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Expired nitric oxide and sputum mycobacterial lipid bodies indicate that pulmonary NO is a double edged sword in tuberculosis

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Background & Aim: The variable occurrence of lipid body rich (fat) and poorly replicating (lazy) *Mycobacterium tuberculosis bacilli* in sputum necessitates an explanation of the environmental signals responsible for these phenotypes. Lipid body rich and poorly replicating *Mycobacterium tuberculosis* bacilli occur at different frequencies in sputum. In vitro, NO stimulates lipid body (LB) accumulation in *M. tuberculosis* via the dormancy-associated regulon [DosR (DevR)]. We hypothesized that the percentage of lipid body-positive acid fast bacilli (%LB+AFB) in sputum correlates with fractional expired NO (FeNO) and that greater LB responses to NO might be associated with poorer responses to chemotherapy.

Methods: In Gondar, Ethiopia, 73 patients with smear positive tuberculosis were recruited and assessed for sputum %LB+AFB, FeNO and HIV status. Weight gain was determined at 7 months in 9 patients as a measure of treatment response.

Results: %LB+AFB in patients' sputum significantly associated with Log10 FeNO concentration ($p<0.001$) with a linear relationship ($r2=0.209$, $p<0.001$). Weight gain showed a negative linear association with %LB+AFB at both 2 ($r2=0.196$) and 7 months ($r2=0.445$) of treatment. Stronger correlations of Log10 FeNO concentration with %LB+AFB were apparent after stratification for HIV status with a shallower negative gradient for HIV positives.\

Conclusions: *M. tuberculosis* LB frequencies in sputum are significantly associated with patient FeNO levels in a manner consistent with bacterial DosR activation by NO in the lung. DosR activation is associated with antibiotic tolerance and may compromise treatment response while bactericidal effects of NO should be beneficial. We suggest that NO is a double-edged sword enabling mycobacterial clearance at high levels but provoking antibiotic tolerance when sub-lethal.

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Direct application of loop mediated isothermal amplification assay for detection of *Mycoplasma bovis* in mastitic milk

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Mycoplasma mastitis is always difficult to control due to lack of rapid and accurate diagnostic tool. The diagnostic methods available are mostly time consuming due to laborious culturing requirement, expensive, non-specific and less sensitive like biochemical tests and conventional PCR assay. A loop mediated isothermal amplification (LAMP) assay was developed for detection of *Mycoplasma bovis* directly from clinical mastitic milk samples. The LAMP assay was developed and validated on clinical samples obtained from *M. bovis* and other mastitis-causing pathogens detected by MALDI-TOF. Three different set of primers were used targeting different gene regions of *M. bovis*. The genes selected were UvrC, 16S rRNA and GyrB region. LAMP conditions were optimized for each of these and the efficiency, sensitivity and specificity of these LAMP primers were evaluated and compared. The result of 16S rRNA primers was more sensitive while GyrB primers were more specific. To confirm the specificity of the developed assay, other bacterial strains used were *Mycoplasma agalactiae, Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis.* No cross reactivity was observed in all of the primer sets. Results were also compared to conventional PCR assay with primers chosen from the same genes and confirmed by sequencing. For the evaluation of LAMP assay sensitivity, culture-positive milk samples were subjected to the assay. LAMP assay detected *M. bovis* in some of those milk samples which were PCR negative. In the present study we have developed, validated and evaluated LAMP assay for detection of *M. bovis* from mastitis milk samples. The assay is authentic, rapid and sensitive.

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