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Reply to "Role of *rpsA* Gene Sequencing in Diagnosis of Pyrazinamide Resistance"

David C. Alexander,[a] Jennifer H. Ma,[a] Jennifer L. Guthrie,[a] Joanne Blair,[a] Pam Chedore,[a] Frances B. Jamieson[a,b]

Public Health Ontario, Public Health Ontario Laboratories, Toronto, Ontario, Canada[a]; University of Toronto, Department of Laboratory Medicine and Pathobiology, Toronto, Ontario, Canada[b]

We appreciate the comments made by Simons and colleagues regarding the value of *rpsA* gene sequencing in the diagnosis of pyrazinamide resistance. The diversity of mutations associated with drug resistance in *Mycobacterium tuberculosis* has been a challenge to the sensitivity and predictive value of molecular diagnostic tools. For most clinical laboratories, full-gene sequencing of all resistance-associated loci is not a feasible approach (1). Until rapid, cost-effective methods for identifying every mutation in every strain are developed, operational demands dictate that routine diagnostic algorithms focus on the most common and informative targets. For example, most molecular assays target only the short "rifampin resistance-determining region" of *rpoB*, even though mutations outside this region can confer resistance to rifampin (2, 3).

Consistently, studies have identified *pncA* mutations in >80% of pyrazinamide-resistant *Mycobacterium tuberculosis* isolates (4). The paper by Shi and colleagues raised the possibility that *rpsA* mutations may explain resistance in wild-type *pncA* ($pncA^{WT}$) strains (5). However, in our clinical collection of pyrazinamide-resistant isolates, no nonsynonymous mutations were observed (6). Although our colleagues report that 1 of the 5 $pncA^{WT}$ strains they examined contained a *rpsA* mutation, they do not indicate if they confirmed the phenotypic impact of the A778→C/Val260→Ile change (e.g., by cloning the mutant *rpsA* gene into a pyrazinamide-sensitive strain and measuring a decrease in susceptibility). To be clear, we do not discount a role for *rpsA*, but the current data indicate that *rpsA* mutations account for resistance in only a small subset of strains. We also concede that a two-step approach, where only $pncA^{WT}$ isolates are subjected to *rpsA* sequencing, may be useful. However, our initial intent was routine analysis of both genes, just as common algorithms for investigating isoniazid resistance target both *katG* and *inhA*.

Irrespective of the possible value of *rpsA* sequencing, it is evident that additional determinants of pyrazinamide resistance remain to be characterized. Notably, two of the isolates described by Simons and colleagues exhibited resistance to both pyrazinamide and isoniazid. We also observed this resistance pattern among $pncA^{WT}$ and $rpsA^{WT}$ isolates. Considering that pyrazinamide and isoniazid have somewhat similar chemical structures and that both are administered as prodrugs, we have wondered if some shared mechanism may mediate resistance to both agents. Thorough analysis of such strains may uncover novel determinants of resistance to pyrazinamide and other antimycobacterial agents and provide useful information for the effective treatment of tuberculosis.

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Address correspondence to Frances B. Jamieson, frances.jamieson@oahpp.ca.

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