Spectrum of non-tuberculous mycobacteria identified using standard biochemical testing versus 16S sequencing.

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SUMMARY

Non-tuberculous mycobacterial isolates from gold miners were speciated using standard biochemical testing (SBT) and 16s rDNA sequencing. Of 237 isolates tested, SBT identified 126 compared with all 237 identified by sequencing. Of 111 isolates unspeciated by SBT but identified by sequencing, 38 (34.2%) were identified as *Mycobacterium gordonae* and 8 (7.2%) were new species. Of 126 isolates speciated by both methods, 37 were discordant, with 14/17 *M. gordonae* isolates incorrectly identified as *M. scrofulaceum* using SBT. The majority of these were the potentially pathogenic strain D *M gordonae*: sequencing is preferable where available to guide treatment.

Identifying non-tuberculous mycobacteria (NTM) is important, especially where HIV is prevalent; to distinguish potential pathogens. In South African gold mines, use of liquid mycobacterial culture media has increased both the yield of positive cultures and the proportion of NTM isolated [1].

Conventionally, NTM are speciated using standard biochemical testing (SBT). 16S ribosomal ribonucleic acid (rRNA) gene sequence determination (16S rDNA sequencing) provides faster, accurate speciation and can identify new species [2].

We compared the spectrum of NTM identified by SBT versus sequencing in a gold-mining population and linked a subgroup of isolates to clinical data. This is the one of the larger clinical studies of NTM reported.

METHODS

This work was part of a sub-study [1] of "Thibela TB", a cluster-randomised trial of community-wide isoniazid preventive therapy (IPT). At pre-IPT screening and follow-up visits, [1] and at routine mine health facilities (restricted to those without prior tuberculosis), we recruited individuals with suspected tuberculosis, between July 2006 and December 2007. Participants gave one sputum specimen; all isolates with results from both SBT and sequencing were included.

Following decontamination, specimens were cultured using both BACTEC MGIT 960 system (BD Diagnostics, Sparks MD) and Löwenstein-Jensen media [1]. *Mycobacterium tuberculosis* complex was distinguished from NTM by detection of MPB64 antigen (Capilia TB, Japan). Phenotypic identification was based on growth rate at 25^0C, 37^0C, 42^0C, 45^0C

and with p-nitrobenzoic acid; pigmentation and colony morphology in light and dark conditions at 37^0C. SBT included Tween hydrolysis; nitrate reduction and the catalase test. For sequencing, heat-killed culture lysates were subjected to 5'-16s rDNA amplification; sequenced [2] and referred to the RIDOM and NCBI GenBank sequence databases for identification [3,4]. GyrB genes of heat killed lysates were sequenced to confirm the absence of *M. tuberculosis*, identify other members of MTB complex and confirm *M. kansasii* identification.

Where SBT and sequencing results were discrepant, sequencing was repeated. SBT was repeated for isolates identified as *M. scrofulaceum* using SBT and *M. gordonae* using sequencing. For a subgroup with discrepant identification, because of uncertainty regarding pathogenicity, routine clinical data were collected retrospectively using a standardised case report form.

RESULTS

237 isolates were included. Dominant species identified using SBT included *M. kansasii* (51isolates), *M. avium* complex (47) and *M. scrofulaceum* (17); and using sequencing, *M.gordonae* (62), *M. kansasii/M. gastri* (53), *M. avium complex* (38) and *M. parascrofulaceum* (20). 28/237 isolates (11.8%) contained mixed NTM species on sequencing but none had mixed NTM/MTB. 111 isolates were not identifiable by SBT, but sequenced as follows: *M. gordonae* (38 isolates), *M. fortuitum* (17), *M. parascrofulaceum* (10), *M. avium* complex (7), *M. kansasii/M. gastri* (5), other NTM species (22), new mycobacterial species (8) or non-mycobacterial species (4).

Among 126 isolates successfully speciated by both methods, 38 (30%) were discordant on initial testing (table 1). Among 17 isolates identified as *M. scrofulaceum* using SBT, most (14/17) were identified as *M. gordonae* by sequencing.

Figure 1 shows a portion of the 16S rRNA sequence of *M. scrofulaceum* and *M. gordonae* strains (positions 392 to 446), indicating one of the few major differences between these species. Differences are visible at positions 411 to 427, including a three base-pair insertion/deletion. Only two minor variations within *M. gordonae* strains are observed among these 10 isolates at position 412 (TC or CC) and position 426 (GC or AT). The sequences of 14 isolates, biochemically identified as *M. scrofulaceum,* are identifiable as *M. gordonae* strains by sequencing. In 9/13 strains (one was not re-sequenced fully), TC replaces CC in position 412.

On repeat SBT, successful for 11/14 isolates originally identified as *M. scrofulaceum*, 10 were *M. gordonae* and one retained the initial identification of *M. scrofulaceum.* Among 38 isolates for which SBT and sequencing were discordant, repeat sequencing produced the same result for 28; one isolate initially identified as *M. szulgai* was identified as *M. parascrofulaceum* on repeat sequencing; the remaining nine isolates had poor and uninterpretable results.

Clinical data were available for 8/10 *M. gordonae* strain D isolates, identified by SBT as *M. scrofulaceum*. Six individuals were recruited at Thibela TB study sites and two at routine health services. 3/8 had a history of previous tuberculosis; all were smear negative; 2/8 reported cough, with one additionally reporting weight loss. An HIV test result was recorded for 1/8, who was HIV negative. 3/8 had cavitation on chest radiograph, only one of whom

had previous tuberculosis. 1/8 (HIV negative with chest cavitation and no prior tuberculosis) was given standard tuberculosis treatment.

DISCUSSION

M. gordonae identified in sputum is generally considered to be non-pathogenic and has frequently been isolated from tap water, whereas *M. scrofulaceum* is considered to cause disease [5]. In nine of our *M. gordonae* isolates, a polymorphism (TC replacing CC at position 412) was shown that is associated with *M. gordonae rpoB* cluster D, which may be more pathogenic than other strains [6]. We note that *M. gordonae* can be pathogenic in the immunocompromised [7, 8] and may be causing disease in some individuals in this population, although relatively low numbers make it difficult to be certain. Accurate distinction between species is therefore important in populations with high HIV prevalence, such as this.

The dominant NTM species were M. *kansasii*, *M. gordonae*, *M. parascrofulaceum* and members of *M. avium* and *M. fortuitum* complexes. *M. kansasii* is known to be prevalent among miners [9]. In previous studies of NTM in miners, SBT was used to identify species mostly cultured on LJ [9, 10]; our data suggest that some *M. gordonae* strains could have been misidentified by SBT as *M. scrofulaceum*, some being associated with features of disease. The importance of this observation lies in the perceived pathogenicity of these two organisms and in our understanding of NTM species distribution in this population.

CONCLUSIONS

Some *M. gordonae* strains can be misclassified by SBT as *M. scrofulaceum*.

Misidentification of NTM may lead to suboptimal clinical management, particularly in

settings with HIV prevalence. Sequencing should be used where available to accurately identify NTM and where SBT is used, the possibility of misidentification should be considered.

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Figure Legend:

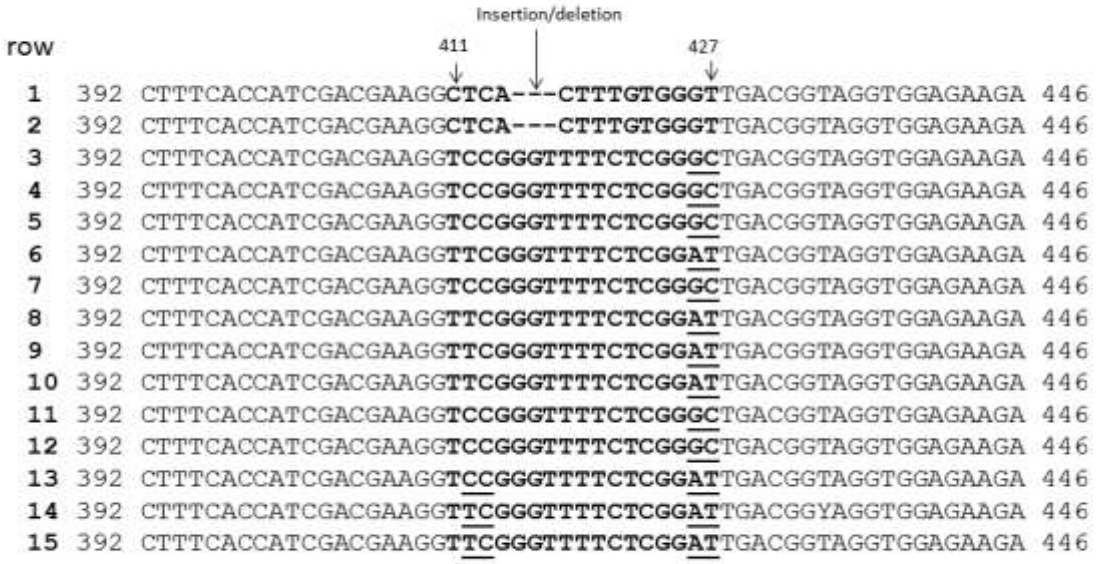
Figure 1: 16S rRNA sequence alignment of *M. gordonae* and *M. scrofulaceum*. Sequences of *Mycobacterium* type strains are shown in row 1-12. *M. scrofulaceum* type strain sequences (ATCC 19981 and DSM 43992) are shown in row 1-2, while row 3-12 show *M. gordonae* type strain sequences (ATCC 14470, DSM 44160, agha3, Tropicalis, NIPHL050404TB, M138, M120, M223, Tropicalis-2 and Tropicalis-3). Rows 13-15 are examples of clinical isolates from our study that were identified as *M. scrofulaceum* on initial standard biochemical testing and *M. gordonae* on sequencing. Two of these clinical isolates (rows 14-15) show TC instead of a CC at position 412.

Tables

Table 1: Identification of non-tuberculous mycobacteria using standard biochemical testing and 16S rDNA sequencing: discordant results on initial testing

Standard biochemical testing	**n**	**16S rDNA sequencing**	**N**
M. scrofulaceum	17	*M. gordonae*	14
		M. szulgai	2
		M. fortuitum	1
M. avium complex	16	*M. parascrofulaceum*	9
		M. paraffinicum	3
		M. fortuitum	1
		M. kyorinense	1
		M. palustre	1
		New mycobacterial species	1
M. kansasii	3	*M. gordonae*	1
		M. parascrofulaceum	1
		M. szulgai	1
M. gordonae	1	*M. asiaticum*	1
M. flavescens	1	*M. gordonae*	1
Total	38	Total	38

Figure 1:



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