O219
Oral Session
Advances in molecular mycology: notes from the bench

CHARACTERIZATION OF A PCR/ESI-MS ASSAY FOR DETECTION AND IDENTIFICATION OF DIVERSE FUNGI IN BRONCHOALVEOLAR LAVAGE SPECIMENS

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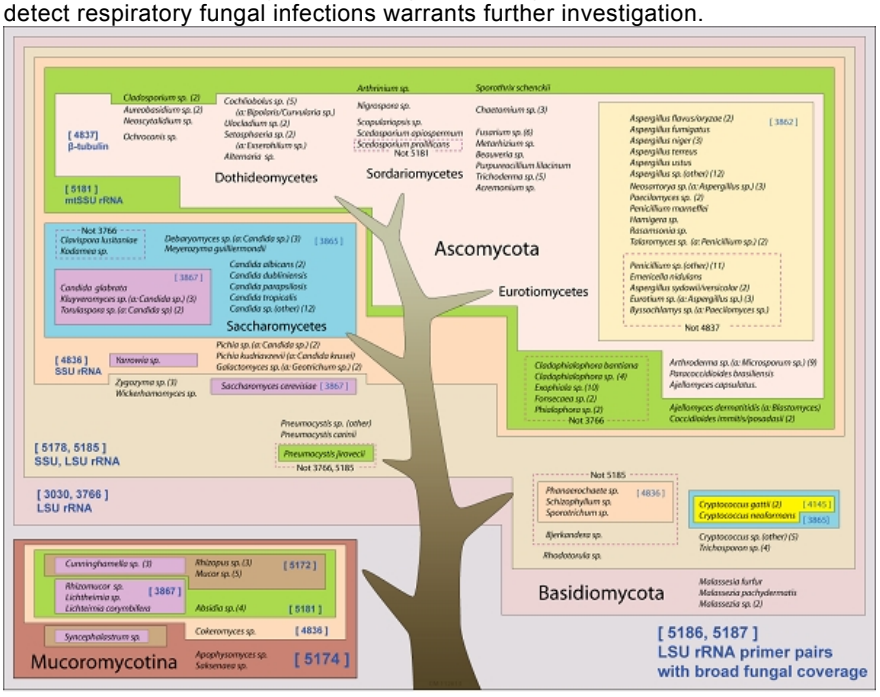
Objective: Respiratory fungal infections are a significant cause of morbidity and mortality among immunocompromised patients. Early and accurate identification of fungal pathogens is central to patient management and improved overall outcome. PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) was evaluated as a novel means for identification of fungal pathogens from bronchoalveolar lavage (BAL) specimens. A broad assay currently in development (not commercially available) to identify common fungal agents, including Ascomycota (e.g. *Aspergillus* spp., *Candida* spp. and *Pneumocystis*), Basidiomycota (e.g. *Cryptococcus* spp.) and Mucorales (e.g. *Mucor* spp. and *Rhizopus* spp.), in a single assay within 6 h was evaluated. By using amplicon base composition as a unique molecular signature ('fingerprint'), the assay is able to identify single or multiple organisms in direct patient specimens without the need for culture.

Methods/Study Design: Fungal nucleic acids were isolated through a combination of bead-beating, chemical lysis and magnetic particle capture. An assay that targets sequences conserved across diverse fungi was used to amplify isolated fungal DNA. PCR amplicons were analyzed using ESI-MS, followed by comparison of signatures to a reference database for identification. The limits of detection (LODs) of the assay were measured by spiking quantified fungal stocks into saline. 75 BAL specimens obtained from consented patients were de-identified and tested by the assay. The results were compared to culture results and to the final clinical diagnosis.

Results: The LODs of the assay were measured to be between 1.25 and 80 CFU/ml, with a median LOD of 5 CFU/ml and an average LOD of 22.8 CFU/ml for 17 fungi. The assay had a sensitivity of 74% and a specificity of 71% compared to culture detection. The accuracy of identification of the assay was 68% when compared to the identifications made by culture in specimens from patients with a high probability (pre-microbiology) of a fungal infection. When the detection results of the assay and culture for a given patient specimen were compared to the final clinical diagnosis, the PCR/ESI-MS assay had a higher sensitivity (78% vs. 68%) and similar specificity to culture (64% vs. 67%).

Conclusion: The PCR/ESI-MS broad fungal assay described here is capable of rapidly and reliably detecting and identifying a variety of fungi from patient specimens in less than 6 hours, without the need to grow organisms in culture and without the need to have a priori knowledge of what organisms might be present. The potential to provide the clinician with a means to rapidly detect respiratory fungal infections warrants further investigation.