CHAPTER 34

MEDICAL SCIENCES
MICROBIOLOGY

Doctoral Theses

01. GIRI (Astha)
Characterization of Genotypic Indicators of Ethambutol Resistance in Clinical Isolate of Mycobacterium Tuberculosis
Supervisor : Prof. Mandira Varma Basil
Th 24127

Abstract
(Not Verified)

Drug resistance against Ethambutol is largely attributed to mutations in embCAB genes. The predominant mutation embB Met306Ile/Val cannot fully account for EMB resistance or predict the EMB MIC. Furthermore, 35-40% of EMB resistant clinical isolates have no mutations in embB. Lately, the importance of efflux pumps, as an alternate mechanism of drug resistance has been recognised. The present study included the collection, identification (by acid-fast staining, biochemical testing, PCR restriction analysis) and drug resistance determination (by Proportion Drug Susceptibility Testing) of 28 EMB resistant and 29 EMB susceptible clinical isolates which were further tested for MIC by MABA assay and Agar dilution. Polymorphisms in nine genes, i.e. dprE1, dprE2, ubiA, aftA, aftB, aftC, embA, embB, embC of the arabinogalactanlipoarabinomannan synthesis pathway were studied. The polymorphism analysis identified two significant polymorphisms w.r.t EMB resistance, i.e. embB Met306Val and a novel mutation aftB Asp397Gly. The novel mutation Asp397Gly upon overexpression in M. tuberculosis H37Rv failed to have any effect on the MIC and was thus not considered to be associated with EMB resistance. To study EMB resistance in a holistic manner, upregulation of efflux pumps in response to EMB stress was studied, which showed that drrABC, jefA, Rv1686c, Rv1687 and Rv0842 were upregulated in EMB resistant isolates only, while the efflux pump Rv0876c was upregulated only in the EMB susceptible isolates. Efflux pumps were found to be up-regulated in HLR strains (carrying canonical mutations in embB) in a linear pattern i.e. an increase in MIC was accompanied with increase in the number of efflux genes upregulated. To conclude, embB306 remains the predominant polymorphism for EMB resistance. Efflux pumps may either act individually to prime clinical isolates to acquire drug resistance or act synergistically with mutations to raise the level of resistance in clinical isolates of Tuberculosis.

Contents

1. Introduction 2. Review of literature 3. To study the inhibitory concentration (MIC) of ethambutol in drug resistant and drug susceptible isolates of M.Tuberculosis 4. To determine the frequency oif mutations in the genes involved in the arabinogalactan liporabinomannan biosynthesis pathway in ethambutol resistant an susceptible isolates of M. Tuberculosis 5. To study the effect of a panel of identified mutations in genes of the arabinogalactan lipoarabinomannan biosynthesis pathway on the MIC of ethambutol in the laboratory strain H37Rv by an overexpression approach 6. To explore the role of efflux pumps in conferring resistance towards ethambutol in clinical isolates anM.Tuberculosis. Summary and Conclusion References, Appendices.

02. INDU BALA

Culture Independent Diagnosis of Mycoplasma Pneumoniae by Using Nucleic Acid Amplification Techniques in Pediatric Lower Respiratory Tract Infections

Supervisor : Dr. Surinder Kumar

Th 24123

Abstract
(Not Verified)

INTRODUCTION: *Mycoplasma pneumoniae* (*M.pneumoniae*) is a major cause of lower respiratory tract infections (LRTIs) causing up to 40% or more cases of community-acquired pneumonias. The detection of antibodies in paired serum samples has been considered the standard laboratory diagnostic method but polymerase chain reaction (PCR) has been found to be useful for rapidly detecting these pathogens in respiratory secretions. Objectives: The aim of the study was culture independent diagnosis of *M.pneumoniae*infections by nucleic acid amplification techniques in pediatric lower respiratory tract infections.Methods: A total of 245 patients 6 months to 12 yrs of age were investigated for LRTIs. Serum specimens were obtained on admission for detection of antibodies to *M. pneumoniae.* Throat swab samples were obtained for detection of *M. pneumoniae* by nucleic acid amplification techniques such as PCR, nested PCR and reverse transcription PCR (RT-PCR).Results & Conclusions*: M. pneumoniae* infection was positive in 76(31%) and negative in 169(68.9%) children. The high prevalence of positive cases were in 5-9 years age group and was statistically significant ($P \le 0.01$). There was no significant association between sex of the patient and incidence of *M.pneumoniae* infection ($P = 0.53$). Presence of dry cough, wheezing and chest pain had statistically significant association with *M. pneumoniae* infection ($P = 0.034$; $P = 0.046$; $P \le 0.01$) respectively. Radiological profile across *M.pneumoniae*positive and negative cases were comparable. Serology, PCR, nested PCR and RT-PCR together were positive in 76(31%) patients. Our data underline the importance of *M. pneumoniae* infection in Indian children particularly in >5 yrs of age. Serology, PCR, nested PCR and RT-PCR in combination are useful for fast, accurate and reliable diagnosis of *M.pneumoniae*in pediatric lower respiratory tract infections.

Contents

03. SINGH (Pradeep Kumar)

Phenotypic and Molecular Characterization, Antifungal Susceptibility Profiles and Clinical Significance of Basidiomycetes Molds Occurring in Patients with Respiratory Disorders.

Supervisors : Prof. Anuradha Chawdhary, Prof. H. S. Randhawa and Prof. S. N. Gaur

Th24122

Abstract
(Not Verified)

Filamentous basidiomycetes have recently emerged as significant pathogens that incite a wide array of clinical manifestations as well as invasive and noninvasive diseases. Definite identification based on conventional methods may be problematic; therefore, sequencing of internal transcribed spacer (ITS) region of small ribosomal subunit and D1/D2 region of large ribosomal subunit are used for the identification. The accurate identification and antifungal susceptibility testing of these etiologic agents is critically important, despite the low frequency of some species that are encountered in clinical specimens. The present study aims to characterize non-sporulatingmoulds (NSM) from respiratory specimens using both phenotypic and molecular methods. Further, clinical significance and antifungal susceptibility profiles of NSMs obtained from patients with varied pulmonary mycoses were studied. Role of basidiomycetousmoulds isolated from clinical specimens of patients with respiratory disorders such as allergic bronchopulmonary mycosis, fungal ball, fungal pneumonia and allergic fungal sinusitis during a 5-year study 2013-2017 was assessed along with therapy and outcome. Basidiomycetousmoulds were identified using ITS and D1/D2 sequencing and phylogenetic analysis of all the basidiomycetousmoulds identified in the study was done using MEGA version6 software. Additionally, species diversity among the *Schizophyllum commune* isolates was studied using multi gene phylogeny. *In vitro* antifungal susceptibility profiles of molecularly confirmed filamentous basidiomycetes isolated from clinical sources were determined against triazoles, amphotericin B, 5- flucytocine, and echinocandins. Role of many other pathogenic filamentous fungi than filamentous basidiomycetes causing respiratory disorders encountered in the study were also analyzed. The study also highlights the importance of antifungal susceptibility testing for treatment and management of basidiomycetous infections and molecular techniques for unambiguous identification of fungi. Finally, patients with chronic pulmonary diseases, for whom basidiomycetes are isolated, should be thoroughly evaluated to rule out an allergic or invasive disease, and the basidiomycetes should not just be ignored as possible contaminants.

Contents

1. A review of filamentous basidiomycetes as agents of human disease 2. Role of basidiomycetousmoulds occurring in patients with respiratory disorders 3. Modular characterization of filamentous basidiomycetes by ITS and D1/D2 sequencing 4. In viro antifungal susceptibility testing filamentous basidiomycetes moulds from clinical sources 5. Other significant pathogenic fungi encountered in the study. Overall summary and conclusion.Appendices.