flucytosine, voriconazole, and caspofungin). Whole genome sequencing of the *C. auris* strain is in progress to determine the clade.

Discussion: The Fungiplex *C. auris* qPCR kit showed good sensitivity and specificity, even for the frequent situation of samples growing with two or three *Candida* species. These results highlight the usefulness of the PCR for surveillance of infected patients as well as for contacts.

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Galactomannan enzyme immunoassay in invasive pulmonary aspergillosis: a cross sectional study in 2021 in a New Delhi

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Objective: This study aims to determine the role of GM EIA in the diagnosis of IPA as per revised EORTC/MSG 2019 criteria.

Material and Methods: A prospective cross-sectional study was performed on 87 children (from 1 to 12 years) admitted to pediatric hematology ward from Oct 2020 to February 2022. Serum of these patients was collected and GM EIA was performed using BIO-RAD Platelia™ Aspergillus Ag. Clinical, mycological workup (potassium-hydroxide mount, fungal culture) was done and furthermore, these patients were classified into proven, probable, and possible IA as per EORTC-MSG guidelines, 2019. Galactomannan indices (GMI) measured in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were validated with revised EORTC -MSG, 2019 of IPA.

Results: A total of 53 patients out of 87 were categorized into probable IPA with routine mycological findings. On culture *Aspergillus flavus* was the most common pathogen, identified in 56% (25), followed by A. *fumigatus* 31.8% (14), A. *niger* 9% (4), etc. 44 (50.5%) had GMI value of >0.7, 9 (10.3%) in the range of 0.5-0.7 and 34 (39%) had GMI <0.5.

The maximal Youden index 'J' [95% confidence interval (CI) of 0.4167-0.6875] was calculated as 0.678 and sensitivity, specificity, PPV, and NPV in IPA were calculated corresponding to a GM index of >1.0, 0.7, 0.5 to determine the best cutoff point. The best cutoff was found to be 0.695 where in the 84.1% and specificity was 83.7% respectively.

Conclusion: GM EIA can prove as an excellent diagnostic test for IPA when done in addition to culture from nonsterile sites in high-risk populations like pediatric patients with hematological malignancy. This study reinforces the definition of the probable category of EORTC-MSG criteria, 2019 in overall diagnosis, prognosis, and management of IPA in pediatric patients with hematological malignancy.