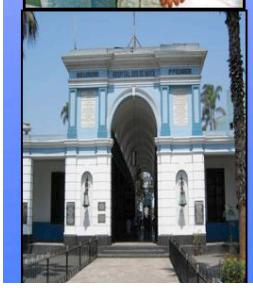

14:30 Session 2: TB Diagnosis
Chair: Bob Gilman

14:30 Proteomic fingerprinting for infectious diseases – *Dan Agranoff*

14:45 Proteomics in TB – *Gurj Sandhu*

15:00 Genetic and molecular correlates of pyrazinamide resistance – *Paty Sheen*

15:15 Unravelling the enigmatic mycobacterial pyrazinamidase – *Mirko Zimic*

15:30 Rapid and sensitive Tuberculosis diagnosis and susceptibility testing without decontamination or centrifugation - *Louis Grandjean*

15:45 Stool testing to diagnose pulmonary TB in adults and children - *Laura Martin*

Genetic and molecular correlates of pyrazinamide resistance

Patricia Sheen, Mirko Zimic, Robert H.Gilman, Melissa Mendez, Carlton A Evans

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Pyrazinamide, isoniazid and rifampicin are the most effective drugs for treating tuberculosis in humans. The addition of pyrazinamide to tuberculosis chemotherapy allows the duration of treatment to be shortened from 9 to 6 months, as benefit that does not apply to tuberculosis that is resistant to pyrazinamide. However, the diagnosis of pyrazinamide resistance is difficult because pyrazinamide is only active in acid media, which is inhibitory to mycobacterial culture causing test failure. In contrast, molecular diagnosis of pyrazinamide resistance does not require culture and therefore may be more reliable. The pncA gene encodes pyrazinamidase, the enzyme that hydrolyzes the pro-drug pyrazinamide to its active form and mutations in the pncA gene are the cause of pyrazinamide resistance . Approximately 96% of reported mutations in the pncA gene have been demonstrated to cause pyrazinamide resistance. We analyzed 186 clinical *M. tuberculosis* strains, 106 of which had mutations in the pncA gene. The unique mutations were: 23 missense; 3 nonsense; 5 insertions; and 4 deletions in the pncA gene. To facilitate clinical application of this finding we optimized the Single Strand Conformational Polymorphism (SSCP) test so that it correctly characterized 96 of 106 mutated strains (sensitivity 92%) and all of 81 non-mutated strains (specificity 100%), reliably determining pyrazinamide resistance directly from sputum samples within 24 hours in most cases. This new test was sufficiently rapid to influence initial tuberculosis therapy in newly diagnosed tuberculosis patients.
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