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CASE REPORT

Diagnostic Aspects of a Bacillus Calmette-Guërin (BCG) Infection Following Intravesical Treatment for Bladder Cancer

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SUMMARY

Background: **Bacillus Calmette-Guérin (BCG) infection is a rare complication of intravesical immunotherapy for bladder cancer. Distinguishing *Mycobacterium bovis* BCG from other *Mycobacterium tuberculosis* complex members is crucial due to intrinsic drug resistance.**
Methods: **We report a case of BCG infection following intravesical immunotherapy and propose three laboratory clues.**
Results: **White, spreading umbonate colony morphology; negative *M. tuberculosis* protein 64 (MPT-64) antigen test; and nearly identical cycle threshold values for insertion sequence 6110 (IS6110) and internal transcribed spacer targets in real-time PCR strongly suggest BCG infection.**
Conclusions: **These findings could facilitate the rapid identification of *M. bovis* BCG and appropriate treatments.**
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KEYWORDS

BCG, bladder cancer, intravesical immunotherapy, MPT64, real-time PCR

CASE PRESENTATION

Intravesical instillation of Bacillus Calmette-Guérin (BCG)-a live attenuated strain of *Mycobacterium bovis* - has proven effective in superficial bladder cancer immunotherapy. The *M. bovis* BCG infection occurs in 3 - 5% of patients following instillation, with dissemination in 1% [1]. Notably, cystitis and general malaise are the most frequently reported complications, accounting for approximately 35% and 15% of cases, respectively. Severe systemic manifestations such as miliary pulmonary tuberculosis, granulomatous hepatitis, and mycotic aneurysms have been reported in cases of disseminated infection [2]. Despite the clinical significance of BCG infection, microbiological confirmation is often difficult as a substantial proportion of *M. bovis* BCG-infected patients lack positive microbiological results.
This study reports a culture-proven and genetically con-

firmed *M. bovis* BCG infection following intravesical BCG immunotherapy. This study was approved by the Institutional Review Board of the Korea University Medical Center, Seoul, Korea (K2024-0374), which waived the need for informed consent. A 75-year-old female diagnosed with bladder cancer underwent transurethral resection of the bladder tumor followed by intravesical BCG immunotherapy (Tice strain; 12.5 mg weekly for 5 weeks) at the Korea University Anam Hospital. The sixth immunotherapy session was discontinued because of a urinary tract infection.

The patient presented with new-onset hematuria and voiding pain after 4 months. Laboratory tests showed proteinuria, hematuria, and pyuria on urinalysis; atypical urothelial cells on urine cytology; and no acid-fast bacteria (AFB) on AFB staining of the urine sample. Urine AFB culture was also performed using a solid medium containing 2% Ogawa agar (Shinyang, Seoul, Korea) and liquid medium from the BACTEC MGIT 960 system (BD, Franklin Lakes, NJ, USA). Dry, wrinkled, and spreading umbonate colonies, suggestive of *M. tuberculosis* complex (MTBC), were isolated from the urine specimen in a solid AFB culture medium (Figure 1a).

To differentiate between MTBC and nontuberculous mycobacteria (NTM), *Mycobacterium tuberculosis* protein 64 (MPT-64) antigen immunochromatography (ICT) was performed using the Bioline MPT-64 Rapid Kit (Abbott, Seoul, Korea). The MPT-64 antigen ICT, which is typically positive for MTBC, yielded a negative result. Subsequently, real-time PCR was performed using AdvanSure TB/NTM real-time PCR (LG Life Sciences, Seoul, Korea), which targeted the insertion sequence 6110 (IS6110) for MTBC and an internal transcribed spacer (ITS) of *Mycobacteria*. The results revealed MTBC positivity with cycle threshold (Ct) values of 24.47, 24.79, and 27.41 for IS6110, ITS, and internal control, respectively.

The antimicrobial susceptibility tests were performed at the Korean Institute of Tuberculosis. The absolute concentration method using an M-kit (The Korean Institute of Tuberculosis, Osong, Korea) revealed cycloserine resistance, and Wayne’s pyrazinamidase test revealed pyrazinamide resistance, which is a common finding in *M. bovis* BCG (Figure 1b, c). To differentiate species among MTBC, multiplex PCR-based genotyping of the RD1, RD4, and RD9 regions was performed. *M. bovis* BCG exhibited deletions from Rv3875 to Rv3879c, Rv1505c to Rv1516c, and Rv2073 to Rv2074c in the RD1, RD4, and RD9 regions, respectively, with different PCR product band sizes for RD1 (212 bp), RD4 (166 bp), and RD9 (205 bp). Detailed interpretations of the multiplex PCR-based genotyping and the patient results are shown in Figure 2.

The bacterium was identified as *M. bovis* BCG. The patient was treated with isoniazid (300 mg/day), ethambutol (1,200 mg/day), levofloxacin (500 mg/day), and pyridoxine (50 mg/day) for 3 months, and rifampicin was discontinued owing to hepatotoxicity. After 3 months, radical cystectomy was performed on the urine high-grade urothelial carcinoma cells, and follow-up urine AFB staining and cultures revealed no signs of *M. bovis* BCG infection.

DISCUSSION

The pathogenic mechanisms underlying the BCG-related infectious complications remain unclear. Previous studies have reported low microbiological detection rates for AFB staining, AFB culture, and PCR-based assays (25.3%, 40.9%, and 41.8%, respectively) [3]. However, recent studies have increasingly reported the presence of *M. bovis* BCG isolates, suggesting ongoing active infections. In this study, we isolated *M. bovis* BCG from AFB cultures and confirmed its presence using PCR-based assays. This finding may contribute valuable insight into the mechanism of complications associated with an active infection.

Differentiating *M. bovis* BCG from other MTBC is critical for appropriate clinical management. BCG strains are intrinsically resistant to pyrazinamide, and tailored treatment regimens distinct from those used for typical MTBC infections are essential. Furthermore, the clinical manifestations and disease course associated with *M. bovis* BCG infections differ from those of tuberculosis, characterized by less frequent lung involvement and less extensive pulmonary damage [4,5]. Despite these therapeutic and clinical distinctions, conventional diagnostic strategies primarily detect MTBC without distinguishing *M. bovis* BCG because of the limited availability of molecular diagnostic tests in routine clinical laboratories. Specifically, line probe assays, which can differentiate MTBC subspecies based on region-specific genomic signatures, are not available for clinical use in South Korea due to the lack of regulatory approval. This limitation was evident in our case, in which genotypic confirmation of *M. bovis* BCG required a referral to a specialized reference laboratory, leading to delays in diagnosis and appropriate treatment.

Given the challenges in distinguishing *M. bovis* BCG from other MTBC members, phenotypic features, such as distinct colony morphology, can serve as useful preliminary clues. As observed in our case, BCG colonies appeared whiter and more dispersed, often exhibiting umbonate morphology, compared with the typically buff-colored and more compact colonies of *M. tuberculosis* sensu stricto [6]. Although not definitive, these morphological differences provide valuable preliminary clues for differentiating *M. bovis* BCG from other MTBC members in culture-based diagnostic workflows.

M. bovis BCG, including the Tice strain, exhibits distinct molecular and antigenic profiles. One notable distinction is the absence of the MPT64 antigen. Certain BCG strains, particularly later derivatives such as Tice, undergo deletions or mutations in *mpt64* during serial subculture [7]. This genomic alteration results in the absence or markedly reduced expression of the MPT64

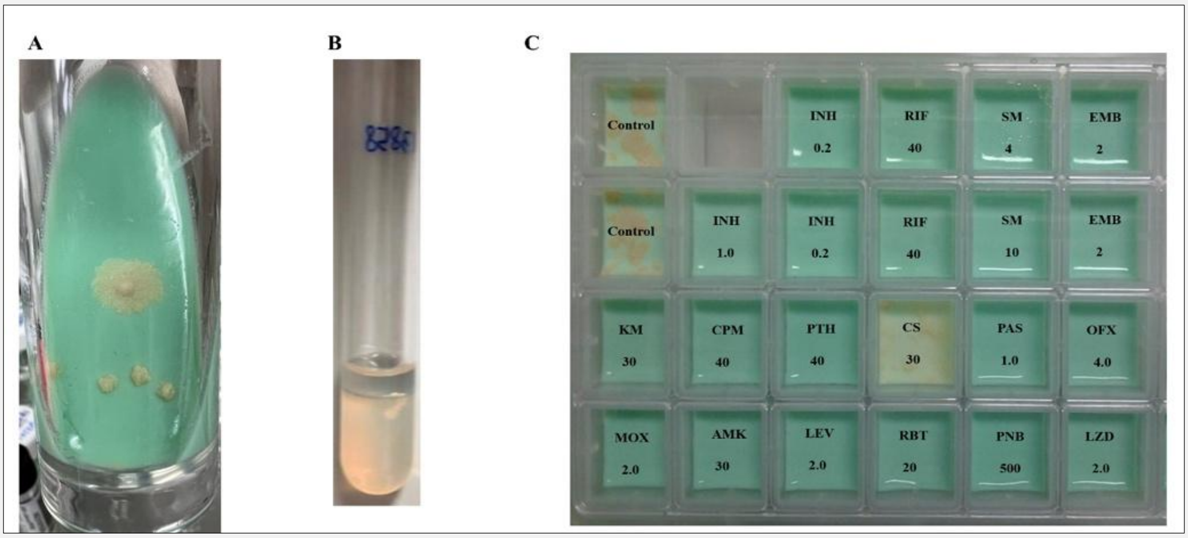

Figure 1. A Colony morphology on a solid medium 6 weeks post-inoculation. B Pyrazinamide resistance was identified using the Wayne method. C Visible colony growth appeared on medium containing cycloserine. The numbers below each antibiotic indicate the concentration of the drug (µg/mL).

INH isoniazid, RFP rifampicin, SM streptomycin, EMB ethambutol, KM Kanamycin, CPM capreomycin, PTH prothiomide, CS cycloserine, PAS para-aminosalicylic acid, OFX ofloxacin, MOX moxfloxacin, LEV levofloxacin, RBT rifabutin, PNB para-nitrobenzoic acid, LZD linezolid.

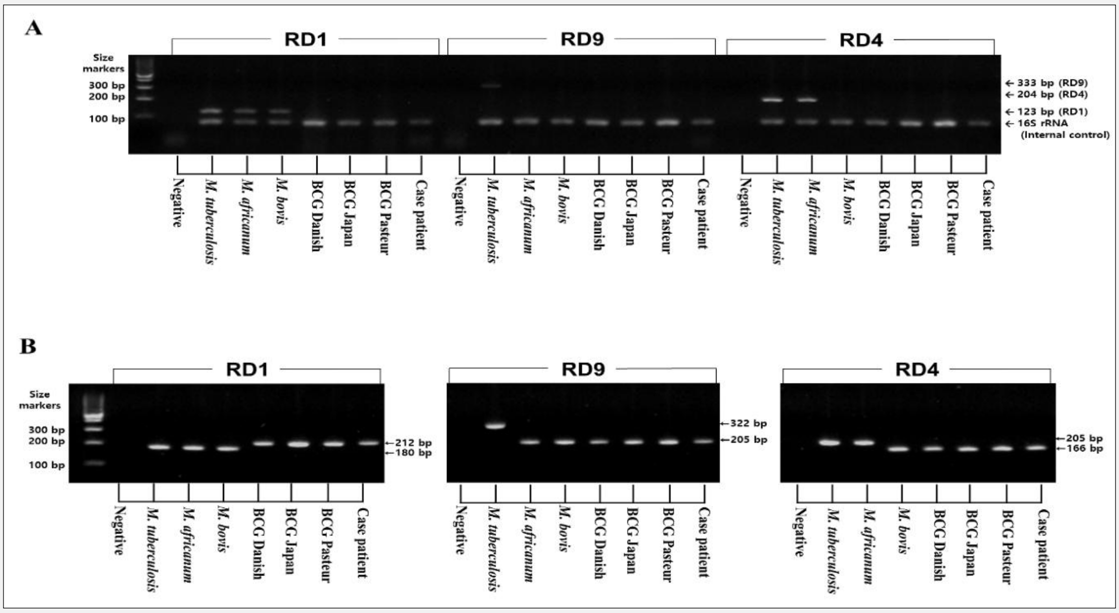

Figure 2. Multiplex PCR-based genotyping results of the isolates showing: A the presence or absence of target genes and B the size of the PCR products.

The PCR products were separated via agarose gel electrophoresis.

protein, leading to false-negative results in MPT64 rapid antigen tests. Supporting these findings, previous studies have reported negative MPT64 antigen ICT results in specific BCG strains, including the Tice, Danish, and Pasteur [8-10]. Our patient, who was treated with intravesical BCG of the Tice strain, also tested negative for the MPT64 antigen.

In nucleic acid-based diagnostics, real-time PCR assays targeting IS6110 and ITS yielded different amplification profiles for *M. bovis* BCG and *M. tuberculosis*. IS6110, a commonly used insertion sequence for detecting MTBC, is typically present in multiple copies of *M. tuberculosis* (generally > 10 copies), whereas most *M. bovis* BCG strains harbor only one or two copies, and some derivatives lack the element entirely [11]. The ITS region, which is located between the *16S* and *23S* rRNA genes, is a highly conserved single-copy locus shared by *M. tuberculosis* and *M. bovis* BCG [12]. As PCR amplification depends heavily on the target copy number, *M. tuberculosis* typically yields significantly lower Ct values for IS6110 than *M. bovis* BCG. In contrast, the Ct values for ITS remain relatively stable, with only minor variations between *M. bovis* BCG and *M. tuberculosis* [13,14].

Among *M. bovis* BCG strains, the Tice strain, characterized by a single IS6110 copy and the ITS region, is most widely used for intravesical BCG therapy [15]. Notably, real-time PCR results for *M. bovis* BCG typically yield comparable Ct values for IS6110 and ITS. This pattern is a novel finding, distinct from *M. tuberculosis*, where a high IS6110 copy number results in markedly lower Ct values compared to ITS. In our study, the Ct values for IS6110 and ITS were nearly identical, supporting *M. bovis* BCG amplification patterns.

In addition to microbiological and molecular findings, the clinical context provides critical information for suspecting *M. bovis* BCG infections. In particular, urine specimens from patients with a history of intravesical BCG instillation or specimens obtained from anatomical sites adjacent to recent BCG vaccinations should raise suspicion of BCG-related infections. Integrating this clinical context with laboratory findings, preliminary identification of *M. bovis* BCG can be performed before definitive genotypic confirmation. Both the MPT64 antigen ICT and TB/NTM real-time PCR targeting the IS6110 and ITS regions are widely used in clinical microbiology laboratories. Although these assays are not specifically designed to differentiate BCG strains, their combined interpretation may enable the preliminary identification of *M. bovis* BCG before referring to specialized reference laboratories.

CONCLUSION

We reported a case of culture-proven and genetically confirmed *M. bovis* BCG infection following intravesical BCG immunotherapy. We also highlighted three useful laboratory clues for differentiating BCG infection when interpreted alongside the clinical history of BCG instillation. Distinctive white, spreading umbonate colony morphology; negative MPT-64 antigen test results; and nearly identical Ct values for IS6110 and ITS targets in real-time PCR strongly suggested BCG infection along with a history of BCG treatment. This diagnostic strategy could facilitate the rapid identification of *M. bovis* BCG infections and guide the initiation of appropriate treatments.

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Declaration of Interest:
The authors have no relevant financial or non-financial interests to disclose.

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