Pulmonary invasive aspergillosis is difficult for many hours and they are inhaled by humans [6]. The spores are released in large numbers ambient air, remain airborne aspergillosis (CPA) while having retreatment for TB [6]. The prevalence of disease is the inefficiency of immune system and the population and use of treatment modalities that prolong the survival are becoming more frequent because of expansion of high risk opportunistic infections have increased tremendously due to increase in cases of secondary aspergilloma [4]. The reason for increased prevalence of disease is the inefficiency of immune system and the use of Antituberculosis Treatment (ATT), which promote growth and reproduction of fungal flora and in turn aggravate the underlying pathology [5]. Invasive aspergillosis infections may manifest in several different forms, but more common in bronchopneumonia [4]. The opportunistic fungus cause disease in immunocompromised patients with pre-existing disease and with long history of antibiotics. In the world more than a million people develop Chronic Pulmonary Aspergillosis (CPA) while having retreatment for TB [6]. The spores are released in large numbers ambient air, remain airborne for many hours and they are inhaled by humans [6]. Aspergillus finds a comfortable abode in damaged or scarred pulmonary tissue like pre existing cavities or bronchietatic areas. Mycelia grows inside the cavity and forms ball like mass, without invading into viable tissue or blood vessels, the predisposing cause may range from TB, sarcoidosis, cavities in rheumatoid lung etc [7]. The pathologic reaction in human beings varies from simple colonization, allergic alveolitis, aspergilloma and invasive aspergillosis [8-10]. Repeated exposure of Aspergillus spore can precipitate and aggravate this immune mediated bronchial pathology and can manifest as asthmatic episodes [10]. The present study was carried out to assess the prevalence of invasive aspergillosis among (PTB). Culture based diagnosis of aspergillosis infection is time consuming and often has diagnostic sensitivity. The Galactomannan assay is to detect pathogens from Aspergillus genus but is susceptible to false type of TB as per Revised National TB Control Programme protocol. 20 patients without TB were taken as control group and sputum samples were collected. Direct smear was done with 10% potassium hydroxide, Lacto Phenol Cotton Blue (LPCB) mount for identifying the fungus and culture on Saburoudes dextrose agar. All samples were processed and identified according to standard protocol. Growth was confirmed by repeated isolation for atleast 3 times. The species level identification was done. All Aspergillus were taken up for PCR. Universal primer and spices specific primer were used. After amplifications the amplicons were visualized on 1.5 % agarose gel for presence of band and the gels were scanned under UV illumination, visualized, and digitized with the Gel Doc documentation system.

RESULTS
Out of 80 (PTB) sputum collected only 26 (32.50%) isolates were grown from 24 patients. All 24 patients showed smear and culture positive. Smear and culture were negative for control group. Universal primer and spices specific primer were used. After amplifications the amplicons were visualized on 1.5 % agarose gel for presence of band and the gels were scanned under UV illumination, visualized, and digitized with the Gel Doc documentation system.

DISCUSSION
The prevalence of PTB with fungal co-infection is well documented in late 1960s. Treated PTB can lead to progressive loss of pulmonary function and chronic Aspergillosis [11]. CPA is a sequelae and is a
differential diagnosis of smear negative PTB after successful ATT [12]. In our present study the percentage of mycotic infection in (PTB) patients was 30.8%, which is comparable with the study of Sunita and Mahendra [3]. Tejal Patel et al., [13] found that Aspergillus is the most common invasive fungal infection in humans. Our study is concordant with the above study that Aspergillus is common isolates. Sunita and Mahendra [3] isolated 46% of Aspergillus from TB patients. In our study we had isolated 30.7% of Aspergillus. Current diagnostic methods such as culture, biopsy and serology lack rapidity and efficiency. PCR has potential to improve fungal diagnosis as it is faster, more sensitive and specific [14]. Guizhen Luo et al., [15] did a multiplex PCR evaluation of fungal colonies and it provided 100% sensitivity. In their study they could amplify only one half of molds directly from mycelia fragments. In our study, among the 8 Aspergillus species, only 2 Aspergillus fumigatus were amplified and in addition we describe the use of whole hyphal cells as template for PCR. Omission of DNA extraction procedure significantly decreases the time required to make an accurate identification by PCR. In our study also only 2 Aspergillus fumigatus were amplified from mycelial fragments. Diagnosis using culture is time consuming and limitations in diagnostic sensitivity. Serology like β-D gluco is non-specific because it is found in many fungal cell walls such as Fusarium, Acremonium species [16]. For diagnosing invasive aspergillosis Galactomannan assay has a sensitivity of 71% and specificity of 80% [17]. False positive and false negative values are common in Galactomannan assay because of certain antibiotics, histoplasma fungal infection and even certain foods can increase values of galactomannan assay [18] PCR can be used as a tool to detect invasive aspergillosis. Lack of standardization has limited its acceptance as a diagnostic tool and preventing its inclusions in disease forming criteria [19].

CONCLUSION
Diagnosis of opportunistic respiratory fungal infections poses a difficult diagnostic challenge due to lack of any pathognomonic clinical syndromes. Our opinion is to suspect fungal infection in all sputum positive patients. Fungal screening is highly recommended as routine investigation in microbiology for all cases of TB for aspergillosis to start antifungal therapy at the right time. PCR is a useful tool to diagnose the invasive aspergillosis and can guide for antifungal therapy.

ABBREVIATION
ATT-Antituberculosis treatment, PTB- (PTB), PCR- Polymerase chain reaction, IA-Invasive aspergillosis

REFERENCES

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