Natural Built Social Environment Health
ISSN 3085-461X
www.nbseh.org

Failure of First-Generation GeneXpert MTB/RIF to Detect Rifampicin Resistance in Cutaneous Tuberculosis: A Case Report

Kholoud Maafi [1,2,3,a], Zakaria Asbai [1,b], Karim Souly [2,3,c], Souad Aboudkhil [1,d]

[1] *Laboratory of Biotechnology, Agri-Food, Materials and Environment,* Hassan II University of Casablanca

[2] *Ibn Sina University Hospital in Rabat ,Rabat, Morocco*

[3] *Faculty of Medicine and Pharmacy of Rabat, Mohamed V University , Rabat, Morocco*

Check for updates

Verify that you are reading the current Version of Record and access any official updates

[a] ***ORCID:*** 0009-*0004*-8128-2149*;* ***Email:*** *kholoud.maafi@pasteur.ma*

[b] ***ORCID:*** *0000-0003-2748-3973****;*** ***Email:*** *zakaria.asbai@etu.fstm.ac.ma*

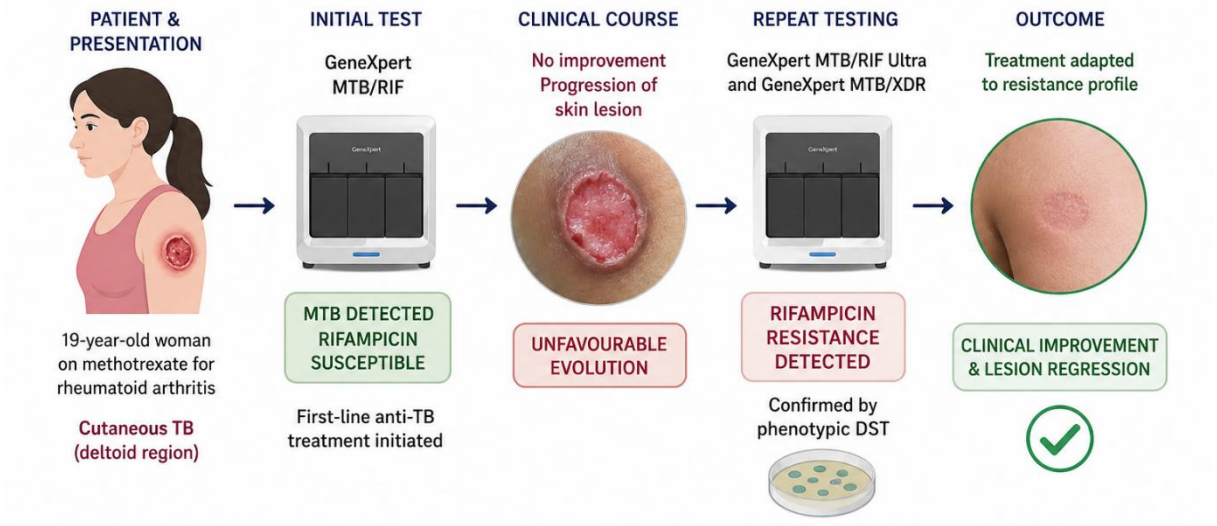
[c] ***ORCID:*** *****-****-****-****; **Email**: sl.karim@hotmail.fr*

[d] ***ORCID****: 0000-0001-5666-0970; **Email:** souadaboudkhil@gmail.com*

*** *Corresponding author :*** Kholoud Maafi ; ***Email*** : *Docteurmaafi@gmail.com*

Graphical Abstract

Cutaneous tuberculosis is a rare extrapulmonary form whose diagnosis remains challenging because of its low bacillary load. We report the case of a 19-year-old Moroccan woman receiving methotrexate for rheumatoid arthritis who developed deltoid cutaneous tuberculosis. Initial GeneXpert MTB/RIF detected *Mycobacterium tuberculosis* but failed to identify rifampicin resistance. GeneXpert MTB/RIF Ultra and GeneXpert MTB/XDR subsequently confirmed rifampicin monoresistance, allowing appropriate treatment modification and rapid clinical improvement. This case highlights the limitations of first-generation GeneXpert MTB/RIF in paucibacillary cutaneous tuberculosis and the value of next-generation molecular techniques for the early detection of drug resistance.



DOI: 10.63095/NBSEH.26.779811

Received: *18 June 2026*
Revised: *19 July 2026*
Accepted: *25 July 2026*
Published online: *07 October 2026*

Citation: Maafi, K., Asbai, Z., Souly, K., & Aboudkhil, S., 2026, Failure of first-generation GeneXpert MTB/RIF to detect rifampicin resistance in cutaneous tuberculosis: A case report. *Natural Built Social Environment Health* **2**(5), 74-94. http://www.doi.org/10.63095/NBSEH.26.779811

Abstract: Cutaneous tuberculosis is a rare form of extrapulmonary tuberculosis. The diagnosis remains challenging because of its clinical polymorphism and low bacillary load. We report the case of a 19-year-old Moroccan woman receiving methotrexate for recently diagnosed rheumatoid arthritis who developed cutaneous tuberculosis in the deltoid region. Initial GeneXpert MTB/RIF analysis detected *Mycobacterium tuberculosis* without identifying rifampicin resistance, leading to first-line anti-tuberculosis treatment. Despite excellent adherence and multidisciplinary follow-up, the lesion progressively worsened. Repeat molecular testing with GeneXpert MTB/RIF Ultra and GeneXpert MTB/XDR revealed rifampicin monoresistance, subsequently confirmed by phenotypic drug susceptibility testing. Adjustment of the treatment regimen according to the resistance profile resulted in rapid clinical improvement and progressive lesion regression. This case highlights the diagnostic limitations of the first-generation GeneXpert MTB/RIF assay in paucibacillary cutaneous tuberculosis and emphasizes the value of next-generation molecular techniques for the early detection of anti-tuberculosis drug resistance, particularly in immunocompromised patients.

Keywords: *Cutaneous tuberculosis, Rifampicin resistance, GeneXpert MTB/RIF Ultra.*

Introduction

Tuberculosis (TB), one of the leading infectious diseases responsible for high global morbidity, remains the deadliest infectious disease, and is among the ten leading causes of death worldwide [1]. Although considerable progress has been made in its diagnosis and management, the World Health Organization (WHO) estimated that approximately 10.7 million people developed TB in 2024, resulting in nearly 1.25 million deaths [1,2]. The pulmonary form is predominant, but *Mycobacterium tuberculosis* (MTB) exhibits systemic tropism and can affect virtually any organ of the body, giving rise to a wide spectrum of clinical manifestations [1,2].

These various anatomical localizations, often characterized by a polymorphic clinical presentation and a low bacillary load (paucibacillary forms), are similar to numerous inflammatory, infectious, and neoplastic diseases. In addition to the considerable overlap between the clinical, radiological, and histopathological manifestations of extrapulmonary tuberculosis (EPTB) and those of many other diseases, the difficulty in obtaining representative clinical specimens and the variable performance of conventional microbiological techniques further complicate the diagnosis, making EPTB a major diagnostic challenge and justifying the use of rapid, accurate, and sensitive molecular methods [2–4].

In the Kingdom of Morocco, TB remains a major public health problem despite the efforts undertaken through the National Tuberculosis Control Programme (PNLAT). According to the World Health Organization (WHO), the estimated incidence has gradually declined over recent decades, from 115 to 92 cases per 100,000 population between 2000 and 2023. Nevertheless, the disease remains active, with approximately 33,000 new cases reported in 2024 [2]. The proportion of extrapulmonary tuberculosis (EPTB) has also increased, accounting for nearly half of all reported cases (49% in 2024), with lymph node (51%) and pleural (26%) forms being the most frequent [2] (Figure 1). This proportion exceeded 53% in 2025, according to the ministerial statement issued on the occasion of World Tuberculosis Day on 24 March 2026, in which the Minister of Health and Social Protection instructed that the screening and management of extrapulmonary tuberculosis (EPTB) should be strengthened [5].

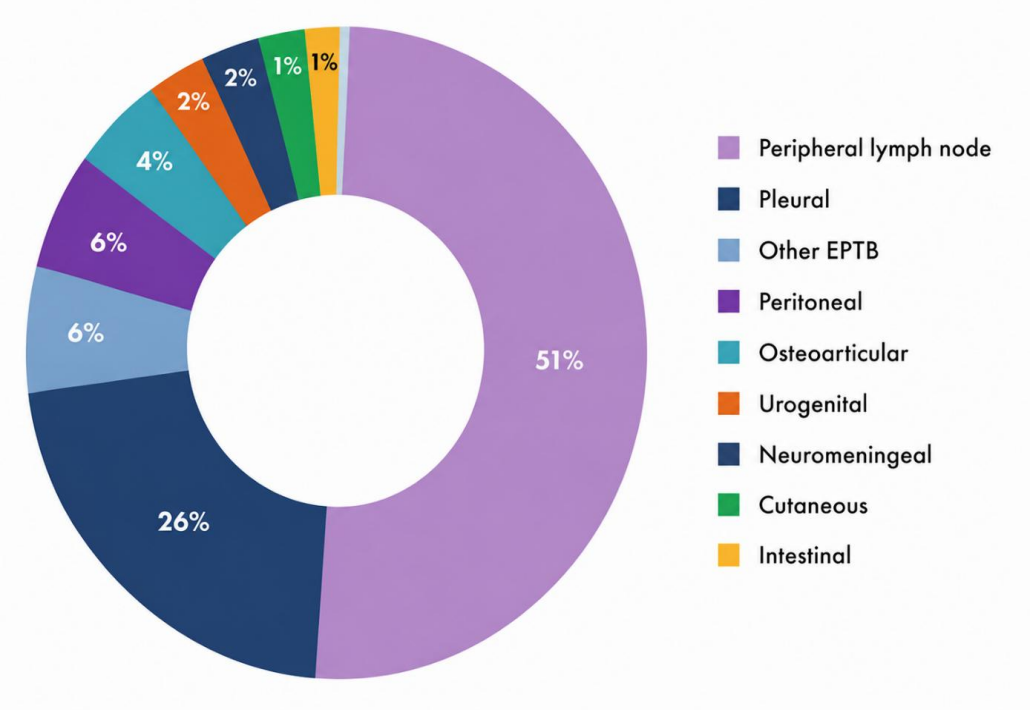


Figure 1. Distribution of newly notified extrapulmonary tuberculosis (EPTB) cases according to anatomical site in Morocco, 2024. *Source:* National Tuberculosis Control Guidelines, Ministry of Health and Social Protection (2025) [2]

Among the extrapulmonary tuberculosis (EPTB) localizations, the cutaneous form remains rare, accounting for approximately 1% of EPTB cases reported in Morocco [2,3] (Figure 1). Cutaneous tuberculosis (cutaneous TB) constitutes a distinct clinical entity, characterized by a wide diversity of dermatological manifestations, which may present as nodular, verrucous, ulcerative, or infiltrative lesions. Its rarity, frequently non-specific and atypical clinical presentation, together with the paucibacillary nature of cutaneous lesions, further delays and complicates the diagnosis [4,6].

This diagnostic difficulty may be worse in immunocompromised patients, particularly those living with HIV or receiving immunosuppressive therapy, in whom the risk of reactivation of latent tuberculosis infection or the development of atypical forms is higher. Furthermore, the emergence of drug-resistant *Mycobacterium tuberculosis* (MTB) strains, particularly rifampicin-resistant tuberculosis (RR-TB), represents an additional threat that may lead to treatment failure and prolonged clinical progression [1,4,7].

In this context, the rapid identification of MTB together with the early determination of the resistance profile is indispensable for the timely adaptation of the therapeutic regimen. Molecular techniques, particularly GeneXpert MTB/RIF, GeneXpert MTB/RIF Ultra, and GeneXpert MTB/XDR, have profoundly transformed the diagnostic approach by enabling the simultaneous

detection of mycobacterial DNA and mutations associated with resistance to anti-tuberculosis drugs, especially those active in rifampicin resistance through mutations in the *rpoB* gene [8].

These high-performance diagnostic tools are of crucial value in the diagnosis of paucibacillary extrapulmonary forms, in which conventional methods often lack sufficient sensitivity, and they allow earlier and more appropriate therapeutic decision-making [1,4,6]. In this report, we describe a rare case of rifampicin-monoresistant cutaneous tuberculosis (RR-TB), initially missed by first-line molecular testing (first-generation GeneXpert MTB/RIF), highlighting the importance of extended molecular diagnosis for revealing resistance profiles and optimizing therapeutic management.

Case Report

The patient was a 19-year-old female from the Kingdom of Morocco who had received Bacillus Calmette–Guérin (BCG) vaccination at birth in accordance with the National Immunization Programme (NIP). She was being followed in the Rheumatology Department for recently diagnosed rheumatoid arthritis (RA), and, in this context, her physician initiated immunosuppressive therapy with methotrexate (MTX), administered as subcutaneous injections as part of a chronotherapeutic treatment strategy, at the standard dose of 15 mg per week.

Four months after the initiation of this therapeutic regimen, the patient noticed the gradual appearance of a small cutaneous lesion over the right deltoid region. Initially, it presented as a small inflammatory nodule, slightly erythematous, mildly painful, and without discharge, with no associated systemic symptoms, including fever, deterioration of the general condition, night sweats, or weight loss. In view of the slow but progressive evolution of the lesion and its persistence despite topical treatment with an H1 antihistamine (chlorphenoxamine), a topical antibiotic (fusidic acid), and an antifungal agent (bifonazole), the patient consulted a dermatologist (Figure 2).

Dermatological examination revealed a well-defined, rounded subcutaneous nodular lesion with a firm inflammatory consistency, covered by stretched, slightly erythematous-violaceous skin, mildly raised and tender on palpation, with a non-specific appearance and measuring approximately 3 × 3 cm. The lesion was characterized by a small central blackish area suggesting early fistulization or superficial necrosis, with no discharge observed at the time of clinical examination (Figure 2).



Figure 2. Initial clinical appearance of cutaneous tuberculosis in a 19-year-old female patient receiving immunosuppressive therapy for rheumatoid arthritis, presenting as a small non-specific inflammatory nodule

Although several differential diagnoses were considered, including an inflammatory dermatosis or a chronic cutaneous infection (bacterial or fungal), the dermatologist rapidly suspected cutaneous tuberculosis based on the combination of several suggestive clinical and epidemiological findings. These included the patient's history of pulmonary tuberculosis (PTB) during childhood (at the age of 6 years, she developed PTB caused by rifampicin-susceptible *Mycobacterium tuberculosis* (MTB), which was treated with a standard first-line anti-tuberculosis regimen, resulting in complete recovery after six months of treatment, with no documented recurrence to date), the state of immunosuppression induced by the immunosuppressive therapy prescribed for the treatment of the autoimmune disease (RA), and the endemic nature of TB in the Kingdom of Morocco, despite the complete absence of clinical signs suggesting pulmonary tuberculosis at the time of consultation.

In this clinical context, a skin biopsy was performed and submitted to the Microbiology Laboratory of Ibn Sina University Hospital in Rabat for specialized mycobacteriological investigations in order to confirm biologically the suspected diagnosis of cutaneous tuberculosis before the initiation of an appropriate therapeutic regimen.

Direct bacteriological examination of the biological specimen by smear microscopy following modified Ziehl–Neelsen staining demonstrated the presence of acid-fast bacilli (AFB). In addition,

culture on a specific solid medium (Löwenstein–Jensen medium) revealed the presence of typical *Mycobacterium tuberculosis* colonies after two weeks of incubation at **37°C**.

Molecular diagnosis of the skin biopsy using the GeneXpert MTB/RIF assay, after homogenization of the biopsy specimen in a sterile mortar with sterile physiological saline in order to release the mycobacterial biological material, confirmed the presence of *Mycobacterium tuberculosis* (MTB) DNA, detected at a low level, corresponding to a Cycle Threshold (Ct) value ranging from 22 to 28, consistent with a moderate-to-low bacillary load. Parallel analysis of the probes targeting the rifampicin resistance-determining region (RRDR) of the *rpoB* gene did not reveal any detectable abnormality, leading to an initial interpretation in favour of a rifampicin-susceptible MTB strain.

On the basis of these biological findings, which were communicated to the dermatologist, the diagnosis of cutaneous tuberculosis was confirmed, and the patient was referred to a Centre for the Diagnosis and Treatment of Respiratory Diseases (CDTMR) in the city of Casablanca, where free first-line anti-tuberculosis treatment was prescribed and initiated in accordance with the national recommendations of the National Tuberculosis Control Programme (PNLAT).

The anti-tuberculosis treatment regimen consisted of a standard four-drug combination comprising rifampicin (R), isoniazid (H), pyrazinamide (Z), and ethambutol (E), prescribed for an initial period of two months (2RHZE), followed by a continuation phase consisting of rifampicin and isoniazid for four months (4RH), in accordance with the national recommendations for the management of drug-susceptible tuberculosis.

The patient adhered rigorously to the prescribed therapeutic regimen, with excellent treatment compliance throughout the course of therapy, ensured through the use of an electronic pill dispenser. She complied with the recommended administration schedule for the different medications (oral anti-tuberculosis drugs and systemic immunosuppressive therapy), particularly the recommended chronotherapeutic regimen, in order to optimize the efficacy of anti-tuberculosis treatment and minimize potential drug interactions. Given the concomitant administration of first-line anti-tuberculosis drugs together with methotrexate as immunomodulatory therapy for rheumatoid arthritis, particular attention was paid to the prevention of adverse drug reactions.

Coordination among the five specialists (the rheumatologist, dermatologist, physician from the National Tuberculosis Control Programme (CDTMR), pharmacist, and medical biologist) made it possible to adapt both the therapeutic and biological monitoring of methotrexate, given its potential hepatic and haematological toxicity, together with therapeutic drug monitoring (TDM) of the different anti-tuberculosis drugs and regular biochemical monitoring of renal function throughout the entire course of anti-tuberculosis treatment.

The Medical Biology Department ensured regular biological assessment through laboratory investigations, including a complete blood count (CBC), liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin; together with monitoring of renal function (urea, creatinine, and estimated glomerular filtration rate (eGFR)). These biological parameters were also essential for monitoring anti-tuberculosis treatment because of its prolonged duration.

Despite this optimal management, including excellent treatment adherence without any reported interruption of therapy, avoidance of drug interactions between the prescribed anti-tuberculosis drugs and methotrexate, correct administration of all medications, and appropriate clinical and biological monitoring, the initial clinical course was unfavourable. At the end of treatment, the lesion had worsened. It was larger, more infiltrative, and more inflammatory, with no improvement after several weeks of therapy. This prompted the dermatologist to investigate an underlying microbiological cause, suspecting the presence of a drug-resistant *Mycobacterium tuberculosis* strain (Figure 3).



Figure 3. Clinical progression of cutaneous tuberculosis despite first-line anti-tuberculosis treatment initiated following an initial GeneXpert MTB/RIF result indicating rifampicin susceptibility (no mutation detected in the *rpoB* gene

A second skin biopsy was subsequently submitted to our laboratory, accompanied by a request for anti-tuberculosis drug susceptibility testing. This time, the GeneXpert MTB/RIF Ultra assay detected *Mycobacterium tuberculosis* at a medium level and also revealed rifampicin resistance (RR) through the detection of a mutation in the *rpoB* gene. In the context of suspected multidrug-resistant tuberculosis (MDR-TB), a GeneXpert MTB/XDR assay, allowing a more comprehensive

anti-tuberculosis drug susceptibility assessment, was also performed. This assay did not reveal resistance to the other anti-tuberculosis agents tested, including Group A fluoroquinolones, Group C second-line injectable agents (the aminoglycosides amikacin, kanamycin, and capreomycin), or the newer Group B anti-tuberculosis drugs (bedaquiline and linezolid), with the exception of rifampicin, thereby confirming a rifampicin-monoresistant tuberculosis (RR-TB) profile.

These molecular findings were subsequently confirmed by phenotypic drug susceptibility testing performed on mycobacterial colonies obtained after 10 days of incubation of the culture from the second biopsy on Löwenstein–Jensen solid medium. This anti-tuberculosis drug susceptibility study confirmed the resistance profile identified by the molecular approaches (RR-TB) by assessing the growth of *Mycobacterium tuberculosis* in the presence of the different anti-tuberculosis drugs tested. The concordance between the molecular and phenotypic findings further strengthened the reliability of the resistance diagnosis, allowing precise characterization of the susceptibility profile of the strain isolated from the cutaneous specimen and, consequently, appropriate guidance of therapeutic management.

These biological findings explained the initial therapeutic failure and demonstrated a diagnostic discrepancy between the first-generation GeneXpert MTB/RIF assay and the complementary analyses performed using GeneXpert MTB/RIF Ultra, GeneXpert MTB/XDR, and conventional phenotypic drug susceptibility testing. These mycobacteriological findings led to a reassessment of the therapeutic strategy in accordance with the national recommendations of the National Tuberculosis Control Programme (PNLAT), with the initiation of a new short-course treatment regimen adapted to the identified rifampicin-monoresistant (RR-TB) profile.

This new therapeutic regimen was based on a four-drug combination comprising three Group A anti-tuberculosis agents (bedaquiline (Bdq), linezolid (LZD), and the fluoroquinolone moxifloxacin (Mfx)), combined with pyrazinamide (Z), a Group C agent. This treatment regimen was prescribed for a duration of six months in our patient. Clinical evolution under this new therapeutic regimen was marked, from the first weeks after its initiation, by progressive clinical improvement, characterized by a reduction in local inflammation, decreased cutaneous infiltration, and gradual regression of the tuberculous lesion during medical follow-up (Figure 4).

This favourable outcome was achieved through appropriate and rigorous therapeutic management based on optimal treatment adherence, correct administration of the different anti-tuberculosis drugs, and strict compliance with the recommended chronotherapeutic regimen in order to optimize pharmacological efficacy and minimize potential drug interactions, particularly those related to the concomitant administration of methotrexate (MTX) prescribed for the underlying autoimmune disease.

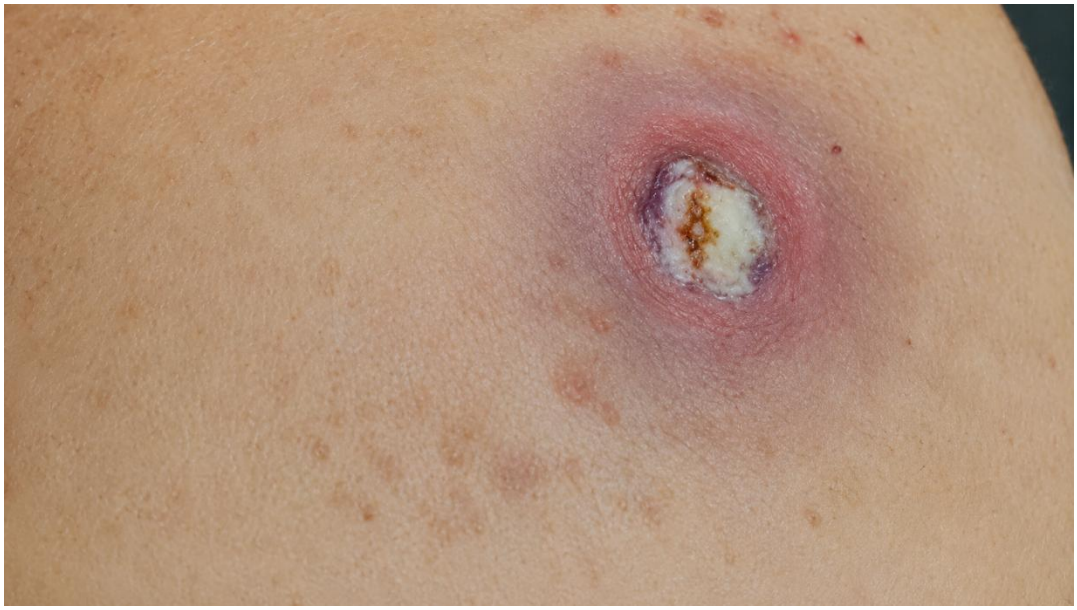


Figure 4. Progressive clinical regression of the cutaneous lesion one month after initiation of second-line anti-tuberculosis treatment

Discussion

Cutaneous tuberculosis constitutes one of the rare forms of extrapulmonary tuberculosis (EPTB), accounting for a small proportion of all extrapulmonary tuberculous manifestations [2,3]. Unlike pulmonary tuberculosis, whose clinical, radiological, and mycobacteriological presentation is generally better characterized and often typical, cutaneous localizations remain particularly difficult to diagnose because of their clinical polymorphism, low bacillary load, and resemblance to numerous dermatological diseases, including psoriasis, chronic eczema, fungal dermatoses, cutaneous sarcoidosis, cutaneous leishmaniasis, and cutaneous squamous cell carcinoma [9].

The skin represents a generally effective natural barrier against the penetration of the tubercle bacillus. But, when skin integrity is disrupted following a wound, trauma, ulceration, or even a surgical procedure, direct inoculation of *Mycobacterium tuberculosis* (MTB) may occur, resulting in cutaneous or mucocutaneous tuberculosis. In addition to direct inoculation, this form of EPTB may also result from contiguous spread or even haematogenous or lymphatic dissemination [6].

Cutaneous lesions may present with highly variable clinical appearances, ranging from an inflammatory nodule to a chronic ulcer, including verrucous or infiltrative forms, making clinical diagnosis frequently difficult and often insufficient [2,6,9]. In our case, the initial presentation as a small, isolated, atypical inflammatory cutaneous nodule, without constitutional symptoms or associated pulmonary manifestations, perfectly illustrates this diagnostic difficulty.

This can be increased in patients with pathological or iatrogenic immunosuppression. In our case, the patient was receiving methotrexate for the treatment of recently diagnosed autoimmune disease, a context known to favour opportunistic diseases such as the reactivation of latent tuberculosis infection and the emergence of atypical forms of the disease [2,10].

A recent meta-analysis published in 2026 confirmed that the introduction of biologic therapies (immunomodulatory treatments), including tumour necrosis factor-alpha (TNF-α) inhibitors, interleukin-17 (IL-17) inhibitors, and ustekinumab (an inhibitor of the p40 subunit shared by interleukins IL-12 and IL-23), which selectively target the immune system, increases the risk of tuberculosis associated with these therapies. This risk differs from that observed with conventional immunosuppressive agents and varies according to the different classes of biologic therapies [2,10].

In any patient receiving immunosuppressive therapy who presents with a chronic cutaneous lesion or a lesion resistant to conventional treatment (topical antifungal agents, H1 antihistamines, topical antibacterial agents, etc.), particularly when aggravated following the application of topical corticosteroids, cutaneous tuberculosis should remain a major differential diagnosis, even in the absence of concomitant active pulmonary tuberculosis, especially in an epidemiological context suggesting tuberculosis endemicity, such as that observed in Morocco.

The main originality of our clinical case lies in the demonstration of rifampicin resistance (RR) that was initially not detected by the first-generation GeneXpert MTB/RIF assay. Although this molecular technique has profoundly transformed the management of tuberculosis owing to its rapidity, sensitivity, and ability simultaneously to detect *Mycobacterium tuberculosis* complex DNA and mutations associated with rifampicin resistance (*rpoB* gene), certain analytical limitations persist. To overcome these limitations, a new version of the GeneXpert MTB/RIF assay was developed under the name **GeneXpert MTB/RIF Ultra**, with the aim of improving, on the one hand, the sensitivity for detecting *Mycobacterium tuberculosis* complex DNA, thereby reducing the limit of detection to **15.6 bacterial CFU/mL**, and, on the other hand, enhancing the sensitivity for detecting rifampicin resistance. The GeneXpert MTB/RIF Ultra assay incorporates an improved cartridge containing two different multicopy amplification targets (IS1081 and IS6110) [11].

The diagnostic performance of GeneXpert MTB/RIF Ultra and GeneXpert MTB/RIF for the detection of rifampicin resistance has been compared in several studies included in a Cochrane systematic review. Regarding GeneXpert MTB/RIF Ultra, five studies evaluated its ability to identify rifampicin resistance, reporting sensitivity values ranging from **50% to 100%**; specificity ranged from 93% to 100% (Table 1) [12].

Difference (Ultra − Xpert) In terms of clinical impact, the predictive analyses from this multicentre study showed that, in a hypothetical population of 1,000 patients, including 100 with rifampicin-resistant tuberculosis (RR-TB), the GeneXpert MTB/RIF Ultra assay would correctly identify all rifampicin-resistant cases without generating either false-positive or false-negative results, according to the estimates of the meta-analysis. By contrast, GeneXpert MTB/RIF would correctly identify the vast majority of rifampicin-resistant cases but could generate a small number of false-positive and false-negative results [12].

Overall, all the studies included in the meta-analysis provided evidence indicating that both assays have excellent diagnostic accuracy for the detection of rifampicin resistance. Although GeneXpert MTB/RIF Ultra demonstrated slightly superior performance in this study, no clinically significant difference was observed between the two techniques [12]. The principal advantage of GeneXpert MTB/RIF Ultra remains its improved detection of *Mycobacterium tuberculosis*, particularly in specimens with a low bacillary load, especially in extrapulmonary tuberculosis, rather than a substantially better identification of rifampicin resistance [12]. Both GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra exhibit excellent technical performance, with comparable sensitivity and specificity for the detection of rifampicin resistance. This meta-analysis also suggests that future researchers should consider the limitations of Löwenstein–Jensen culture as the reference standard for paucibacillary specimens and explore alternative approaches to overcome these limitations [12]. (Table 1)

A prospective multicentre study evaluating the diagnostic performance of GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra for the detection of rifampicin resistance (RR) showed that both molecular techniques exhibit excellent diagnostic accuracy and overall comparable performance. The authors reported a sensitivity of **95%** (95% CI: 91–98%) for both assays, together with a specificity of **98%** (95% CI: 96–99% for GeneXpert MTB/RIF and 97–99% for GeneXpert MTB/RIF Ultra) [13] (Figure 7). These findings indicate that the introduction of the Ultra technology has not significantly improved the performance of the assay for the identification of rifampicin resistance. Its principal contribution is that it increases the sensitivity for detecting the *Mycobacterium tuberculosis* complex, particularly in paucibacillary specimens. With regard to the detection of rifampicin resistance, both analytical methods provide equivalent diagnostic reliability and constitute effective tools for the early guidance of therapeutic management [36].

In contrast, a study investigating discordant rifampicin resistance detection between GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra demonstrated excellent reproducibility of both techniques for all isolates tested at moderate and low bacterial concentrations, with the exception

of two weakly enriched specimens. GeneXpert MTB/RIF Ultra produced a **"trace-positive"** result for one specimen harbouring the **H445D** mutation, thereby preventing determination of the melting temperature (Tm), whereas GeneXpert MTB/RIF failed to detect the *Mycobacterium tuberculosis* complex in another specimen carrying the **P439L** mutation [37] (Table 2).

Table 1. Forest plots comparing the diagnostic accuracy of GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra for the detection of rifampicin resistance. Each row represents an individual study. Blue squares indicate the estimated sensitivity or specificity, and horizontal lines represent the corresponding 95% confidence intervals. TP: true positive; FP: false positive; FN: false negative; TN: true negative.

Rifampicin resistance – Xpert MTB/RIF							
Study	**Year**	**TP**	**FP**	**FN**	**TN**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Biadglegne *et al.* [13]	2014	2	1	0	26	1.00 [0.16, 1.00]	0.96 [0.81, 1.00]
Dhasmana *et al.* [14]	2014	1	0	0	26	1.00 [0.03, 1.00]	1.00 [0.87, 1.00]
Bera *et al.* [15]	2015	1	0	0	1	1.00 [0.03, 1.00]	1.00 [0.03, 1.00]
Al-Ateah *et al.* [16]	2012	2	0	0	14	1.00 [0.16, 1.00]	1.00 [0.77, 1.00]
Hanif *et al.* [17]	2011	1	0	0	10	1.00 [0.03, 1.00]	1.00 [0.69, 1.00]
Friedrich *et al.* [18]	2011	1	0	0	4	1.00 [0.03, 1.00]	1.00 [0.40, 1.00]
Gu *et al.* [19]	2015	6	0	0	18	1.00 [0.54, 1.00]	1.00 [0.81, 1.00]
Nhu *et al.* [20]	2014	3	0	0	104	1.00 [0.29, 1.00]	1.00 [0.97, 1.00]
Meldua *et al.* [21]	2014	1	0	0	4	1.00 [0.03, 1.00]	1.00 [0.40, 1.00]
Wang *et al.* [22]	2020	5	0	0	21	1.00 [0.48, 1.00]	1.00 [0.84, 1.00]
Rufai *et al.* [23]	2015	1	0	0	17	1.00 [0.03, 1.00]	1.00 [0.80, 1.00]
Peter *et al.* [24]	2016	1	0	0	28	1.00 [0.03, 1.00]	1.00 [0.88, 1.00]
Rufai *et al.* [25]	2017	39	0	0	22	1.00 [0.91, 1.00]	1.00 [0.85, 1.00]
Vadwai *et al.* [26]	2011	39	5	1	80	0.97 [0.87, 1.00]	0.94 [0.87, 0.98]
Nataraj *et al.* [27]	2016	28	0	1	121	0.97 [0.82, 1.00]	1.00 [0.97, 1.00]
Sharma *et al.* [28]	2014	26	3	1	211	0.96 [0.81, 1.00]	0.99 [0.96, 1.00]
Li *et al.* [29]	2017	11	0	1	47	0.92 [0.62, 1.00]	1.00 [0.92, 1.00]
Du *et al.* [30]	2015	9	2	1	31	0.90 [0.55, 1.00]	0.94 [0.80, 0.99]
Ligthelm *et al.* [31]	2011	1	0	1	26	0.50 [0.01, 0.99]	1.00 [0.87, 1.00]
Rifampicin resistance – Xpert MTB/RIF Ultra							
Study		**TP**	**FP**	**FN**	**TN**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Wang *et al.* [32]	2019	6	0	0	23	1.00 [0.54, 1.00]	1.00 [0.85, 1.00]
Sun *et al.* [33]	2019	9	0	0	38	1.00 [0.66, 1.00]	1.00 [0.91, 1.00]
Wu *et al.* [34]	2019	4	0	0	23	1.00 [0.40, 1.00]	1.00 [0.85, 1.00]
Wang *et al.* [35]	2020	5	0	0	21	1.00 [0.48, 1.00]	1.00 [0.84, 1.00]

Reproduced from Kohli *et al.* [12]

Table 2. Comparison of the diagnostic performance of GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra for the detection of tuberculosis and rifampicin resistance. Adapted from Kohli *et al.* [36]

Assay	Sensitivity: All culture-positive	Sensitivity: Smear-negative	Sensitivity: HIV-negative	Sensitivity: HIV-positive	Specificity	Sensitivity for rifampicin resistance	Specificity for rifampicin resistance
Xpert MTB/RIF	83% (79–86; 383/462)	46% (37–55; 63/137)§	90% (84–94; 143/159)	77% (68–84; 88/155)	98% (97–99; 960/977)	95% (91–98; 167/175)	98% (96–99; 369/376)
Xpert MTB/RIF Ultra	88% (85–91; 408/462)	63% (54–71; 86/137)§	91% (86–95; 145/159)	90% (83–95; 103/115)	96% (94–97; 934/977)	95% (91–98; 166/175)	98% (97–99; 370/376)
Difference (Ultra − Xpert)	5.4% (3.3–8.0; 25/162)	17% (10–24; 23/137)	1.3% (−1.8 to 4.9; 2/159)	13% (6.4–21; 15/115)	−2.7% (−3.9 to −1.7; 36/977)	−0.6% (−3.2 to 1.6; 1/175)	0.3% (−0.7 to 1.5; 1/376)
Non-inferiority margin	Not predefined	−7%	Not predefined	Not predefined	Not predefined	−3%	−3%

Values are expressed as % (95% CI; n/N). CI, confidence interval; n, number of positive results; N, total number of samples

This study, based on the use of experimental **mock samples**, specifically evaluated the performance of rifampicin resistance (RR) detection, thereby further supporting the relevance of GeneXpert MTB/RIF Ultra in this context [14]. This improved version (GeneXpert MTB/RIF Ultra) showed a trend towards higher sensitivity than GeneXpert MTB/RIF in a high-resource setting with an intermediate incidence of tuberculosis, although this difference did not reach statistical significance. Its specificity was comparable to that of GeneXpert MTB/RIF, despite the occurrence of a small number of false-positive results among the **"trace-positive"** and **"very low-positive"** categories. Furthermore, certain mutations located within the region targeted by the **rpoB1** probe resulted in indeterminate rifampicin resistance results [37] (Table 3)

Table 3. Comparison of the performance of GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra for the detection of different *rpoB* mutations associated with rifampicin resistance [37]

rpoB mutation(s)	Phenotypic DST (RIF)	Xpert MTB/RIF	Xpert MTB/RIF Ultra
Q432P (Q513P)	R	Detected	Detected
D435Y (D516Y)	R	Detected	Detected
P439L (P520L)	S	Detected	Detected
H445D (H526D)	R	Detected	Detected
H445Y (H526Y)	R	Detected	Detected
S450L (S531L)	R	Detected	Detected
I491F (I572F)	R	Detected	Detected
D545E (D626E)	S	Detected	Detected
Q429H + H430P + H445Q	R	Detected	Detected
Q432K + H445D	R	Detected	Detected
D435N + H445N	R	Detected	Detected
Q432_D435delinsHI	R	Detected	Detected

Abbreviations: DST, drug susceptibility testing; RIF, rifampicin; R, resistant; S, susceptible.

The discordance observed in our clinical case between the results of GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra also raises the issue of the management of rare rifampicin-resistant strains. Resistance to this first-line anti-tuberculosis drug (R) is mainly caused (in approximately 95% of cases) by genetic variants within the rifampicin resistance-determining region (RRDR) of the *rpoB* gene, which encodes the β-subunit of RNA polymerase [38].

Rifampicin resistance is of particular importance because it constitutes an indirect marker strongly associated with multidrug-resistant tuberculosis. Early detection of rifampicin-

resistant tuberculosis (RR-TB) is therefore essential for the prompt adaptation of the therapeutic strategy, thereby avoiding the prolonged use of ineffective treatment regimens that may favour the acquisition of additional drug resistance while increasing the risk of adverse effects associated with anti-tuberculosis drugs administered for at least six months. In our case, the delayed identification of rifampicin resistance resulted in initial exposure to a treatment regimen to which the infecting strain was not susceptible, probably explaining the absence of clinical improvement despite appropriate therapeutic management based on the mycobacteriological findings obtained from the first biopsy

Conclusion

This case highlights the diagnostic challenges and potential limitations associated with cutaneous tuberculosis, a rare form of extrapulmonary tuberculosis whose atypical clinical presentation, often combined with a low bacillary load, frequently delays diagnosis. In patients receiving immunosuppressive therapy, the occurrence of chronic or unusual cutaneous lesions should raise suspicion of cutaneous tuberculosis, even in the absence of pulmonary manifestations, particularly in an endemic setting. This clinical case also illustrates that an initial rifampicin-susceptible result obtained by GeneXpert MTB/RIF does not definitively exclude rifampicin resistance when the clinical course is unfavourable. The use of next-generation molecular techniques, particularly GeneXpert MTB/RIF Ultra and GeneXpert MTB/XDR, combined with reference phenotypic methods, constitutes an essential strategy for the accurate and early identification of drug resistance profiles, thereby allowing prompt adaptation of the therapeutic regimen.

References

[1] World Health Organization, 2024, Global tuberculosis report 2020. World Health Organization (October, 2024). https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024

[2] Ministère de la Santé et de la Protection Sociale, 2025, Guide National de la lutte antituberculeuse au Maroc. Programme National de Lutte Antituberculeuse, Direction de l'Épidémiologie et de Lutte contre les Maladies. https://fr.scribd.com/document/939446213/Guide-du-PNLAT-2025-251008-095850

[3] Hailu, S., Hurst, C., Cyphers, G., Thottunkal, S., Harley, D., Viney, K., Irwin, A., Dean, J., & Nourse, C., 2024, Prevalence of extra-pulmonary tuberculosis in Africa: A

systematic review and meta-analysis. *Tropical Medicine & International Health* **29**(4), 257-265. https://doi.org/10.1111/tmi.13970

[4] Ministry of Health and Social Protection of Morocco, 2026, The Ministry of Health and Social Protection strengthens screening and management efforts for all forms of tuberculosis on the occasion of World Tuberculosis Day 2026 [Translated from the original French]. Ministerial communiqué. (Rabat: Ministry of Health and Social Protection). https://www.sante.gov.ma/Pages/communiqués.aspx?communiqueID=485&

[5] Jain R., Gupta G., Mitra D. K., & Randeep Guleria R., 2024, Diagnosis of extra pulmonary tuberculosis: An update on novel diagnostic approaches. *Respiratory Medicine* **225**, 107601. doi: 10.1016/j.rmed.2024.107601

[6] de Brito A. C., de Oliveira C. M., Unger D. A-A., & de Jesus Semblano Bittencour M., 2022, Cutaneous tuberculosis: epidemiological, clinical, diagnostic and therapeutic update. *Anais Brasileiros de Dermatologia* **97**(2), 129-144. https://doi.org/10.1016/j.abd.2021.07.004

[7] Qiu B., Wu Z., Tao B., Li Z., Song H., Dan Tian D., Wu J., Zhan M., & Jianming Wang Send J. W., 2022, Risk factors for types of recurrent tuberculosis (reactivation versus reinfection): A global systematic review and meta-analysis. *International Journal of Infectious Diseases* **116,** 14–20. https://doi.org/10.1016/j.ijid.2021.12.344

[8] World Health Organization, 2024, Manuel opérationnel de l'OMS sur la tuberculose. Module 3 : diagnostic - outils de diagnostic rapide pour le dépistage de la tuberculose. https://www.who.int/fr/publications/b/58221

[9] Mexico, U. C. A. A., González, J. F. M., González, A. L. R., Del Estado de Puebla Puebla Mexico, U. P. A., Servín, M. M., De Guadalajara Zapopan Mexico, U. A., Ávila, P. I. N., De Guadalajara Zapopan Mexico, U. A., Almanza, A. M., & De México Celaya Mexico, U. L., 2025), Cutaneous Tuberculosis : A Comprehensive Review of Etiopathogenesis, Clinical Subtypes, Diagnostic Modalities, and Contemporary Therapeutic Approaches. *International Journal of Medical Science and Clinical Research Studies* **5**(05). https://doi.org/10.47191/ijmscrs/v5-i05-06

[10] Kaixuan Liu K., Zeng Z., MD; Lu W., Yang Y., Lin T., 2026, Tuberculosis-Related Outcomes in Dermatologic Patients, Receiving Systemic Therapy A Systematic Review and Meta-Analysis. *JAMA Dermatology*. www.doi.org/10.1001/jamadermatol.2026.2365

[11] Jiang J., Yang J., Shi Y., Jin Y., Tang S., Zhang N., Lu Y., & Sun G., 2020, Head-to-head comparison of the diagnostic accuracy of Xpert MTB/RIF and Xpert MTB/RIF Ultra for tuberculosis: a meta-analysis. *Infectious Infectious Diseases* **52**(11):763-775. https://doi.org/10.1080/23744235.2020.1788222

[12] A. Kohli, M., Schiller, I., Dendukuri, N., Yao, M., Dheda, K., Denkinger, C. M., Schumacher, S. G., & Steingart, K. R., 2021, Xpert MTB/RIF Ultra and Xpert MTB/RIF assays for extrapulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database of Systematic Reviews* (1), CD012768. https://doi.org/10.1002/14651858.cd012768.pub3

[13] Biadglegne, F., Tesfaye, W., Sack, U., & Rodloff, A. C., 2013, Tuberculous Lymphadenitis in Northern Ethiopia : In a Public Health and Microbiological Perspectives. *PLoS ONE* **8**(12), e81918. https://doi.org/10.1371/journal.pone.0081918

[14] Dhasmana, D. J., Ross, C., Bradley, C. J., Connell, D. W., George, P. M., Singanayagam, A., Jepson, A., Craig, C., Wright, C., Molyneaux, P. L., Wickremasinghe, M., Lalvani, A., Cooke, G. S., & Kon, O. M., 2014, Performance of Xpert MTB/RIF in the Diagnosis of Tuberculous Mediastinal Lymphadenopathy by Endobronchial Ultrasound. *Annals of the American Thoracic Society* **11**(3), 392-396. https://doi.org/10.1513/annalsats.201308-250oc

[15] Bera, C., Michael, J. S., Burad, D., Shirly, S. B., Gibikote, S., Ramakrishna, B., Goel, A., & Eapen, C. E., 2015, Tissue XpertTM MTB/Rif assay is of limited use in diagnosing peritoneal tuberculosis in patients with exudative ascites. *Indian Journal of Gastroenterology* **34**(5), 395-398. https://doi.org/10.1007/s12664-015-0599-7

[16] Al-Ateah S. M., Al-Dowaidi M. M, El-Khizzi N. A., 2012, Evaluation of direct detection of Mycobacterium tuberculosis complex in respiratory and non-respiratory clinical specimens using the Cepheid Gene Xpert(R) system. *Saudi Medical Journal* **33** (10):1100-5. https://pubmed.ncbi.nlm.nih.gov/23047207/

[17] Hanif, S. N. M., Eldeen, H. S., Ahmad, S., & Mokaddas, E., 2011, GeneXpert® MTB/RIF for rapid detection of Mycobacterium tuberculosis in pulmonary and extra-pulmonary samples [Correspondence]. *The International Journal of Tuberculosis and Lung Disease* **15**(9), 1274-1275. https://doi.org/10.5588/ijtld.11.0394

[18] Friedrich S.O., Von Groote-Bidlingmaier F., & Diacon A. H., 2011, Xpert MTB/RIF assay for diagnosis of pleural tuberculosis. *Journal of Clinical Microbiology* **49**(12):4341-2. https://doi.org/10.1128/jcm.05454-11

[19] Gu Y., Wang G., Dong W., Li Y., Ma Y., Shang Y., Qin S., & Huang H., 2015, Xpert MTB/RIF and GenoType MTBDRplus assays for the rapid diagnosis of bone and joint tuberculosis. *International Journal of Infectious Diseases* **36**, 27-30. https://doi.org/10.1016/j.ijid.2015.05.014

[20] Nhu N. T., Heemskerk D., Thu do D.A., Chau T. T., Mai N.T., Nghia H. D., Loc P. P., Ha T. M., Merson L., Thinh T. V., Day J., Chau N. V., Wolbers M., Farrar J., & Caws M., 2014, Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. *Journal of Clinical Microbiology* **52**(1):226-33. www.doi.org/10.1128/JCM.01834-13

[21] Meldau, R., Peter, J., Theron, G., Calligaro, G., Allwood, B., Symons, G., Khalfey, H., Ntombenhle, G., Govender, U., Binder, A., Van Zyl-Smit, R., Dheda, K., 2014, Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN-γ for the evaluation of pleural tuberculosis : a prospective cohort study. *BMC Pulmonary Medicine* **14**(1), 58. https://doi.org/10.1186/1471-2466-14-58

[22] Wang G., Wang S., Yang X., Sun Q., Jiang G., Huang M., Huo F., Ma Y., Chen X., Huang H., 2020, Accuracy of Xpert MTB/RIF Ultra for the diagnosis of pleural TB in a multicenter cohort study. *Chest* 157(2), 268-275. https://pubmed.ncbi.nlm.nih.gov/31437432/

[23] Rufai, S. B., Singh, A., Kumar, P., Singh, J., & Singh, S., 2015, Performance of Xpert MTB/RIF Assay in Diagnosis of Pleural Tuberculosis by Use of Pleural Fluid Samples. *Journal of Clinical Microbiology* **53**(11), 3636-3638. https://doi.org/10.1128/jcm.02182-15

[24] Peter JG, Zijenah LS, Chanda D, Clowes P, Lesosky M, Gina P, Mehta N., Calligaro G., Lombard C. J., Kadzirange G., Bandason T., Chansa A., Liusha N., Mangu C., Mtafya B., Msila H., Rachow A., Hoelscher M., Mwaba P., Theron G., Keertan Dheda K., 2016, Effect on mortality of point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group, multicountry, open-label, randomised controlled trial. *Lancet* **387**(10024):1187-97. https://pubmed.ncbi.nlm.nih.gov/26970721/

[25] Rufai, S. B., Singh, A., Singh, J., Kumar, P., Sankar, M. M., Singh, S., Nigam, K., Sharma, D., Gupta, A. K., &Rawat, V. S., 2017, Diagnostic usefulness of Xpert MTB/RIF assay for detection of tuberculous meningitis using cerebrospinal fluid. *Journal of Infection* **75**(2), 125-131. https://doi.org/10.1016/j.jinf.2017.04.010

[26] Vadwai V., Boehme C., Nabeta P., Shetty A., Alland D., & Rodrigues C., 2011, Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *Journal of Clinical Microbiology* **49**(7), 2540-5. https://pubmed.ncbi.nlm.nih.gov/21593262/

[27] Nataraj G., Kanade S., Mehta P., 2016, Xpert((R)) MTB/RIF for improved case detection of extra-pulmonary TB in a tertiary care setting in urban India. International *Journal of Tuberculosis and Lung Disease* **20**(7), 890-894. https://www.researchgate.net/publication/303892896_XpertW_MTBRIF_for_improved_case_detection_of_extra-pulmonary_TB_in_a_tertiary_care_setting_in_urban_India

[28] Sharma S. K., Kohli M., Chaubey J., Yadav R. N., Sharma R., Singh B. K., Sreenivas V., Sharma A., Bhatia R., Jain D., Seenu V., Dhar A., & Soneja M., 2014, Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. *European Respiratory Journal* **44**(4):1090-3. https://pubmed.ncbi.nlm.nih.gov/25063241/

[29] Li, Y., Pang, Y., Zhang, T., Xian, X., Wang, X., Yang, J., Wang, R., Chen, M., & Chen, W., 2017, Rapid diagnosis of extrapulmonary tuberculosis with Xpert Mycobacterium tuberculosis/rifampicin assay. *Journal of Medical Microbiology* **66**(7), 910-914. https://doi.org/10.1099/jmm.0.000522

[30] Du, J., Huang, Z., Luo, Q., Xiong, G., Xu, X., Li, W., Liu, X., & Li, J., 2015, Rapid diagnosis of pleural tuberculosis by Xpert MTB/RIF assay using pleural biopsy and pleural fluid specimens. *Journal of Research in Medical Sciences* **20**(1):26–31. https://pmc.ncbi.nlm.nih.gov/articles/PMC4354061/

[31] Ligthelm, L. J., Nicol, M. P., Hoek, K. G. P., Jacobson, R., Van Helden, P. D., Marais, B. J., Warren, R. M., & Wright, C. A., 2011, Xpert MTB/RIF for Rapid Diagnosis of Tuberculous Lymphadenitis from Fine-Needle-Aspiration Biopsy Specimens. *Journal of Clinical Microbiology* **49**(11), 3967-3970. https://doi.org/10.1128/jcm.01310-11

[32] Wang, G., Wang, S., Jiang, G., Yang, X., Huang, M., Huo, F., Ma, Y., Dai, G., Li, W., Chen, X., & Huang, H., 2019, Xpert MTB/RIF Ultra improved the diagnosis of

paucibacillary tuberculosis: A prospective cohort study. *Journal of Infection* **78**(4), 311-316. https://doi.org/10.1016/j.jinf.2019.02.010

[33] Sun, Q., Wang, S., Dong, W., Jiang, G., Huo, F., Ma, Y., Huang, H., & Wang, G., 2019, Diagnostic value of Xpert MTB/RIF Ultra for osteoarticular tuberculosis. *Journal of Infection* **79**(2), 153-158. https://doi.org/10.1016/j.jinf.2019.06.006

[34] Wu, X., Tan, G., Gao, R., Yao, L., Bi, D., Guo, Y., Yu, F., & Fan, L., 2019, Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis. *International Journal of Infectious Diseases* **81**, 91-96. https://doi.org/10.1016/j.ijid.2019.01.050

[35] Wang, G., Wang, S., Yang, X., Sun, Q., Jiang, G., Huang, M., Huo, F., Ma, Y., Chen, X., & Huang, H., 2020, Accuracy of Xpert MTB/RIF Ultra for the diagnosis of pleural TB in a multicenter cohort study. *Chest* **157**(2), 268-275. https://pubmed.ncbi.nlm.nih.gov/31437432/

[36] Dorman, S. E., Schumacher, S. G., Alland, D., Nabeta, P., Armstrong, D. T., King, B., Hall, S. L., Chakravorty, S., Cirillo, D. M., Tukvadze, N., Bablishvili, N., Stevens, W., Scott, L., Rodrigues, C., Kazi, M. I., Joloba, M., Nakiyingi, L., Nicol, M. P., Ghebrekhristos, Y., Anyango, I., Murithi, W., Dietze, R., Peres, R. L., Skrahina, A., Auchynka, V., Chopra, K. K., Hanif, M., Liu, X., Yuan, X., Boehme, C. C., Ellner, J. J., & Denkinger, C. M., 2018, Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *The Lancet Infectious Diseases* **18**(1), 76-84. www.doi.org/10.1016/S1473-3099(17)30691-6

[37] Suh, E., Jung, S., Lee, J., Jhun, B. W., Kim, T. Y., Huh, H. J., & Lee, N. Y., 2025, Prospective Comparative Evaluation of the Xpert MTB/RIF and Xpert MTB/RIF Ultra Assays for Detecting Mycobacterium tuberculosis and Rifampin Resistance in High-resource, Intermediate-burden Settings. *Annals of Laboratory Medicine* **45**(6), 583-590. https://doi.org/10.3343/alm.2025.0101

[38] Van Rie, A., Whitfield, M. G., De Vos, E., Scott, L., Da Silva, P., Hayes, C., Heupink, T. H., Sirgel, F. A., Stevens, W., & Warren, R. M., 2019, Discordances between molecular assays for rifampicin resistance in Mycobacterium tuberculosis: frequency, mechanisms and clinical impact. *Journal of Antimicrobial Chemotherapy* **75**(5), 1123-1129. https://doi.org/10.1093/jac/dkz564