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DOI:

[10.1007/s00408-019-00241-4](https://doi.org/10.1007/s00408-019-00241-4)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Barac, A., Karimzadeh-Esfahani, H., Pourostadi, M., Taghi Rahimi, M., Ahmadpour, E., Rashedi, J., Mahdavi poor, B., Samadi Kafil, H., Spotin, A., Hassen Abate, K., Mathioudakis, A., & Asgharzadeh, M. (2019). Laboratory cross-contamination of *Mycobacterium tuberculosis*: a systematic review and meta-analysis. *Lung*.
<https://doi.org/10.1007/s00408-019-00241-4>

Published in:

Lung

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Lung

Laboratory cross-contamination of Mycobacterium tuberculosis: a systematic review and meta-analysis --Manuscript Draft--

Manuscript Number:	LUNG-D-19-00061R2	
Full Title:	Laboratory cross-contamination of Mycobacterium tuberculosis: a systematic review and meta-analysis	
Article Type:	Original Research	
Keywords:	Mycobacterium tuberculosis; laboratory diagnosis; cross-contamination; false positive; systematic review; genotyping	
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Funding Information:	University of Tabriz (IR) (37876)	Dr Ehsan Ahmadpour
	Iranian National Sciences Foundation (843599)	Dr Ehsan Ahmadpour
	National Institute for Health Research (NIHR Manchester BRC)	Dr. Alexander G. Mathioudakis
	Ministry of Education, Science and Technology of the Republic of Serbia (III45005)	Dr Aleksandra Barac
Abstract:	Background: Microbiological cultures are the mainstay of the diagnosis of tuberculosis (TB). False positive TB results lead to significant unnecessary therapeutic and economic burden and are frequently caused by laboratory cross-contamination. The aim of this meta-analysis was to quantify the prevalence of laboratory cross-contamination.	

	<p>Methods: Through a systematic review of five electronic databases, we identified studies reporting rates of laboratory cross-contamination, confirmed by molecular techniques in TB cultures. We evaluated the quality of the identified studies using the National Institute of Health (NIH) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies, and conducted a meta-analysis using standard methodology recommended by the Cochrane Collaboration.</p> <p>Results: Based on 31 eligible studies evaluating 29,839 TB cultures, we found that 2% (95% confidence intervals [CI]: 1-2%) of all positive TB cultures represent false positive results secondary to laboratory cross-contamination. More importantly, we evaluated the rate of laboratory cross-contamination in cases where a single positive TB culture was available in addition to at least one negative TB culture, and we found a rate of 15% (95%CI: 6-33%). Moreover, 9.2% (91/990) of all patients with a preliminary diagnosis of TB had false-positive results and received unnecessary and potentially harmful treatments.</p> <p>Conclusions: Our results highlight a remarkably high prevalence of false positive TB results as a result of laboratory cross-contamination, especially in single-positive TB cultures, leading to the administration of unnecessary, harmful treatments. The need for the adoption of strict technical standards for mycobacterial cultures cannot be overstated.</p>
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Laboratory cross-contamination of *Mycobacterium tuberculosis*: a systematic review and meta-analysis

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40 **Keywords:** *Mycobacterium tuberculosis*, laboratory diagnose, cross-contamination, false
41 positive, systematic review, genotyping

42

43 **Word count:**

44 Manuscript: 2374

45 Abstract: 222

46 Tables: 1

47 Figures: 4

48

49 **Abbreviations:**

50 HIV – Human Immunodeficiency Virus

51 MDR-TB – Multidrug Resistant Tuberculosis

52 NIH – National Institute of Health

53 PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

54 TB – Tuberculosis

55 WHO – World Health Organization

56

57 **Abstract**

58 **Background:** Microbiological cultures are the mainstay of the diagnosis of tuberculosis (TB).

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60 are frequently caused by laboratory cross-contamination. The aim of this meta-analysis was to
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69 confidence intervals [CI]: 1-2%) of all positive TB cultures represent false positive results
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71 laboratory cross-contamination in cases where a single positive TB culture was available in
72 addition to at least one negative TB culture, and we found a rate of 15% (95%CI: 6-33%).
73 Moreover, 9.2% (91/990) of all patients with a preliminary diagnosis of TB had false-positive
74 results and received unnecessary and potentially harmful treatments.

Conclusions: Our results highlight a remarkably high prevalence of false positive TB results as a result of laboratory cross-contamination, especially in single-positive TB cultures, leading to the administration of unnecessary, harmful treatments. The need for the adoption of strict technical standards for mycobacterial cultures cannot be overstated.

Introduction

Despite global efforts to control tuberculosis (TB), the incidence of the condition is growing¹. According to the World Health Organization (WHO) 2018 report, “TB is one of the top 10 causes of death worldwide, and the leading cause from a single infectious agent”². In 2017, 10.4 million people were diagnosed with TB and 1.6 million died from the disease (including 0.3 million patients with concomitant HIV infection)^{1,2}. Over 95% of TB deaths occur in low- and middle-income countries. Five countries account for 56% of the total number of cases, with India leading the count, followed by Indonesia, China, the Phillipines, and Pakistan. In 2016, the estimated incidence of TB in children exceeded 1 million. In 2016, an estimated 490 000 people developed multidrug-resistant TB (MDR-TB) worldwide². TB diagnosis is confirmed by the isolation and identification of *M. tuberculosis* bacillus in microbiological cultures³. The accuracy of mycobacterial microbial cultures is limited by the prevalence of false positive and false negative results. Laboratory cross-contamination causing false positive results is not infrequent and has important medical and psychological implications for patients and their families, as well as financial and public health ramifications for the healthcare system⁴. Over the years, different methods have been utilized to limit the burden of laboratory cross-contamination. More than one decade ago, it was recommended to consider a result false positive if there were 5 or less colonies grown on a specific growth media⁵. Since the above method was not reliable, molecular techniques are now used for the

confirmation of TB⁶⁻⁷. IS6110-based restriction fragment length polymorphism typing (RFLP) is a standard method to assess the cross-contamination and transmission of tuberculosis. IS6110-RFLP which is based on the number and genomic site of IS6110⁸⁻⁹. In the cases that the copy number of IS6110 is less than 6 bands, the use of other methods can be helpful¹³. Approaches based on next generation sequencing (NGS) may offer a more accurate assessment¹⁴. However, the exact burden of false positive mycobacterial cultures resulting from laboratory cross-contamination is unknown. The aim of this systematic review and meta-analysis was to estimate this prevalence in order to facilitate planning accurate, cost-effective diagnostic strategies.

Methods

Inclusion criteria

We included studies reporting on the prevalence of TB-laboratory cross-contamination, detected by genotyping and confirmed by clinico-epidemiological analyses. We did not apply any geographical limitations. We only included studies published during the last 20 years (since 1997), as culturing methods and standards have been changing, and we considered that older studies would not reflect current practice. We excluded studies exploring non-TB mycobacteria, those that solely used genotyping to explore cross-contamination without taking into account clinic-epidemiological data, case reports, specific organ TB and those with very limited sample size (≤ 60). We only included studies written in the English language.

Outcome Measures

The outcomes of this meta-analysis include: The proportion of TB laboratory cross-contamination among (a) all positive TB cultures or smears, (b) single positive TB cultures or

smears, in cases where the results of at least one additional negative TB culture was available, and (c) all TB cultures or smears (positive or negative). In addition, we assessed the proportion of false-negative results in the same groups.

Search Strategy and Study Selection

We systematically reviewed the electronic databases of Medline, PubMed, Scopus, ScienceDirect and Cochrane Controlled Register of Trials (CENTRAL), using appropriate controlled vocabulary and free search terms to identify studies evaluating the prevalence of tuberculosis (use TB instead) laboratory cross-contamination, including the following terms: “tuberculosis”, “mycobacterium”, “mycobacterial”, “cross-contamination”, “laboratory diagnosis”, “false positive”, “culture” and “genotyping”. Databases were searched from January 1997 to Jan 2019. Two authors independently screened abstracts and full texts (when appropriate), for eligibility for all identified studies. The study selection process was detailed in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

Data extraction

Relevant data including the full reference and study identifiers, study date, study design, eligibility, predefined outcomes, number and characteristics of the participants and details on the outcomes of interest were extracted by two authors independently. Disagreement was resolved through discussion and adjudication by a third investigator.

Quality of the included studies

We used the National Institutes of Health (NIH) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to assess the risk of bias of each included study (available

from: <https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools>). Two authors evaluated risk of bias of the included studies independently. We used funnel plots to assess for publication bias when appropriate.

Analysis

We used I^2 statistic to assess statistical heterogeneity within the studies included in each analysis. We conducted prespecified subgroup analyses to explore the causes of heterogeneity in cases with substantial heterogeneity ($I^2 > 50\%$).

We expected significant heterogeneity in our analyses, due to the differences in the standards used in different laboratories and the accuracy of the methodologies used to confirm laboratory cross-contamination in the primary studies. For this reason, we conducted our meta-analyses using the random effects mode. We considered it imperative to present overall estimates, even if the heterogeneity was particularly significant and to declare the limitations. Meta-analyses were performed using R version 3.4.4 and the relevant Comprehensive R Archive Network (CRAN) packages for meta-analysis (meta and metafor).

In different prespecified sensitivity analyses for all outcomes (i) we included only studies with low risk of bias and (ii) we divided the studies according to the methodology used to identify TB laboratory cross-contamination. In an additional sensitivity analysis, we also excluded one of the identified studies that found unexpectedly high levels of cross-contamination, which, as reported by the investigators, reflected laboratory specific problems.

Results

Our search results and study selection process are summarized in a PRISMA flowchart (figure 1). Briefly, our systematic searches yielded 1,033 records of which we included 32 records

reporting on 31 studies evaluating n = 29,839 positive cultures for *Mycobacterium tuberculosis*^{3,6,11-39}. Basic study characteristics are available in table 1.

Study characteristics

The study population of the included studies ranged between 61 and 8,889 participants. The proportion of positive cultures as a result of laboratory cross-contamination ranged from 0.3% to 7.84%, with the exception of one study⁶, which reported significantly larger proportion (18.2%), as a result of an extensive episode of cross-contamination involving numerous samples.

Different genotyping methods were used to identify possible laboratory cross-contamination. Most studies (n = 19) used IS6110-RFLP. Others used 12 or 24-loci mycobacterial interspersed repetitive units (MIRU) typing, variable numbers of tandem repeats (VNTR), polymorphic GC-rich sequence (PGRS), direct repetitive element (DRE), spoligotyping, and direct repeat (DR)-RFLP. In the majority of studies more than one method was performed for genotyping.

Risk of Bias Assessment

We deemed all of the included studies to be of good (n = 21) or fair (n = 10) methodological quality (figure 2). Specific limitations included: (i) None of the included studies provided a sample size justification, (ii) The study population was poorly defined in 6 studies, (iii) the participation rate was less than 50% of the eligible persons in one study and (iv) four studies recruited heterogeneous populations. In addition, our funnel plots suggest the presence of publication bias (figure 3). These may have led to a slight overestimation of the prevalence of laboratory cross-contamination.

Data Synthesis

Overall effect estimates for each outcome are presented in figure 4. The proportion of positive samples secondary to laboratory cross-contamination as a proportion of all positive samples was evaluated by 30 studies, with an overall study population of $n = 29,022$. We found a mean proportion of 0.02 with 95% confidence intervals (95% CI) between 0.01 and 0.02 (figure 4a). There was significant heterogeneity ($I^2 = 88\%$) which was resolved by removing the study with unexpectedly high levels of cross-contamination⁶ and separating the studies according to the methods used to identify cross-contamination.

We defined single positive a TB culture or smear, in cases where the results of at least one additional TB culture were available and negative. Eight studies reported on cross-contamination as a proportion of single-positive samples. The mean proportion was 0.15 (95% CI: 0.06 – 0.33, figure 4b). The significant heterogeneity was resolved by the exclusion of two studies reporting unexpectedly high proportions of cross-contamination. The mean proportion of the remaining, homogeneous studies was 0.10 (95% CI: 0.06, 0.15, figure 4c).

Finally, 20 studies provided data on false-positive results and allowed us to assess the number of false-positive results as a proportion of all positive results. The mean proportion was 0.03 (95% CI 0.02, 0.04, figure 4d). The significant heterogeneity was resolved by removing the study with unexpectedly high levels of cross-contamination⁶ and separating the studies according to different methodologies used to identify false positives and cross-contamination.

In addition, 9.2% (91/ 990) of patients with a preliminary diagnosis of TB had false-positive results and consequently received the incorrect treatment^{3,11-25} (16 studies), which led to a fatal outcome in eight cases^{17,23-25} (4 studies).

Discussion

In a meta-analysis of 31 studies evaluating 29,839 positive cultures of *Mycobacterium tuberculosis*, using genotyping and clinico-epidemiological analyses to identify false positive

cultures, we evaluated the global prevalence of false positive Mycobacterial cultures. Our findings point out a remarkably high prevalence of false-positive TB results secondary to laboratory cross-contamination. Specifically, 2% of all positive TB cultures and, more importantly, one in six (15%) of all single positive TB cultures, are the results of laboratory cross-contamination. False positive results lead to the unnecessary administration of anti-tubercular medications, which are associated with side effects and could result in avoidable harm to numerous patients¹². Indeed, in our meta-analysis we found that up to 9.2% of patients with a preliminary diagnosis of TB had a false-positive result and received inappropriate treatment. This poses a significant health and economic burden and the need to impose strict standards to reduce the rate of cross-contamination in the laboratory cannot be overstated. It is repeatedly demonstrated that coherent planning; experienced technicians and preparation of the appropriate facilities will be effective in contamination prevention³. For this reason, the WHO has produced comprehensive technical standards for mycobacteriology laboratories (<http://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf>). Simple measures to limit laboratory cross-contamination include the use of separate areas for the handling of positive and negative TB smears. In addition, first time sampling of swabs from patients also should be conducted in separate room/part of the laboratory reserved for these activities⁴⁰. Education of laboratory staff, strict conduction of epidemiological measures, external controls and follow-up of proposed guidelines could reduce rate of TB cross-contamination¹¹. Additional measures are required for the identification of false-positive results and avoidance of the administration of unnecessary treatments to subjects with false-positive cultures. Our study demonstrated a remarkable 15% incidence of false-positive results among single positive TB cultures, suggesting it is a prime target for interventions aimed to reduce the unneeded administration of anti-tubercular medications. The American Thoracic Society, Infectious

Disease Society of America and Centers for Disease Control and Prevention recently issued Guidelines for the diagnosis of tuberculosis, suggesting the need of confirmatory tests following an initial positive mycobacterial culture, acknowledging that false-positive results are common⁴³. Based on our findings, it appears appropriate to delay the initiation of anti-tubercular treatment until the acquisition of a second, confirmatory TB culture, especially in atypical presentations if the clinical condition of the patient allows such a delay. Moreover, when the obtained smear is negative and culture results are inconsistent with clinical symptoms, laboratory staff should perform genotyping methods aimed to distinguish if the result is a true or false-positive. Since this method is not precise for strains with less than 6 copies of IS6110, other methods including mycobacterial interspersed repetitive units (MIRU) and spoligotyping could be helpful⁴¹⁻⁴². Contaminated specimens could be detected by genotyping, as well as identification of the source of contamination and detection of re-infection with same strain¹⁵. Although NGS remains cost prohibitive for resource-challenged countries, this approach overcomes many of the significant challenges associated with limitations of other less comprehensive molecular tests by providing rapid, detailed sequence information for multiple gene regions or whole genomes of interest. However, the uptake of these technologies for DR-TB diagnosis has been hindered by concerns regarding costs, integration into existing laboratory workflows, technical training and skill requirements for utilization of the technology and clinical interpretation of sequencing data^{14,44}. The genetic analysis using NGS has enabled rapid genome analysis with minimal sample preparation time (1 to 2 days) at relatively moderate costs when multiple samples are analyzed per run. Reagent expenses can be marginally decreased by combining 24 to 48 samples per sequencing run. Additionally, a qPCR specific for *M. tuberculosis* and used prior to NGS can be employed for the prediction of genomic sequencing success, a helpful strategy for reducing costs⁴⁴.

Obtaining false-positive results causes a delay in the correct diagnosis being reached, which further delays starting the appropriate treatment, or not receiving the necessary management. It also leads to increased costs due to hospitalization length, nursing expenses and non-medical costs³. By including the false-positive TB cases in the overall number of positive cases, the prevalence of TB is falsely elevated. In addition, published results with false-positive TB cases contribute to incorrect interpretation of epidemiological data. Consequently, there is no proper estimation of TB risk in many countries.

An additional complication is drug resistant TB, especially if such strains are present in the body of the “contaminator”. In this case, the wrong patient with false-positive results, is treated with second-line TB drugs, that additionally increase financial and health costs. From 1998 to 1999 in the United States of America, every false diagnosis cost the health care system 10,873 dollars⁴⁵. It is estimated that annually approximately 10 million dollars of excessive costs are imposed to health system, not only for incorrect TB treatment, but also for physical harm after use of anti-TB drugs and psychological pressures – this data could not be easily ignored².

Although complete elimination of false-positive results obtained from the culture is the main goal of every mycobacteriological laboratory, several factors still cause false-positivity of the culture plate. In the current meta-review, several factors were highlighted as the most significant for causing false-positivity of TB culture (Supplementary table). These include, error in the performance of the laboratory technician, reagent contamination and the presence of aerosols in the workplace. Aerosols containing live *M. tuberculosis* are created during the removal of samples which can survive for a long period in harsh environmental conditions and small areas. They have a major role in the contamination of reagents and instruments such as pipettes or lids of containers^{5,46}. In addition, inadequately sterilized bronchoscope may lead to false positive results as well as transmission of the infection³³.

One of the included studies found unexpectedly high levels of cross-contamination (18.2%)⁶. The authors reported several large clusters of false-positive samples as a result of cross-contamination. Characteristically, they identified two clusters of 9 and 5 false-positive samples (9% of all included samples) that were contaminated in the laboratory by a single true positive culture each. This study was an outlier and the laboratory performance was below standards. For this reason, we excluded this study in a sensitivity analysis. Our findings were not changed by the omission of this study.

Our study has several strengths. Firstly, we conducted an extensive systematic review of five online databases and our findings are based on a large number of studies, evaluating almost 30,000 TB cultures. However, we did not identify any studies using NGS, which may offer a more accurate assessment to identify possible laboratory cross-contamination. This is unlikely to affect our estimates, as the identified studies implemented rigorous methods for identifying laboratory cross-contamination. The quality of the available evidence was good and all included studies adequately reported on the methodology used to identify laboratory cross-contamination. Our results are at risk of publication bias and this may have led to a slight overestimation of the prevalence of laboratory cross-contamination. Many were specifically conducted to evaluate the incidence of cross-contamination and employed exhaustive methods to identify false-positive results and cross-contamination. On the other hand, the variability in methods used among different studies led to a significant (but expected) heterogeneity in our results. When heterogeneity (I^2) is higher than 75%, the quality of the pooled estimate is very limited. However, in our sensitivity analyses, we were able to resolve the observed heterogeneity and that did not lead to significant alterations, supporting the robustness of our results. In addition, our results are at risk of publication bias, as evident by our funnel plot (figure 3). Finally, we did not prospectively register the protocol of this meta-analysis, but we

used standard methodology suggested by the Cochrane Collaboration, to prospectively address a clearly defined research question.

Conclusion

To our knowledge, this is the first comprehensive systematic review and meta-analysis evaluating the incidence of *M. tuberculosis* laboratory cross-contamination. We found a remarkably high incidence, 2% of all positive TB cultures and 15% of all single-positive TB cultures represent false-positive results due to laboratory cross-contamination. This is associated with a significant therapeutic and economic burden. Therefore, there is an urgent need for the adoption of a strict technical standard aiming to prevent or identify laboratory cross-contamination and false positive TB results.

Ethics approval and consent to publish: Not applicable (Meta review article).

Consent for publication: Not applicable.

Availability of data and material: Please contact author for data requests.

Competing interests: The authors declare that they have no conflict of interest relevant to this work.

Authors' contributions: MP, HK, MTR, EA, AB, JR, BM, HSK, AGM, MA carried out the systematic review and meta-analyses analyses and drafted the manuscript. AGM provided methodological expertise. AS, KHA, MA, EA participated in the design of the study and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgment

This study was supported by the Iranian National Sciences Foundation (Grant No: 843599) and Tabriz University of Medical Sciences (Grant No: 37876). AGM is supported by the National

Institute for Health Research Manchester Biomedical Research Centre (NIHR Manchester BRC). Dr Aleksandra Barac's scientific work and research is supported by the Project of Ministry of Education, Science and Technology of the Republic of Serbia (No. III45005).

Figure and Table Legends:

Figure 1. PRISMA flow diagram of the systematic review process

Figure 2. Risk of Bias of the included studies using the National Institutes of Health (NIH) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. Domains: (1) Was the research question or objective in this paper clearly states? (2) Was the study population clearly specified and defined? (3) Was the participation rate of eligible persons at least 50%? (4) Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants? (5) Was a sample size justification, power description or variance and effect estimates provided? (6) For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured? (7) Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed? (8) For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)? (9) Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? (10) Was the exposure(s) assessed more than once over time? (11) Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? (12) Were the outcome assessors blinded to the exposure status of participants? (13) Was loss to follow-up after baseline 20% or less? (14)

Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Figure 3. Funnel plots evaluating the publication bias of the included studies

Figure 4. Forest plot diagram of the meta-analyses: (a) Incidence of laboratory cross-contamination among all positive cultures, (b) Incidence of laboratory cross-contamination among single positive culture samples, (c) Incidence of laboratory cross-contamination among single positive culture samples, after excluding two studies with unexpectedly high proportions. (d) Incidence of false-positive results among all positive results.

Table 1. Characteristics of the included studies

Table 2. Causes of *M. tuberculosis* cross-contamination that were identified in the included studies

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Figure 3

Figure 4b

Figure 4c

Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A

Ruddy 2002
Thumamo 2012
Yan 2005

Study	Typing method	total tested	Total positives	Single positive culture	Cross-contamination	Total number of false positives
Globan 2016	24-locus MIRU VNTR		2298		17	
Lee 2012	100% identical DRE-PCR	14462	458	71	6	5
Thumamo 2012	Spoligotyping and 12-loci MIRU		103		3	10
Asgharzadeh 2010	12-locus MIRU VNTR		156		2	1
Lai 2010	24-loci MIRU VNTR		400		3	5
Ribeiro 2009	RAPET (Rapid PCR based epidemiological typing)	2399	106	82	2	1
Alix-Beguec 2008	24-loci MIRU VNTR and Spoligotyping		974		7	
Martinez 2006	IS6110- RFLP and spoligotyping		154		28	28
Yan 2005	VNTR and MIRU		515	82	8	15
Fujikane 2004	IS6110- RFLP		227		2	
Hernandez 2004	IS6110- RFLP		793		2	
Glynn 2004	IS6110- RFLP		930		16	

Jasmer 2004	IS6110- RFLP		1244			6	6
Drobniewski 2003	IS6110- RFLP		972			37	40
Dahle 2003	IS6110- RFLP and Spoligotyping		552			13	
Hayward 2001	Spoligotyping HPCR		563			11	
McConkey 2002	IS6110- RFLP and pTBN12		61			1	
Maguire 2002	IS6110- RFLP		2779			10	
Nitta 2002	IS6110- RFLP		102			7	8
Jasmer 2002	IS6110- RFLP	21835	988		27	6	10
De Boer 2002	IS6110- RFLP and polymorphic GC-rich sequence		8889			187	213
Ruddy 2002	IS6110- RFLP		2042			19	11
Gascoyne Binzi 2001	VNTR	4751	397			9	34
Dahle 2001	IS6110- RFLP		718			20	20
Breese 2001	DNA Fingerprinting	13940	652		63	4	4
Godfrey Fausett 2000	IS6110- RFLP		429			10	10
Behr 1999	IS6110- RFLP		1599			25	
Gutierrez 1998	IS6110- RFLP		306			24	24

Braden 1997	DNA fingerprinting	259			9	9
Burrman 1997	IS6110- RFLP	696		44	6	13
Bauer 1997	IS6110- RFLP	1439			49	

1

No.	Factors	No.	Reference
1.	Inappropriate technician's laboratory work	34	15
2.	Contamination of reagents	25	15, 26, 31, 32, 27
3.	Creation of aerosols	24	3, 26
4.	Mislabeling	16	3, 5, 28, 18, 22, 26, 37, 32
5.	Contaminated equipment	11	3, 27
6.	Poor laboratory techniques	10	26
7.	Contaminated bronchoscope	4	3, 26
8.	Splashing	2	27
9.	Reprocessing of contaminated	2	32
10.	BACTEC needle carryover	2	12

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