Nontuberculous mycobacterium (NTM) is a group of bacteria, which are present in our natural environment habitat. They are present in soil, dust, natural water and water damaged walls, biofilms, and drinking water supplies. There are more than 100 species, of which 60 species are pathogenic to healthy as well as immunocompromised humans. They cause a variety of infections, broadly divided into pulmonary and extrapulmonary infections. In Pakistan, there was a 7.7-fold increase in NTM infections from 21 cases in 2012 to 163 cases in 2018. An earlier study evaluating the distribution of NTM species across Pakistan suggested geographical variation across different regions, every area having its own distribution spectrum. There are no data available especially in developing countries such as Pakistan regarding PJI due to NTM following primary TKA. The purpose of our study was to determine treatment outcomes of two-stage revision surgery following NTM infection.
into pulmonary and extrapulmonary NTM infections.⁵ Our institutional experience regarding risk factors and common organisms associated with prostatic joint infection (PJI) following primary total knee arthroplasty (TKA) was already published.⁵

NTM is a rare cause of PJI and mostly rapid-growing mycobacterium (RGM) is responsible for PJI, rather than slow-growing mycobacterium (SGM).⁶,⁷ Organisms such as Mycobacterium abscessus, Mycobacterium fortuitum, and Mycobacterium chelonae are considered RGM because they have the ability to culture within one week.⁸ SGM takes at least 6–8 weeks to grow. Among SGM, Mycobacterium avium complex (MAC) is the most clinically relevant species, which includes M. avium, Mycobacterium chimaera, and Mycobacterium intracellulare.⁹,¹⁰ NTM infection can result from inoculation of the surgical field during surgical or medical procedures and environmental contamination in the early postoperative period. The potential source of infection such as contaminated water is not always identified despite thorough investigation. Other less common sources of NTM infection include transient colonization of human hair and body sites.¹¹

In Pakistan, there was a 7.7-fold increase in NTM infection from 21 cases in 2012 to 163 cases in 2018. Previously, research was conducted in Pakistan demonstrating various species of NTM among different regions of Pakistan. Extrapulmonary infections are less common than pulmonary infections. Data from one of the private national laboratories revealed that 42% of the extrapulmonary NTM were nosocomial in nature.¹²

To the authors' best knowledge, there are no data from Pakistan on NTM in PJI. The purpose of our study was to determine treatment outcomes of two-stage revision surgery following NTM infection. We hypothesized that two-stage revision surgery along with prolonged antibiotic treatment would provide favorable outcome following NTM infection.

**METHODS**

This is a single-center, retrospective study conducted in the Department of Orthopedics. Patients who underwent TKA between June 2008 and December 2018 were enrolled. Data were retrieved from hospital records. The study was approved by the Ethics Review Committee of Liaquat National Hospital (0222-2018). Written informed consent was taken from all participants. All patients who underwent primary TKA with subsequent revision surgery for PJI due to NTM infection was included in the study, whereas patients with PJI due to tuberculous mycobacterium, gram-positive, gram-negative, or culture-negative bacteria, and fungal species were excluded from the study. PJI caused by any organism following revision surgery were also excluded from our study. PJI was diagnosed in 58 patients who underwent primary TKA between June 2008 and December 2018. Out of the 58 patients, 8 patients were diagnosed with NTM infection. Primary TKA had been performed somewhere else in the patients who had PJI due to NTM. All patients with NTM originated from a single center. All patients were female with a mean age of 62.8 ± 7.9 years. The mean body mass index (BMI) was 25.6 ± 2.8 kg/m². The detailed demographic characteristics of the patients with NTM infection are presented in Table 1.

Factors examined include age, sex, comorbidities including hypertension, diabetes mellitus, rheumatoid arthritis, and asthma, American Society of Anesthesiology score, BMI, causative microorganism, culture medium, and their antibiotic susceptibility.

NTM infection is defined as the presence of traditional criteria for diagnosing PJI plus growth of NTM cultured from a joint aspirate or deep periprosthetic tissue specimen using Löwenstein-Jensen (LJ) and Mycobacteria Growth Indicator Tube (MGIT).¹³ Tsukayama classification was used to determine time interval between the index operation and PJI. All PJIs were identified by a trained infection control practitioner (SI) by using standard methods.

It was very difficult to make any presumptive diagnosis of NTM infection simply on the basis of clinical symptoms because patients present with symptoms very much similar to those presenting with PJI due to common organisms. Pain on weight-bearing, fever, and swelling were commonly associated with RGM, whereas purulent discharge and sinus formation were the most commonly encountered symptoms associated with SGM. The detailed description of antibiotic sensitivities is shown in Table 2.

Routine laboratory workup including inflammatory markers and joint aspiration was performed to diagnose PJI. Tissue samples were obtained from three standard surgical sites such as synovium and medullary canal of the femur and tibia. Pus and deep tissue were sent for routine microbiological culture and sensitivity along with histological examination of infected tissue. If no organism was revealed in the first 48–72 hours, then we asked the microbiologist (SSN) to further extend the culture time for 2 weeks.

Before making any definitive diagnosis, we used 3 g of vancomycin plus 2 g of tobramycin as a cement spacer (non-articulating) in all cases. The diagnosis and treat-
ment of NTM infection were directed mainly by an arthroplasty surgeon (SSN) and an infectious disease specialist (SI). All patients were initially kept on vancomycin 1 g twice a day until results of conventional cultures were negative (6/6 samples) for at least 2–3 weeks. Additional laboratory investigation such as Acid-Fast Bacilli smear with Ziehl-Neelson staining was then carried out in patients who remained culture negative or did not respond with antibiotics till 2–3 weeks. Both solid medium (LJ) and liquid medium (MGIT) were used to culture microorganisms from clinical specimens. The microscopic characteristics along with the rate of growth of organisms were useful for making any presumptive diagnosis. Red bacilli with Ziehl-Neelsen stain is suggestive of genus mycobacteria.

Search engines such as PubMed were used to get access to medical literatures related to PJI due to NTM following primary TKA. The purpose of this literature search was to identify antibiotics sensitivities, duration of antibiotic regime in case of PJI due to NTM. Delphi consensus criteria were used in all patients to determine eradication of infection prior to revision surgery. According to the Delphi consensus criteria, eradication of infection is characterized by healed wound with no draining sinus and pain. During revision surgery, the antibiotic cement spacer was removed. Bone loss was assessed via Anderson Orthopaedic Research Institute classification and managed accordingly. An extensile approach such as tibial tubercle osteotomy (TTO) was performed in cases when the patella was unable to evert with the knee at 90 degree flexion. A constrained implant was used during revision surgery. Knee Society Score (KSS) was assessed at final follow-up in all patients who underwent revision surgery. Patients were asked to visit the clinic for follow-up at 6 weeks, 3 months, 6 months, and annually thereafter till 2 years.

Second-stage revision surgery was performed in patients who had favorable outcome following antimicrobial treatment. All patients underwent revision surgery. Inflammatory markers were used to assess the response of antimicrobial treatment initially performed twice weekly followed by monthly. During revision surgery, after making thick flaps to prevent skin necrosis, fluid was aspirated and sent for culture and sensitivity. The antibiotic cement spacer was removed. The aspirated fluid revealed no organism. Out of the 8 patients, the Legacy constrained condylar knee was used in 7 patients, whereas 1 patient had a rotating hinge knee (RHK) implant. Bone loss was managed with a wedge in 2 patients. Extensile approaches

| Age (yr) | Sex | Method to diagnose | Positive culture (wk) | Comorbid | BMI (kg/m²) | Organism | Antibiotic regime | Duration of antibiotic use (mo) |
|----------|-----|--------------------|-----------------------|----------|-------------|-----------|-------------------|-------------------------------|
| 57       | F   | LJ + MGIT medium   | 12                    | DM, HTN  | 24.3        | Mycobacterium avium | Clarithromycin + azithromycin + rifampicin + ethambutol | 10–12                         |
| 64       | F   | LJ + MGIT medium   | 9                     | DM       | 28.6        | M. avium   | Clarithromycin + azithromycin + rifampicin + ethambutol | 10–12                         |
| 61       | F   | LJ + MGIT medium   | 5                     | No comorbid | 23.3       | Mycobacterium abscessus | Clarithromycin + rifampicin | 4–6                           |
| 56       | F   | LJ + MGIT medium   | 5                     | DM       | 25.8        | Mycobacterium fortuitum | Clarithromycin + moxifloxacin + linezolid | 6–8                           |
| 60       | F   | LJ + MGIT medium   | 4                     | No comorbid | 31.2       | M. fortuitum | Clarithromycin + doxycycline | 6–8                           |
| 67       | F   | LJ + MGIT medium   | 5                     | No comorbid | 24.8       | Mycobacterium chelonae | Clarithromycin + linezolid + moxifloxacin | 6–9                           |
| 59       | F   | LJ + MGIT medium   | 5                     | DM, HTN, RA | 23.4       | M. abscessus | Clarithromycin + rifampicin + linezolid + meropenem | 12–18                         |
| 61       | F   | LJ + MGIT medium   | 4                     | DM       | 23.9        | M. abscessus | Clarithromycin + cefoxitin + linezolid + rifampicin | 12–18                         |

PJI: prosthetic joint infection, NTM: nontuberculous mycobacterium, TKA: total knee arthroplasty, BMI: body mass index, LJ: Löwenstein-Jensen, MGIT: mycobacteria growth indicator tube, DM: diabetes mellitus, HTN: hypertension, RA: rheumatoid arthritis.
such as TTO were performed in 1 patient only, in which the RHK was used. Drain was not used in revision surgery. Full weight-bearing mobilization was allowed from the very next day of surgery. Antibiotics were continued postoperatively according to culture and sensitivity as shown in Table 1. Patients were followed up at 2 weeks, 3 months, 6 months, and annually thereafter till 2 years. Functional outcome was assessed using KSS at the final follow-up.

Two patients with *M. abscessus* infection required multiple surgical debridements at an interval of 4–6 weeks along with antimicrobial treatment. Inflammatory markers such as CRP and ESR remained elevated despite multiple surgical debridements. Antibiotics for NTM infection were given according to culture and sensitivity. We found RGM in 6 patients, whereas SGM was found in 2 patients only. The antibiotic regimen and duration of antibiotics varied depending on the nature of microorganism. Histopathology revealed microabcesses along with necrotizing granulomas in all cases. The antibiotics were used in combination in all NTM infections. Generally, clarithromycin was the standard antibiotic used in all cases of NTM infection.

### Table 2. NTM Organisms and Their Antibiotic Sensitivity

| Organism                  | Antibiotic sensitivity |
|---------------------------|------------------------|
| **RGM**                   |                        |
| *Mycobacterium abscessus* | Clarithromycin         |
|                           | Cefoxitin              |
|                           | Rifampicin             |
|                           | Linezolid              |
|                           | Meropenem              |
| *Mycobacterium fortuitum* | Clarithromycin         |
|                           | Trimethoprim-sulfamethoxazole |
|                           | Azithromycin           |
|                           | Moxifloxacin           |
|                           | Linezolid              |
|                           | Doxycycline            |
| *Mycobacterium chelonae*  | Clarithromycin         |
|                           | Meropenem              |
|                           | Doxycycline            |
|                           | Moxifloxacin           |
|                           | Trimethoprim-sulfamethoxazole |
| **SGM**                   |                        |
| *Mycobacterium avium complex* | Clarithromycin | Rifampicin | Ethambutol | Azithromycin | Meropenem |
| *Mycobacterium avium*     | Clarithromycin         |
|                           | Rifampicin             |
|                           | Ethambutol             |
|                           | Azithromycin           |
|                           | Meropenem              |

NTM: nontuberculous mycobacterium, RGM: rapid-growing mycobacterium, SGM: slow-growing mycobacterium.

Statistical Analysis

The data were analyzed statistically using statistics IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Data regarding numerical variables are presented as mean ± standard deviation. Categorical variables were compared through chi-square and Fisher Exact test. The level of significance was set at *p*-value < 0.05.

### RESULTS

Treatment outcomes were categorized into favorable and unfavorable. Favorable outcomes were categorized further into remission and stable cases. Remission was when wound was healthy and no signs of local sepsis were present with normal inflammatory markers, whereas stable was when the patient was clinically healthy and there were no signs of local sepsis but inflammatory markers such as ESR or CRP did not return to normal. Unfavorable outcomes were further categorized into failure of treatment, relapse, reinfection, or death related to PJI that occurred during the course of treatment. Failure of treatment was defined when there was no clinical or microbiological response to treatment or when side effects of antimicrobial treatment prevented the patient from continuing treatment for a prolonged period. Relapse was persistent or recurrent infection by the same microorganism during or after completion of antimicrobial treatment. Any recurrent infection by new organism during or after completion of antimicrobial treatment was considered reinfection.

Second-stage revision surgery was performed in patients who had favorable outcome following antimicrobial treatment. All patients underwent revision surgery. Out of 8 patients, *M. abscessus* was the main culprit for early PJI in 2 cases, whereas it caused late infection in only 1 case. *M. fortuitum* caused late PJI in 2 patients. *M. chelonae* caused late PJI in only 1 patient. SGM such as *M. avium* caused PJI in only 2 patients.

Functional outcome was determined by the KSS
preoperatively and postoperatively at 2 years. There was significant difference in both clinical and functional scores at final follow-up with \( p \)-value < 0.05.

**DISCUSSION**

To the best of our knowledge, this study is the first from a developing country (Pakistan) to show results of treatment of PJI due to NTM organism. NTM is a rare cause of PJI. A previous study showed that RGM is mainly responsible for PJI following primary TKA.\(^{17}\) Multiple studies have already shown factors associated with PJI other than NTM infection following primary TKA.\(^{18,19}\) Only a few case reports addressed this unusual cause of PJI. Factors associated with NTM infection following primary TKA have yet to be determined. All patients in our study were female. Previous epidemiological data also suggest that NTM infections are most commonly seen in elderly female patients and in patients with low BMI.\(^{20}\) Another study was conducted in the United States (US) to find out the prevalence of NTM infections and demographic characteristics associated with NTM infections. It reported that the prevalence of NTM infections in US actually surpassed that of mycobacterium tuberculosis infections and it was 1.1 to 1.6 fold higher in women than men in different states of US. This shows that female sex is more prone to develop NTM infection following primary TKA. The increasing trend of NTM infection in US might be due to the improvement in diagnostic methods such as the availability of 16S rRNA gene sequencing to identify mycobacteria species along with the awareness of important species of NTM causing infections in healthy as well as immunocompromised patients.\(^{21}\) The situation is different in developing countries including Pakistan where mycobacterium tuberculosis infection has a rising trend as compared to NTM infection. According to the 2017 World Health Organization report, approximately 68% new cases were diagnosed with mycobacterium tuberculosis in Pakistan. Out of the 68% cases, 20% patients had extrapulmonary tuberculosis.\(^{22}\) Lack of awareness along with the decreasing trend of NTM infections might cause difficulty in diagnosing NTM infection following primary TKA in our region.

The spectrum of diseases caused by NTM is the same as mycobacterium tuberculosis, but generally they respond poorly to the standard and classic anti-tuberculous therapy. A misdiagnosis of mycobacterium tuberculosis can lead to a treatment failure, especially in the resource-limited countries due to the inadequate diagnostic infrastructure.\(^{23,24}\) Furthermore, NTM infection often leads to a chronic disease that requires lengthy, complex, and sometimes poorly tolerated drug regimens over many months to years and following treatment, patients can experience relapse from incomplete treatment or reinfection. The main purpose of our study was to highlight challenges in diagnosing NTM infections. There was an increasing trend of culture-negative PJI in our region due to the use of inappropriate antibiotics prior to taking cultures that warrants further investigation to isolate an organism, especially in circumstances where the patient failed to respond even after aggressive surgical debridement and a prolonged antibiotic course. A major obstacle in determining the true burden of disease and identifying patients with NTM infections is limited diagnostic capacity (both clinical and laboratory) in many low- and middle-income countries. We used liquid medium such as LJ medium and solid medium such as MGIT to isolate NTM in our study. LJ medium requires 23–25 days to isolate an NTM organism, whereas Middlebrook \( H^1 \) agar only takes 12 days to isolate an NTM organism.\(^{25}\) The unavailability of rapid diagnostic Middlebrook \( H^1 \) agar medium leads to unnecessary surgical debridement and prolonged antibiotic treatment, which actually increases financial burden to patients. Therefore, PJI due to NTM following primary TKA must not be overlooked in developing countries like Pakistan.\(^{26}\) Previously, two cultures were compared for isolation of mycobacteria such as LJ medium and Ogawa 1% with egg yolk medium. Although Ogawa with egg yolk medium is well known for isolation of NTM, the study revealed that LJ medium is superior in diagnosing NTM as compared to Ogawa medium, which was particularly used to isolate mycobacterium tuberculosis. Their study revealed that 95% clinical specimens were positive for mycobacterium tuberculosis on Ogawa medium as compared to 88.4% on LJ medium.\(^{27}\) Based on our findings, it was very difficult to make any firm consensus regarding patients who were at risk of developing NTM infection. It warrants further studies to determine predisposing factors associated with NTM infection. We believe that PJI due to NTM must be considered in patients who remain culture negative for initial 2–3 weeks and appropriate investigations must be performed in order to isolate an NTM organism.\(^{28}\)

Regarding treatment of PJI due to NTM, clarithromycin is the choice of antibiotic for both slow-growing and rapid-growing nontuberculous mycobacterial infections. Linezolid can be used as an alternative. Among injectable drugs, amikacin is mostly active against RGM infection, whereas imipenem or meropenem shows limited activity against species of nontuberculous mycobacterial infection in developing countries like Pakistan. Previous
literature also showed that nosocomial infections are more resistant to injectable antibiotics, whereas community-acquired infections are more resistant to oral antibiotics. The antibiotic treatment of PJI due to NTM is variable and prolonged as well. There are no clear guidelines available on which combination of drugs is effective for treating NTM infection.29,30

There are several limitations of our study. This is a retrospective study with a small number of patients diagnosed with NTM infection following primary TKA. However, the findings of this study were statistically robust enough to facilitate evidence-based decision making for the treatment of PJI with the unusual cause by the arthroplasty surgeon and the infectious disease consultant. Another drawback of our study is that it was not a comparative study involving PJI caused by other common organisms in a similar population. A short follow-up will eventually fail to determine the long-term survival of the implant used in revision surgery. We were also unable to determine the exact incidence of PJI due to NTM as primary TKA was performed somewhere else. Therefore, preoperative factors of causing PJI due to NTM could not be determined. Although our study is not a comprehensive study to determine complete guidelines for managing this unusual cause of PJI, we believe that this study will provide a useful guideline to help arthroplasty surgeons and infectious disease consultants make decisions for treating this unusual cause of PJI.

Our study concludes that meticulous surgical debridement and prolonged antibiotic treatment course are the only hope of cure to combat this unusual cause of PJI following primary TKA. PJI due to NTM infection must be considered in patients who remain culture negative for at least 2 to 3 weeks.

**CONFLICT OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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