

insurance reimbursement from total cost was 8.8%, the proportion of TB control project reimbursement from total cost was 33.8%, out-of-pocket payment was account for 44.8% of total cost and 5.8% of disposable household income in 2012. But there was still about 6.5% of PTB reached catastrophic health expenditure (>40% of disposable income).

**Conclusion:** The direct medical costs of whole course of treatment for PTB patients in Shanghai were relatively high, especially the costs of hepatoprotectants, liver function laboratory test and CT examination. Current TB control project reduced the economic burden of PTB patients in a certain extent, but the item and proportion of reimbursement, and the procedure of the cost reduction still need to be optimized.

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#### Confirmation of silent mutations in the *rpoB* gene locus of *M. tuberculosis* isolates using pyrosequencing and phenotypic DST



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**Background:** More than 95% cases of rifampicin resistance in *M.tuberculosis* strains can be attributed to mutations in 81-bp hotspot region of *rpoB* gene locus called Rifampicin Resistance Determining Region. These mutations can be detected by rapid molecular diagnostic techniques such as Line Probe Assay (LPA), but detection of silent mutations on LPA may not necessarily confer rifampicin resistance and result in false rifampicin resistance reporting. These silent mutations can be confirmed by phenotypic Drug Sensitivity Testing (DST) and/or by DNA sequencing.

Following study was undertaken to confirm silent mutations using phenotypic DST (Gold standard) and pyrosequencing, a rapid real time method for sequencing small DNA segments by synthesis.

**Methods & Materials:** Total 300 DNA extracts comprising of 101 silent mutation strains; 101 pan-sensitive, 96 MDR-TB and 02 rifampicin mono-resistant strains were processed for pyrosequencing following detection by LPA.

Pyrosequencing was performed with sequence analysis mode of PyroMark Q96 ID system (Qiagen, Valencia, CA).

All 300 sputum concentrates were also processed for phenotypic DST, using solid and liquid culture methods.

**Results:** Pyrosequencing detected mutations in all 101 silent mutation strains, but 02 strains did not produce amino acid change (true silent mutations) and were also rifampicin sensitive phenotypically. Maximum mutations were seen in 526 codon(35 strains) followed by 511 codon region(30 strains). Two novel mutations were reported: substitution in 529 codon CGA"CAA and 517-518 codon deletion.

However, only 51/99 true rifampicin resistant strains on Pyrosequencing were rifampicin resistant by MGIT 960 and 57/99 by solid DST. This proves that low level rifampicin resistance linked to specific *rpoB* mutations could be missed by phenotypic DST and this could be attributed to the critical concentration of rifampicin used.

101 pan-sensitive, 96 MDR-TB and 02 rifampicin mono-resistant strains used as controls were confirmed by pyrosequencing and showed 100% concordance with phenotypic DST.

**Conclusion:** Pyrosequencing is a confirmatory tool for detection of rifampicin resistance in silent mutation cases in *M. tuberculosis* and reiterates the fact that liquid DST may miss some rifampicin resistance compared to solid DST conferring mutations further suggesting that the gold standard for rifampicin resistance be reconsidered. Treatment outcome with these mutations is not well known.

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#### Phytochemical and antimycobacterial analysis of aqueous and ethanolic extracts of *Annona muricata* Linn (Soursop)



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**Background:** Against the backdrop evidenced in the threat Tuberculosis poses to developing economies, especially its prevalence among people in their productive (15–45) years; this preliminary study examined the phytochemical constituents and antimycobacterial effect of four (4) aqueous and ethanolic extracts from the fruit skin (epicarp) and leaf of *Annona muricata* Linn.

**Methods & Materials:** Extracts were prepared with distilled water and 95% ethanol according to methods previously described. Phytochemical analysis of the extracts were carried out following standard protocols while the antimycobacterial activity was assayed by employing the Drug susceptibility testing (DST) procedure in a Biosafety Level 3 facility. Lowenstein Jensen (LJ) media were prepared with extracts at three concentrations (1, 40 and 250 µg/ml) following the project design and subsequently inoculated with 10<sup>-3</sup> and 10<sup>-5</sup> suspensions of both control (H37Rv) strain and a clinical isolate (MTB-584) of *Mycobacterium tuberculosis*. LJ media prepared with Rifampicin at 40 µg/ml was used as the standard drug for positive control while plain media with respective inoculum served as negative control. Four Ziehl-Neelsen's stain slides were also prepared to confirm the presence of organisms in the two suspensions employed for the two strains tested. Plain media inoculated with distilled water were employed as normal control to check for possible contaminant. The inoculated media and control slants were placed in an incubator at 37°C and observed every seven days for a period of 4 – 6 weeks.



Subcultured strains of MTB on LJ slants



#### Preparing LJ for DST

**Results:** The phytochemical analysis collectively revealed the presence of tannins, saponins, flavonoids, anto- and betacyanins, terpenoids, phenols and steroids. The *M. tuberculosis* strains exhibited resistance to all the four extracts at tested concentrations as there was substantial growth with typical creamy non-pigmented morphology on all the LJ media prepared with extracts though with varied rate compared to the control. However, there was no growth on the media with standard drug and the media with distilled water as expected.

**Conclusion:** It can therefore be inferred from the result that aqueous and ethanolic extracts from the fruit skin and leaf of *A. muricata* at tested concentrations have no antimycobacterial activity.

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#### Prevalence of culture-positive mycobacteria among suspected cases of pulmonary tuberculosis in Ahmadu Bello University Teaching Hospital, Zaria, Northern, Nigeria

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**Background:** Tuberculosis (TB) is a major cause of death and disability globally. Microscopy of Acid Fast Bacilli (AFB) is the routine method of diagnosis of tuberculosis in many developing countries and although faster and cheaper, requires a high bacterial

load to obtain a positive result. The advent of Gen-Xpert has revolutionized diagnosis of tuberculosis though Mycobacteria other than tuberculosis (MOTT) are not often captured by this technology. The Gene-Xpert cannot also be used for follow up. Therefore culture still remains the gold standard. This research set out to determine the prevalence of culture positive Mycobacteria among suspected cases of pulmonary tuberculosis in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria

**Methods & Materials:** Patients aged ten years and above presenting with clinical features suggestive of pulmonary tuberculosis at ABUTH were recruited for this descriptive study. Three sputum samples were collected from each patient for Ziehl Neelsen staining and one early morning sputum from each patient was inoculated on Lowenstein Jensen media. Isolated Mycobacteria were identified as MOTT or Mycobacterium tuberculosis complex using immunochromatographic method. HIV status was also determined. A structured questionnaire was used to obtain demographic details of the patients. Descriptive statistics are presented.

**Results:** Of the total Mycobacteria isolated, 17(65.4%) were *Mycobacterium tuberculosis* complex (MTBC) while 9(34.6%) were Mycobacteria other than tuberculosis (MOTT). Out of 270 suspected pulmonary tuberculosis patients enrolled, 127(40%) were male and 60 (22.2%) were HIV positive. AFB microscopy was positive for 19 (7%) smears while 26 (9.6%) were culture-positive. Fifteen (11.8%) males and 3 (5%) of the HIV patients were also culture-positive

**Conclusion:** The need for culture in the diagnosis of pulmonary tuberculosis is important as there was obvious difference in prevalence of Mycobacteria detected by culture versus microscopy in this study, though not significant. Occurrence of MOTT among suspected TB cases further corroborates the urgent need for culture in our facilities.

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#### Vitamin D deficiency, CNS inflammation, and clinical outcome in tubercular meningitis



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**Background:** Tubercular (TB) meningitis results in high mortality (about 40%) and neurological sequelae despite early treatment with anti-TB drugs and dexamethasone. Hence, adjunctive treatments are needed to improve the outcome. Vitamin D deficiency is associated with poor treatment outcomes in pulmonary TB. But, its effect in patients with TB meningitis is unknown. We tested the hypothesis that low serum 25-OH vitamin D levels would be associated with poor clinical outcome in patients with TB meningitis.

**Methods & Materials:** We prospectively studied 40 consecutive HIV-negative patients aged > 12 years with TB meningitis (based on the international consensus criteria) up to treatment completion,