P423

Recombinase polymerase amplification for Mucormycosis

Vinaykumar Hallur[1], Mukund Sable[2], Preetam C.[3], Supriya Sahu[1], Malati Tudu[1]
[1]Department of Microbiology, AIIMS Bhubaneswar, Sijua, Patrapada, India
[2]Department of Pathology & Laboratory Medicine, AIIMS Bhubaneswar, Sijua, Patrapada, India
[3]Department of ENT, AIIMS Bhubaneswar, Sijua, Patrapada, India

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: (1) To develop a Recombinase polymerase amplification (RPA)-based assay to diagnose mucormycosis. (2) To determine the analytical sensitivity and cross-reactivity of the developed assay. (3) To validate the assay in clinical samples. (4) To determine the turnaround time for the developed assay.

Methods: Multiple alignments of the ITS region of *Rhizopus arrhizus* were used to identify conserved sequences and design 30-32 bp long primers and 45 bp long Exo probes for RPA-based assay as per TwistDX guidelines (Cambridge, UK) using the PrimedRPA program (Higgins et al. 2019) and subject to in silico specificity check using NCBI primer blast. Genomic DNA and plasmid clone of the target region were spiked into human DNA and were used to determine analytical sensitivity. DNA from 35 clinically relevant fungi and bacteria were used to determine the cross-reactivity. A total of 40 sinus tissue samples from patients with microscopy and culture-confirmed mucormycosis and 40 sinus tissue samples from patients without mucormycosis were used to determine the diagnostic sensitivity and specificity of the assay. DNA from relevant fungi, bacteria and molds were extracted using the MPbio FastDNA kit as per the manufacturer's instructions with an elution volume of 100 μl. All extracted DNA samples were subjected to GAPDH PCR before RPA testing to rule out the presence of inhibitors as per established protocols. A 50 μl RPA reaction using Twistamp Exo kit as per manufacturer's instruction but using 10 μl template instead of 1 μl. The reactions were set up and inserted into a portable Qiagen ESE quant TS2.4 fluorometer incubated at 39°C and fluorescence was acquired every 30 sec over 20 min. The tubes were removed from the fluorometer mixed well and reinserted after 4 min as per kit instructions. Positive control and no template control were set up with each reaction. All relevant data were entered in Microsoft excel and diagnostic sensitivity and specificity were calculated using the Medcalc free online statistical software.

Results: A total of 1246 primer-probe combinations were designed and checked for in silico specificity using NCBI primer blast. Of these 1246 primer probes, 8 primers and 1 probe was finalized for *in vitro* testing to determine the best primer and probe set. The finalized primers had analytical sensitivity of 10pg and 10 copies and showed no cross-reactivity with any of the 35 clinically relevant fungi and bacteria. All extracted DNA showed amplified GAPDH gene demonstrating the absence of PCR inhibitors in extracted DNA. The sensitivity and specificity of the assay were 97.5% (95% CI: 86.8-99.9) and 97.6% (95% CI: 87.1-99.9). The turnaround time for RPA was 6 h.

Conclusion: Real-time RPA can be used to rapidly and reliably diagnose mucormycosis.

P424

Comparative analysis of Galactomannan Lateral Flow Assay, Galactomannan Enzyme Immunoassay and BAL culture for diagnosis of COVID-19-associated pulmonary aspergillosis

Mohammad Hedayati[1], Mona Ghazanfari[1], Jamshid Yazdani Charati[1], Lotfollah Davoodi[1], Amir Arastehfar[2], Maryam Moazeni[1], Mahdi Abastabar[1], Iman Haghani[1], Sabah Mayahi[1], Martin Hoenigl[3], Weihua Pan[4]
[1]Mazandaran University of Medical Sciences, Sari, Iran
[2]Center for Discovery and Innovation, Hackensack Meridian Health, NJ, USA
[3]Division of Infectious Diseases and Global Public Health, Department of Medicine, University of California, San Diego, CA 92093, USA
[4]Medical Mycology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: Invasive pulmonary aspergillosis is a well-known complication in COVID-19 patients. A reliable and early diagnosis is essential for the early initiation of antifungal therapy. On the other hand, conventional Bronchoalveolar lavage (BAL) culture may lack the specificity of differentiating between colonization and infection. Here, we aimed a comparative analysis of Galactomannan Lateral Flow Assay (GM-LFA), Galactomannan Enzyme Immunoassay (GM-EIA), and BAL culture for diagnosis of COVID-19-associated pulmonary aspergillosis (CAPA).

Methods: BAL ($n = 105$) and serum samples ($n = 101$) from COVID-19 patients who were mechanically ventilated for $\geq$4 days in intensive care units (ICUs) were evaluated by GM-LFA and GM-EIA. All BAL samples were cultured on Sabouraud-Chloramphenicol dextrose Agar. Diagnostic performance of GM-LFA and GM-EIA in BAL (GM indexes $\geq$1) and serum (GM indexes >0.5) were evaluated and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and Roc curve compared with BAL culture calculated.

Results: The *Aspergillus* GM-LFA for serum and BAL samples showed a sensitivity of 46.9% and 54.5%, specificity of 100% and 91.7%, PPV of 100% and 75%, and NPV of 80.2% and 81.5%, when compared with BAL culture, respectively. GM-EIA showed sensitivities of 56.3% and 60.6%, specificities of 94.2% and 88.9%, PPVs of 81.8% and 71.4%, and NPVs of 82.3% and 83.1% for serum and BAL samples, respectively.

Conclusion: According to our results BAL GM detection using both EIA and LFA may be a promising approach for early diagnosis of CAPA and differentiating between colonization and invasive infection.

P425

Approach to identify and isolate *C. dubliniensis* in laboratory and comparison of various methods for same

Abiroo Jan
GMC Anantnag, Anantnag, India

Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Introduction: *C. dubliniensis*, a newly recognized fungal pathogen, is clinically significant as it develops a stable fluconazole-resistant phenotype on exposure to this antifungal in vitro. *C. dubliniensis* is overlooked and misidentified as it shares many phenotypic traits with *C. albicans*. Molecular methods developed to distinguish the two species are not readily applicable. So, an easy-to-perform and an accurate phenotypic test would be a valuable tool.

Aim: This study first of its kind from Kashmir Valley (conducted in SKIMS-1200 bedded tertiary care hospital) evaluated seven phenotypic tests with PCR-RFLP as a gold standard. The best methodology for routine identification of this emerging pathogen was developed.

Materials: A total of 206 *Candida* spp. were tested which included 186 in-house *C. albicans* strains and 20 *C. dubliniensis* strains. The in-house 186 *C. albicans* strains were isolated from cancer patients. A total of 500 patients with various malignancies were screened, patients on antifungal drugs and screened once were excluded. All strains were sensitive to both fluconazole and voriconazole. Among 22°C *dubliniensis* strains, 14 were provided by Dr. Ziauddin Khan (Professor and Chairman, Department of Microbiology, Kuwait University) and 6 by VPCI institute Delhi.

Reference strains included in the study:

C. albicans 90028; (National culture collection of pathogenic fungi (NCCPF), Department of Medical Microbiology PGIMER Chandigarh)

C. dubliniensis (CD36) and (CBS 7987); provided by Dr. Ziauddin Khan

Methods: PCR-RFLP using BlnI (AvrII) enzyme was done to confirm the identification of all isolates (Fig. 1). Seven phenotypic tests that were evaluated include Growth on hypertonic Sabouraud Dextrose Agar (SDA), Colony color on Crome *Candida* Differential Agar, Growth at 45°C, Assimilation of xylose (XYL), Colony color and Chlamydospore formation on Tobacco Agar, Germ tube formation at 39°C and Fluorescence on methyl blue SDA. The variety of tests applied makes our study one of the most detailed studies for the identification of *C. dubliniensis* till date.

Results: No *C. dubliniensis* was found among the isolates recovered from cancer patients by PCR-RFLP.

Phenotypic tests that showed the best results were

1. Growth on Hypertonic SDA
2. Colony color on Crome *Candida* Differential Agar
3. Relative growth at 45°C.
4. Assimilation of xylose (XAM as well as DISCs)

Germ tube formation at 39°C was most rapid. Phenotypic methods proved to be very cost-effective than PCR-RFLP. Based on our results of growth on Hypertonic SDA and XAM and also knowing the fact that 5% of *C. dubliniensis* assimilate xylose and give false results of xylose assimilation, we developed a novel medium 'Hypertonic xylose agar medium' (HXAM). HXAM proved to be 100% accurate, simple, easy to perform, and cost-effective.

Conclusion: For routine identification of *C. dubliniensis* we recommend colony color on Crome Agar and growth on HXAM as they are simple, reliable, inexpensive, reproducible, and readily applicable methods. For retrospective evaluation of stored cultures, we recommend HXAM.

Based on our observations, we propose a scheme for the identification of *C. dubliniensis* (Fig. 2).

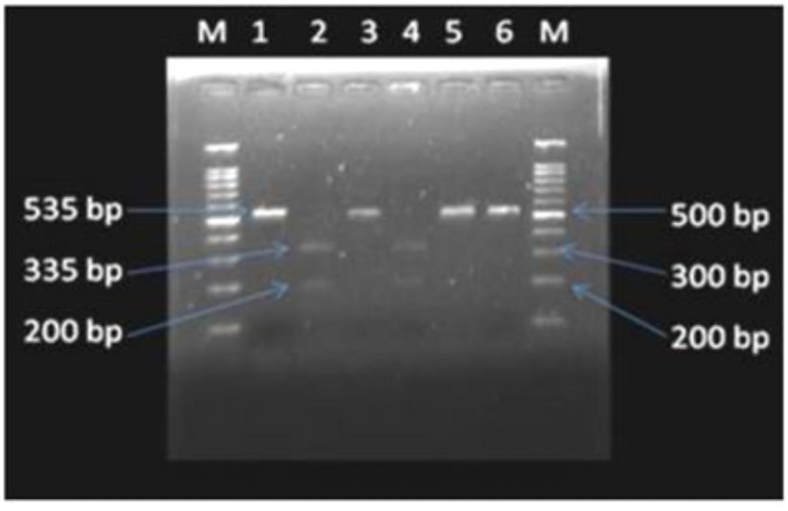

Figure 1. Refrence strains. Lane M: 100 bp DNA ladder. Lanes 1, 3: PCR products of *C. dubliniensis*, Lanes 2, 4: RFLP products of *C. dubliniensis*, Lanes 5: PCR product *C. albicans*, Lanes 6: RFLP product of *C. albicans*.