Lung (2019) 197:651–661
https://doi.org/10.1007/s00408-019-00241-4

TUBERCULOSIS

Laboratory Cross-Contamination of *Mycobacterium tuberculosis*: A Systematic Review and Meta-analysis

Aleksandra Barac[1,2] · Hannah Karimzadeh-Esfahani[3] · Mahya Pourostadi[4] · Mohammad Taghi Rahimi[5] · Ehsan Ahmadpour[6,7] · Jalil Rashedi[8] · Behroz Mahdavipoor[9,10] · Hossein Samadi Kafil[11,12] · Adel Spotin[12] · Kalkidan Hassen Abate[13] · Alexander G. Mathioudakis[3,14] · Mohammad Asgharzadeh[15]

Received: 4 February 2019 / Accepted: 24 May 2019 / Published online: 15 June 2019

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.

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.

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.

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.

Keywords *Mycobacterium tuberculosis* · Laboratory diagnose · Cross-contamination · False positive · Systematic review · Genotyping

Abbreviations

HIV	Human immunodeficiency virus
MDR-TB	Multidrug-resistant tuberculosis
NIH	National Institute of Health
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
TB	Tuberculosis
WHO	World Health Organization

Introduction

Despite global efforts to control tuberculosis (TB), the incidence of the condition is growing [1]. 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

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00408-019-00241-4) contains supplementary material, which is available to authorized users.

Aleksandra Barac and Hannah Karimzadeh-Esfahani have contributed equally to this work.

✉ Ehsan Ahmadpour
ehsanahmadpour@gmail.com; ahmadpoure@tbzmed.ac.ir

✉ Alexander G. Mathioudakis
alexander.mathioudakis@manchester.ac.uk

Extended author information available on the last page of the article

for 56% of the total number of cases, with India leading the count, followed by Indonesia, China, the Philippines, 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) worldwide [2].

TB diagnosis is confirmed by the isolation and identification of *M. tuberculosis* bacillus in microbiological cultures [3]. 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 system [4]. 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 five or less colonies grown on a specific growth media [5]. Since the above method was not reliable, molecular techniques are now used for the confirmation of TB [6, 7]. IS6110-based restriction fragment length polymorphism typing (RFLP) is a standard method to assess the cross-contamination and transmission of tuberculosis. IS6110-RFLP is based on the number and genomic site of IS6110 [8, 9]. In the cases that the copy number of IS6110 is less than 6 bands, the use of other methods can be helpful [10]. Approaches based on next-generation sequencing (NGS) may offer a more accurate assessment [11].

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 following: 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 (Fig. 1). Briefly, our systematic searches yielded 1033 records of which we included 32 records reporting on 31 studies evaluating $n = 29{,}839$ positive cultures for *Mycobacterium tuberculosis* [3, 6, 10–38]. Basic study characteristics are available in Table 1.

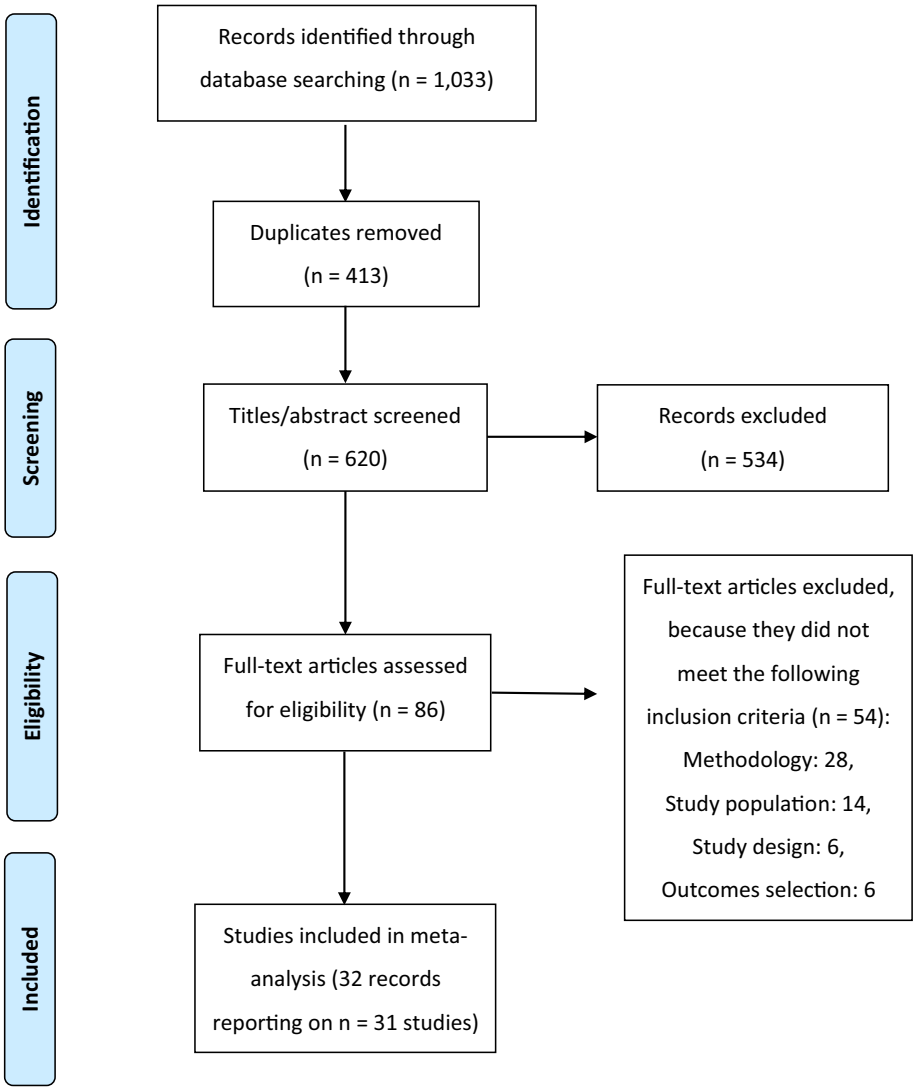

Fig. 1 PRISMA flow diagram of the systematic review process

Table 1 Characteristics of the included studies

Study	Typing method	total tested	Total positives	Single-positive culture	Cross-contamination	Total number of false positives
Globan et al. [27]	24-Locus MIRU VNTR		2298		17	
Lee et al. [12]	100% identical DRE-PRCR	14,462	458	71	6	5
Thumamo et al. [38]	Spoligotyping and 12-loci MIRU		103		3	10
Asgharzadeh et al. [21]	12-Locus MIRU VNTR		156		2	1
Lai et al. [33]	24-Loci MIRU VNTR		400		3	5
Ribeiro et al. [22]	Rapid PCR-based epidemiological typing (RAPET)	2399	106	82	2	1
Allix-Beguec et al. [23]	24-Loci MIRU VNTR and spoligotyping		974		7	
Martinez et al. [6]	IS6110-RFLP and spoligotyping		154		28	28
Yan et al. [24]	VNTR and MIRU		515	82	8	15
Fujikane et al. [19]	IS6110-RFLP		227		2	
Hernandez et al. [20]	IS6110-RFLP		793		2	
Glynn et al. [28]	IS6110-RFLP		930		16	
Jasmer et al. [32]	IS6110-RFLP		1244		6	6
Drobniewski et al. [25]	IS6110-RFLP		972		37	40
Dahle et al. [16]	IS6110-RFLP and spoligotyping		552		13	
Hayward et al. [30]	Spoligotyping HIPCR		563		11	
McConkey et al. [34]	IS6110-RFLP and pTBN12		61		1	
Maguire et al. [36]	IS6110-RFLP		2779		10	
Nitta et al. [35]	IS6110-RFLP		102		7	8
Jasmer et al. [31]	IS6110-RFLP	21,835	988	27	6	10
De Boer et al. [3]	IS6110-RFLP and polymorphic GC-rich sequence		8889		187	213
Ruddy et al. [37]	IS6110-RFLP		2042		19	11
Gascoyne Binzi et al. [26]	VNTR	4751	397		9	34
Dahle et al. [18]	IS6110-RFLP		718		20	20
Breese et al. [11]	DNA Fingerprinting	13,940	652	63	4	4
Godfrey Fausett et al. [29]	IS6110-RFLP		429		10	10
Behr et al. [15]	IS6110-RFLP		1599		25	
Gutierrez et al. [10]	IS6110-RFLP		306		24	24
Braden et al. [17]	DNA fingerprinting		259		9	9
Burrman et al. [13]	IS6110-RFLP		696	44	6	13
Bauer et al. [14]	IS6110-RFLP		1439		49	

Study Characteristics

The study population of the included studies ranged between 61 and 8889 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 study [6], 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 (Fig. 2). Specific limitations included (i) none of the included studies provided a sample size justification, (ii) the study population was poorly defined in six 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 (Fig. 3).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Allix-Beguec 2008	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Asgharzadeh 2010	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Bauer 1997	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Behr 1999	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Braden 1997	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Bresse 2001	Yes	No	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Burrman 1997	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Dahle 2001	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Dahle 2003	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
De Boer 2002	Yes	No	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Drobniewski 2003	Yes	No	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
Fujikane 2004	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
Gascoyne Binzi 2001	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Globan 2016	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Glynn 2004	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Godfrey fausett 2000	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Gutierrez 1998	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Hayward 2001	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Hernandez 2004	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Jasmer 2002	Yes	No	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Jasmer 2004	No	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
Lai 2010	No	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Lee 2012	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Martinez 2006	Yes	No	No	Yes	No	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
McConkey 2002	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Nitta 2002	Yes	No	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
Nivin 1998	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Ribeiro 2009	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Ruddy 2002	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A
Thumamo 2012	No	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	No	N/A	N/A	N/A
Yan 2005	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	N/A	Yes	N/A	N/A	N/A

Fig. 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 stated? (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)?

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 Fig. 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 (Fig. 4a). There was significant heterogeneity ($I^2 = 88\%$) which was resolved by removing the study with unexpectedly high levels of cross-contamination [6] 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, Fig. 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, Fig. 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

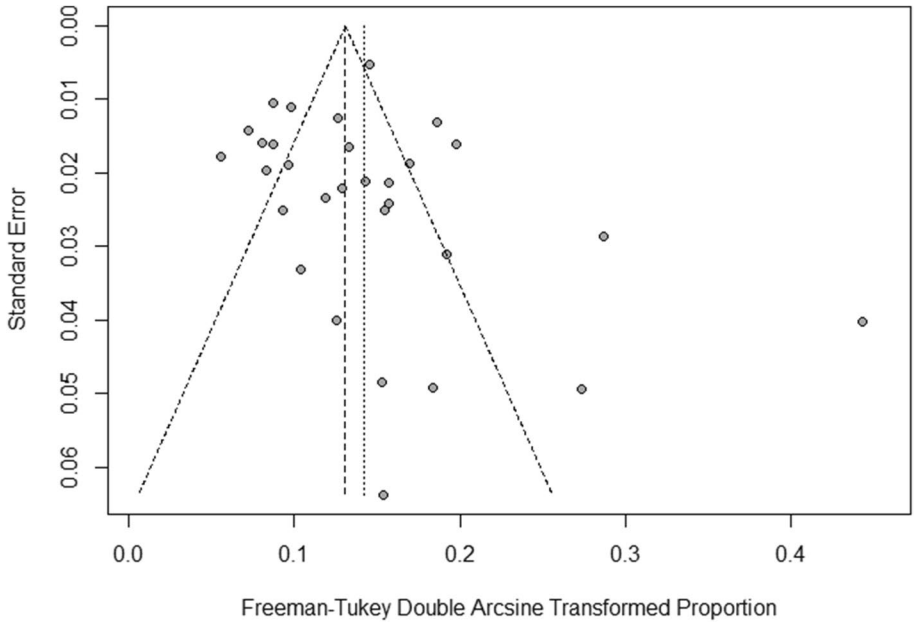

Fig. 3 Funnel plots evaluating the publication bias of the included studies

was 0.03 (95% CI 0.02, 0.04, Fig. 4d). The significant heterogeneity was resolved by removing the study with unexpectedly high levels of cross-contamination [6] 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, 10–24] (16 studies), which leaded to a fatal outcome in eight cases [16, 22–24] (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 patients [13]. 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 prevention [3]. 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 activities [39]. Education of laboratory staff, strict conduction of epidemiological measures, external controls, and follow-up of proposed guidelines could reduce the rate of TB cross-contamination [12].

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 common [40]. 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

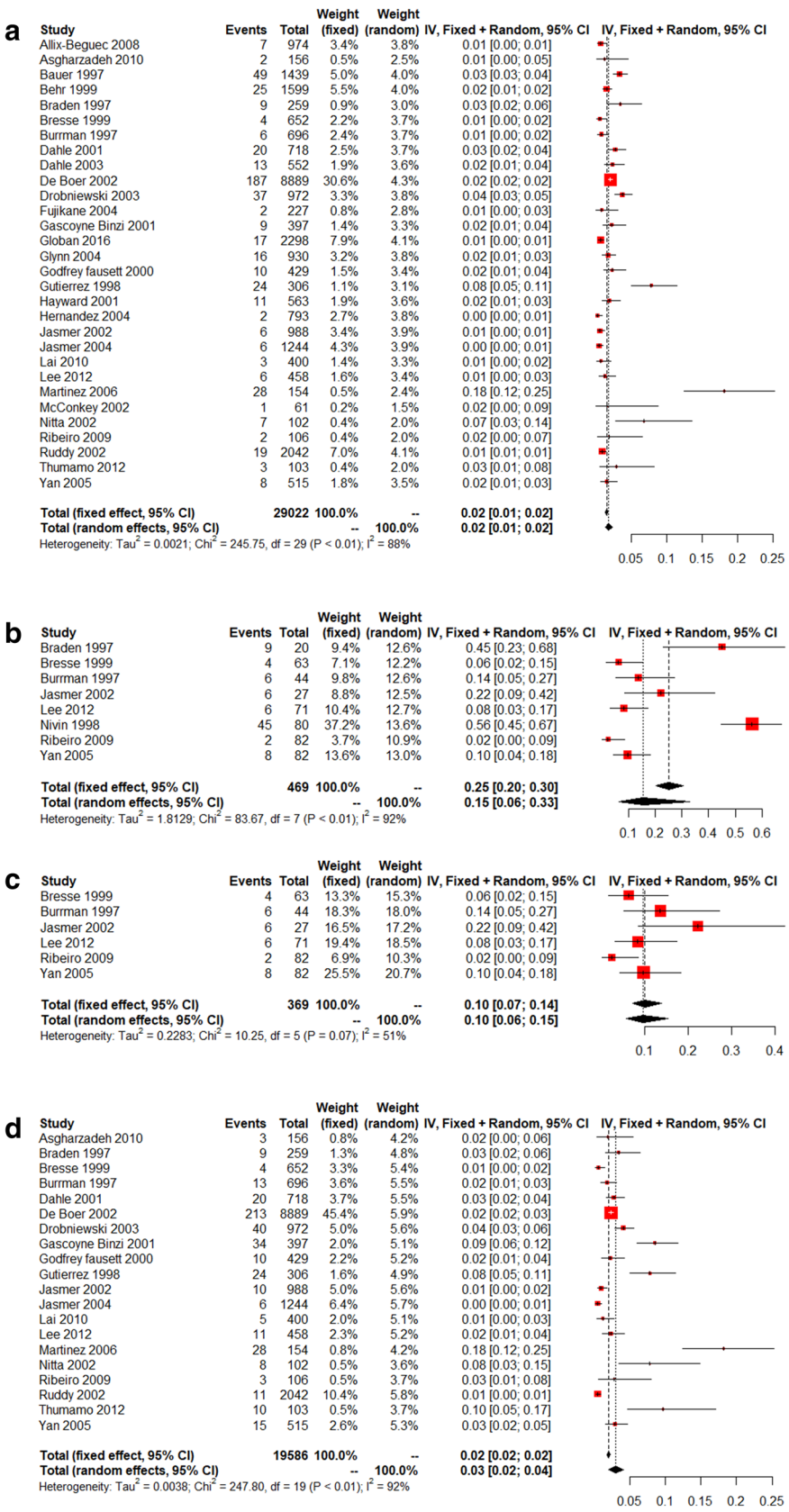

Fig. 4 Forest plot diagram of the meta-analyses: **a** Incidence of laboratory cross-contamination among all positive cultures. **b** Incidence of laboratory cross-contamination among single-positive culture samples. **c** Incidence of laboratory cross-contamination among single-positive culture samples,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

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 six copies of IS6110, other methods including mycobacterial-interspersed repetitive units (MIRU) and spoligotyping could be helpful [41, 42]. Contaminated specimens could be detected by genotyping, as well as identification of the source of contamination and detection of re-infection with same strain [14]. 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 [11, 43]. The genetic analysis using NGS has enabled rapid genome analysis with minimal sample preparation time (1–2 days) at relatively moderate costs when multiple samples are analyzed per run. Reagent expenses can be marginally decreased by combining 24–48 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 costs [43].

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 costs [3]. 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 dollars [44]. 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—these data could not be easily ignored [2].

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, 45, 46]. In addition, inadequately sterilized bronchoscope may lead to false-positive results as well as transmission of the infection [32].

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 (Fig. 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.

Acknowledgement 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).

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

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest relevant to this work.

References

1. Rashedi J, Mahdavi Poor B, Rafi A, Asgharzadeh M, Abdolalizadeh J, Moaddab SR (2015) Multidrug-resistant tuberculosis in north-west of Iran and Republic of Azerbaijan: a major public health concern for Iranian people. J Res Health Sci 15(2):101–103
2. Organization WHO (2018) Global tuberculosis report 2018
3. de Boer AS, Blommerde B, de Haas PE et al (2002) False-positive *Mycobacterium tuberculosis* cultures in 44 laboratories in The Netherlands (1993 to 2000): incidence, risk factors, and consequences. J Clin Microbiol 40(11):4004–4009
4. Ramos MC, Soini H, Roscanni GC, Jaques M, Villares MC, Musser JM (1999) Extensive cross-contamination of specimens with *Mycobacterium tuberculosis* in a reference laboratory. J Clin Microbiol 37(4):916–919
5. Burman WJ, Reves RR (2000) Review of false-positive cultures for *Mycobacterium tuberculosis* and recommendations for avoiding unnecessary treatment. Clin Infect Dis 31(6):1390–1395
6. Martinez M, Viedma D, Alonso M et al (2006) Impact of laboratory cross-contamination on molecular epidemiology studies of tuberculosis. J Clin Microbiol 44(8):2967–2969
7. Small PM, McClenny NB, Singh SP, Schoolnik GK, Tompkins LS, Mickelsen PA (1993) Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. J Clin Microbiol 31(7):1677–1682
8. Asgharzadeh M, Shahbabian K, Majidi J et al (2006) IS6110 restriction fragment length polymorphism typing of *Mycobacterium tuberculosis* isolates from East Azerbaijan Province of Iran. Mem Inst Oswaldo Cruz 101(5):517–521
9. van Embden JD, Cave MD, Crawford JT et al (1993) Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 31(2):406–409
10. Gutierrez M, Vincent V, Aubert D et al (1998) Molecular fingerprinting of *Mycobacterium tuberculosis*and risk factors for tuberculosis transmission in Paris, France, and surrounding area. J Clin Microbiol 36(2):486–492
11. Breese PE, Burman WJ, Hildred M et al (2001) The effect of changes in laboratory practices on the rate of false-positive cultures for *Mycobacterium tuberculosis*. Arch Pathol Lab Med 125(9):1213–1216
12. Lee MR, Chung KP, Chen WT et al (2012) Epidemiologic surveillance to detect false-positive *Mycobacterium tuberculosis* cultures. Diagn Microbiol Infect Dis 73(4):343–349
13. Burman WJ, Stone BL, Reves RR et al (1997) The incidence of false-positive cultures for *Mycobacterium tuberculosis*. Am J Respir Crit Care Med 155(1):321–326
14. Bauer J, Thomsen VO, Poulsen S et al (1997) False-positive results from cultures of *Mycobacterium tuberculosis* due to laboratory cross-contamination confirmed by restriction fragment length polymporphism. J Clin Microbiol 35(4):988–991
15. Behr MA, Warren SA, Salamon H et al (1999) Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. Lancet 353:444
16. Dahle UR, Sandven P, Heldal E, Caugant DA (2003) Continued low rates of transmission of *Mycobacterium tuberculosis* in Norway. J Clin Microbiol 41(7):2968–2973
17. Braden CR, Templeton GL, Stead WW et al (1997) Retrospective detection of laboratory cross-contamination of *Mycobacterium tuberculosis* cultures with use of DNA fingerprint analysis. Clin Infect Dis 24:35–40
18. Dahle UR, Sandven P, Heldal E et al (2001) Molecular epidemiology of *Mycobacterium tuberculosis* in Norway. J Clin Microbiol 29(5):1802–1807
19. Fujikane T, Fujiuchi S, Yamazaki Y et al (2004) Molecular epidemiology of tuberculosis in the north Hokkaido district of Japan. Int J Tuberc Lung Dis 8(1):39–44
20. Hernandez-Garduno E, Cook V, Kunimoto D, Elwood RK, Black WA, FitzGerald JM (2004) Transmission of tuberculosis from smear negative patients: a molecular epidemiology study. Thorax 59(4):286–290
21. Asgharzadeh M, Kafil HS, Roudsary AA, Hanifi GR (2011) Tuberculosis transmission in Northwest of Iran: using MIRU-VNTR, ETR-VNTR and IS6110-RFLP methods. Infect Genet Evol 11(1):124–131
22. Ribeiro FK, Lemos EM, Hadad DJ et al (2009) Evaluation of low-colony-number counts of *Mycobacterium tuberculosis* on solid media as a microbiological marker of cross-contamination. J Clin Microbiol 47(6):1950–1952
23. Allix-Beguec C, Fauville-Dufaux M, Supply P (2008) Three-year population-based evaluation of standardized mycobacterial interspersed repetitive-unit-variable-number tandem-repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol 46(4):1398–1406
24. Yan JJ, Jou R, Ko WC, Wu JJ, Yang ML, Chen HM (2005) The use of variable-number tandem-repeat mycobacterial interspersed

repetitive unit typing to identify laboratory cross-contamination with *Mycobacterium tuberculosis*. Diagn Microbiol Infect Dis 52(1):21–28
25. Drobniewski FA, Gibson A, Ruddy M et al (2003) Evaluation and utilization as a public health tool of a national molecular epidemiological Tuberculosis outbreak database within the United Kingdom from 1997 to 2001. J Clin Microbiol 41(5):1861–1868
26. Gascoyne-Binzi DM, Barlow REL, Frothingham R et al (2001) Rapid identification of laboratory contamination with *Mycobacterium tuberculosis* using variable number tandem repeat analysis. J Clin Microbiol 39(1):69–74
27. Globan M, Lavender C, Leslie D et al (2016) Molecular epidemiology of tuberculosis in Victoria, Australia, reveals low level of transmission. Int J Tuberc Lung Dis 20(5):652–658
28. Glynn JR, Yates MD, Crampin AC et al (2004) DNA fingerprint changes in Tuberculosis: reinfection, evolution or laboratory error? J Infect Dis 190:1158–1166
29. Godfrey-FAussett P, Sonnenberg P, Shearer SC et al (2000) Tuberculosis control and molecular epidemiology in a South African gold-mining community. Lancet 356:1066
30. Hayward AC, Goss S, Drobniewski F et al (2002) The molecular epidemiology of tuberculosis in inner London. Epidemiol Infect 128:175–184
31. Jasmer RM, Roemer M, Hamilton J et al (2002) A prospective multicenter study of laboratory cross0contamination of *Mycobacterium tuberculosis* cultures. Emerg Infect Dis 8(11):1260–1263
32. Jasmer RM, Bozeman L, Schwartzman K et al (2004) Recurrent tuberculosis in United States and Canada: relapse or reinfection? Am J Respir Crit Care Med 170(12):1360–1366
33. Lai CC, Tan CK, Lin SH et al (2010) Molecular evidence of false-positive cultures of *Mycobacterium tuberculosis* in a Taiwanese hospital with a high incidence of TB. Chest 137(5):1065–1070
34. McConkey SJ, Williams M, Weiss D et al (2002) Prospective use of molecular typing of *Mycobacterium tuberculosis* by use of restriction fragment-length polymporphism in a public tuberculosis-control program. Clin Infect Dis 34:612–619
35. Nitta AT, Knowles LA, Kim J et al (2002) Limited transmission of multidrug-resistant Tuberculosis despite a high proportion of infectious cases in Los Angeles County, California. Am J Respir Crit Care Med 165:812–817
36. Maguire H, Dale JW, McHugh TD et al (2002) Molecular epidemiology of tuberculosis in London 1995-7 showing low rate of active transmission. Thorax 57:617–622
37. Ruddy M, McHugh TD, Dale JW et al (2002) Estimation of the rate of unrecognized cross-contamination with *Mycobacterium tuberculosis* in London Microbiology Laboratories. J Clin Microbiol 40(11):4100–4104
38. Thumamo BP, Asuquo AE, Abia-Bassey LN et al (2012) Molecular epidemiology and genetic diversity of *Mycobacterium tuberculosis* complex in the Cross River State, Nigeria. Infect Genet Evol 12(4):671–677
39. Carroll NM, Richardson M, Engelke E, de Kock M, Lombard C, van Helden PD (2002) Reduction of the rate of false-positive cultures of *Mycobacterium tuberculosis* in a laboratory with a high culture positivity rate. Clin Chem Lab Med 40(9):888–892
40. Lewinsohn DM, Leonard MK, LoBue PA et al (2017) Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of tuberculosis in adults and Children. Clin Infect Dis 64(2):111–115
41. Jonsson J, Hoffner S, Berggren I et al (2014) Comparison between RFLP and MIRU-VNTR genotyping of *Mycobacterium tuberculosis* strains isolated in Stockholm 2009 to 2011. PLoS ONE 9(4):e95159
42. Kamerbeek J, Schouls L, Kolk A et al (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 35(4):907–914
43. Daum LT, Konstantynovska OS, Solodiankin OS et al (2018) Next-generation sequencing for characterizing drug resistance-conferring *Mycobacterium tuberculosis* Genes from Clinical Isolates in the Ukraine. J Clin Microbiol 56(6):e00009-18
44. Northrup JM, Miller AC, Nardell E et al (2002) Estimated costs of false laboratory diagnoses of tuberculosis in three patients. Emerg Infect Dis 8(11):1264–1270
45. Larson JL, Lambert L, Stricof RL, Driscoll J, McGarry MA, Ridzon R (2003) Potential nosocomial exposure to *Mycobacterium tuberculosis* from a bronchoscope. Infect Control Hosp Epidemiol 24(11):825–830
46. Asgharzadeh M, Khakpour M, Salehi TZ, Kafil HS (2007) Use of mycobacterial interspersed repetitive unit-variable-number tandem repeat typing to study *Mycobacterium tuberculosis* isolates from East Azarbaijan province of Iran. Pak J Biol Sci 10(21):3769–3777

Affiliations

Aleksandra Barac[1,2] · Hannah Karimzadeh-Esfahani[3] · Mahya Pourostadi[4] · Mohammad Taghi Rahimi[5] · Ehsan Ahmadpour[6,7] · Jalil Rashedi[8] · Behroz Mahdavipoor[9,10] · Hossein Samadi Kafil[11,12] · Adel Spotin[12] · Kalkidan Hassen Abate[13] · Alexander G. Mathioudakis[3,14] · Mohammad Asgharzadeh[15]

1 Clinic for Infectious and Tropical Diseases, Clinical Centre of Serbia, Belgrade, Serbia

2 Faculty of Medicine, University of Belgrade, Belgrade, Serbia

3 Division of Infection, Immunity and Respiratory Medicine, The University of Manchester, Manchester, UK

4 Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

5 School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

6 Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

7 Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

8 Tuberculosis and Lung Disease Research Center, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran

[9] Department of Laboratory Science, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran

[10] Department of Medical Parasitology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

[11] Department of Microbiology, Tabriz University of Medical Sciences, Tabriz, Iran

[12] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[13] Institute of Health Sciences, Jimma University, Jimma, Ethiopia

[14] North West Lung Centre, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Manchester, UK

[15] Faculty of Paramedicine, Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran