Candida DNA extraction from positive blood culture bottles

Uthra Balakumar, Anitha Subramaniam, Anupama Jyoti Kino
Sri Ramaswami Institute of Higher Education and Research, Chennai, India

Poster session 5, September 23, 2022, 12:30 PM - 1:30 PM

Background: Molecular technique of detecting candida from blood is a quick method to identify candida. A major limitation of the molecular method is the difficulty associated with breaking fungal cell wall, the DNA extraction step still requires more than half of a working day.

The successful extraction of DNA involves effective disruption of cells, denaturation of protein, and nucleoprotein complexes, and inactivation of nucleases such as DNases. The extracted DNA should have low contamination of proteins.

Objectives:
1. To perform DNA extraction using phenol-chloroform method.
2. To speculate candida using PCR/RFLP.

Methods: Candida DNA is isolated using phenol-chloroform method. The PCR products of different candida species obtained directly from blood samples were subjected to restriction enzyme (MspI).

Results: DNA was extracted from 50 samples, out of which 40 samples were from known positive blood culture bottles and the remaining 10 were known negatives for candida. Of the 40 known positive blood culture bottles, 34 produced bands of various molecular weights and all 10 negative cultures did not show bands. Representative amplification is shown in the gel picture (Figure 1).

Out of 40 candida-positive blood culture samples, 7 species have been identified using PCR-RFLP method.

Conclusion: The turn-around time can be highly reduced by extracting DNA from positive blood culture bottles and then performing PCR and RFLP for speciation compared with the conventional method.

Figure 1, Gel picture.

Pneumocystis jirovecii pneumonia: Clinical profile and diagnostic modalities

P Bansidhar Tarai
Max Hospital, Delhi, India

Poster session 5, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: In developing countries like India, the diagnosis of Pneumocystis jirovecii infection is often made either by conventional staining or clinically. This study was planned to know the utility of polymerase chain reaction (PCR) in diagnosing P. jirovecii pneumonia (PJP), to compare the PCR results with that of the staining technique, and also to correlate the results with the radiological condition of patients from a tertiary care center in India.

Method: A retrospective study included 3100 adult in-patients with symptoms of lower respiratory tract infections. Sputum and bronchoalveolar lavage samples were processed for both staining and PCR for multicopy nucleolar organellar (nu) genes and single copy DHPS (fe) gene of P. jiroveci.

Results: The present study was carried out in the Department of Microbiology, Max Hospital, Saket, New Delhi, India between the years 2019 and 2022, the patients with clinically suspected pneumocystis pneumonia who attended outpatient or admitted to the Department of Medicine, Gastroenterology, and Pulmonology, etc. of our hospital were retrospectively analyzed. The details of these patients were analyzed as per a well-structured protocol that included the detailed clinical history and laboratory data from the hospital records. A total of 3100 patients were included in the present study. Of these 3100 patients, 1,424, (45.84%) patients of pneumocystis pneumonia were confirmed, based on positivity by at least any two of the above-mentioned techniques. The age of the patients ranged from 29 to 80 years (mean 52 years). Among these 3100 patients, 54 (1.73%) were males and 16 (52%) were females and the male-to-female ratio was 2:1. A total of 10 patients had human immunodeficiency virus (HIV) infection, while the other 23 were HIV negative. The immune status of five patients was unknown. Fever and dyspnea (n = 44, 88%), non-productive cough and abnormal auscultation sound (n = 30, 60%), and also chilli and weight loss (n = 10, 20%) were the documented clinical symptoms of PJP. Radiological diagnosis of Pneumocystis pneumonia was noticed in 54 cases. Foamy alveolar casts were present in 19 cases. Silvery mottled opacities spread in the rounded, helmet, or cleft forms of opacities. Inflammatory infiltrate was mainly polymorphonuclear. All the cases were confirmed as P. jiroveci by PCR amplification.

Conclusion: Clinicians often face the diagnostic dilemmas presented in the study. Individual modalities of the diagnosis are available, but all have drawbacks with varied sensitivity and specificity. Combining the available clinical, radiological, and microbiological modalities to reach early diagnosis can go a long way to avoid undiagnosed and unnecessary delays in treatment.