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Application of nested multiplex PCR to detect Mycobacterium tuberculosis DNA from clinical samples

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Tuberculosis remains a major public health problem worldwide with a global mortality of 1.5 million in 2014. Though early diagnosis is mandatory in control of TB especially for pulmonary TB as it is transmissible, it has remained enigmatic. A major hindrance to the diagnosis of EPTB is the atypical presentation, often simulating neoplasia and or inflammatory disorders. The conventional techniques used in TB diagnosis like AFB smear microscopy lack sensitivity and the gold standard, culture test takes time. The sensitivity and specificity were compared with AFB smear examination, Lowenstein-Jensen culture test and single step PCR. In order to find a sensitive and rapid technique nested multiplex PCR (nMPCR) targeting the IS6110 and MTP40 gene of *Mycobacterium tuberculosis* was evaluated for detection of *M. tuberculosis* DNA directly from clinical specimens of pulmonary and extra-pulmonary origin. A total of 200 clinical specimens from clinically suspected cases of extra-pulmonary tuberculosis and 20 control specimens of non-tuberculous aetiology were processed by smear, culture, single step and by nested multiplex PCR technique for detection of *M. tuberculosis*. The conventional culture was positive only in 150 (75%) of 200 specimens and 162 (81%) were single step PCR positive. The overall positivity of nested multiplex PCR was 100% (200/200). All the 20 control specimens were negative by nested multiplex PCR. Nested multiplex PCR increased the sensitivity of PCR and will be useful in diagnosing smear negative samples. Further, it can also be used to detect samples with *M. tuberculosis* strains lacking IS6110.

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Microbial involvement in cause and treatment of Alzheimer's disease

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A complex ecosystem formed by the microorganisms that reside within human organs is gastrointestinal tract, urogenital tract, skin and nasal and oral mucosa. Albeit, largest pool of microbiome in the human body is gastrointestinal tract that comprising 99% anaerobic bacteria and remaining are fungi, protozoan and archaebacteria. The ratio of prokaryotic microbial cells within the human body to eukaryotic human cells is 100:1 and gene ratio is 150:1. Recently, it has been identified that Microbiome resides in human predict four major division of bacterium resides in GI tract namely actinobacteria (3%), bacteroidetes (23%), firmicutes (64%) and proteobacteria (8%). Remainder 2% consists of diverse minor taxonomic division. Further, such kind of microbial cells has been found to involve in the progression of neurodegenerative disorders. Alzheimer's disease is one of the most lethal disorders which befalls several damages in the brain due to accumulation of toxic Amyloid Beta (Aβ), directed by mainly dementia, cognitive disabilities and tauopathy. Interestingly, amyloid is secreted by various species of microbiome including bacteria and fungi. Blood examination of AD patients has also revealed through the presence of disperse Mycoses and amyloidogenic fungal protein which was found to link with increased risk of AD due to chronic fungal infection. Molecular mimicry is another factor underlying mechanism for neurodegeneration through bacterial amyloid. In spite of this, many plant and animal viruses are also associated with molecular mimicry and altered protein expression in AD. Based on this ground, we demonstrated the microbial source of amyloid causing AD, illustrated underlying mechanism of AD due to microbial amyloid, elucidated the amyloid protein interaction with other proteins and finally, analyzed the microbial product for AD treatment using *in silico* techniques.

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