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# A Blind Smear From LJ Medium Revealing *Pseudomonas aeruginosa* Talus Osteomyelitis After Seeking for Osteotuberculosis in a 13-Year-Old Teenager: A Case Report

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## ABSTRACT

The Lowenstein-Jensen is an egg-based microbiology medium that was designed for selective isolation of *Mycobacterium spp*. The main inhibitory composition in this medium is a synthetic dye called malachite green. *Mycobacterium spp*. is resistant to the dye by de-colorization and oxidation of it. Some non-tuberculosis organisms may also overcome this inhibitory ingredient by similar enzymatic activity. In the final inspection of the bone TB culture of a teenage patient, the blind smear revealed the *Pseudomonas* talus osteomyelitis. According to the present and similar experiences, the preparation of 1-2 blind smears from TB culture-negative specimens taken from sterile sites of the body may unmask mycobacteria with the atypical colony and non-tuberculosis microorganisms.

## Introduction

Although tuberculosis (TB) has reminded a high incidence of pathology in the last 30 years, localization in talus is rarely reported in children [1-3]. The diagnosis of skeletal TB is based on physical examination and para-clinical findings [1]. Isolation of *Mycobacterium tuberculosis* (MTB) and Non-Tuberculous Mycobacteria (NTM) has remained the "gold standard" method for laboratory diagnosis [4].

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The Lowenstein-Jensen (LJ) is the most common medium for isolation and drug sensitivity tests of MTB [4]. The composition of the LJ medium prevents the growth of other organisms that may mask MTB or NTM [5]. The main anti-microbial agent in LJ ingredient is a synthetic azo dye, known as malachite green (MG) [6]. To improve selectivity, some antibiotics may be added to the medium [4]. Moreover, to inhibit the overgrowth of other organisms, known as contaminants, there are specific procedures for collecting, handling, transport, and processing of the specimen [5, 7]. The processing is not essential for the specimen that is collected from sterile sites, except for the concentration of fluid to maximize the yield of MTB [6, 7]. Talking about MTB resistance to MG, the *Pseudomonas spp* also inactivates azo dye through discoloration and reduction [6, 8].

In the presented case and according to our previous experiment, at the time of final inspection, two smears that were prepared from melted LJ medium was studied with Gram and methylene blue staining techniques. Unexpectedly, many Gram-negative bacilli were seen. They were finally identified as *Pseudomonas aeruginosa*.

## Case presentation

A previously healthy 13-year-old girl complained of pain around the right ankle. She could not remember any stroke except a mild twisting during getting out of bed. The pain gradually got worse, especially at bedtime. In the plain x-ray, there were no pathologic changes in the ankle. The patient refused foot fixation by the plastic cast. In the next two months, the swelling and pain got worse. She was referred to a hospital for Magnetic Resonance Imaging (MRI). An effusion was noted in tibiotalar and subtalar joints. The bone bruise of the talus was added to the report.

**Figure 1.** Melted LJ medium in the final inspection

The results of all routine laboratory blood tests were within the normal ranges. Erythrocyte Sedimentation Rate (ESR) was 12 mm/h and C-reactive protein (CRP) 3.9 mg/L. However, the ANA-screen test was 1.9 IU/mL. For close inspection, curettage, and debridement of bone injury, the patient was admitted to the orthopedic ward and underwent surgery. The curetted materials were sent for histopathology, routine bacterial culture, and MTB culture in our lab. The ankle was put at rest in a plaster for 3 weeks. The initial empirical antibiotic therapy began with Cefazolin 1 g every 8 h and Gentamicin 60 mg every 8 h. After hospital discharge, the medication was continued with cephalexin 500 for two weeks. The co-prescription was calcium and aspirin. The result of the conventional microbiology test was reported negative. In histopathology analysis, inflammatory cell infiltration with no evidence of malignancy was reported.

The TB culture was carried out in class II laminar air flow [Jal Teb- Iran]. The material that was sent in a sterile container, was aseptically transferred into the LJ medium container [Conda, pronadisa - Spain]. They were suspended in the extra water that was accumulated at the bottom of the LJ slop. For the spread and fixation of material on slop, the container was gently swinging every day. To complete 4 weeks incubation time, after 10 days, at the time of the New Year holiday, the TB culture was left in the incubator (35°C) [Behdad - Iran].

At the end of the incubation period, no visible colony appeared. According to our previous experiences, two blind smears were prepared from melted medium (Figure 1) for Gram and methylene blue staining. In the Gram stain, many Gram-negative bacilli were seen. In the subculture of material in aerobic conditions, *Pseudomonas*

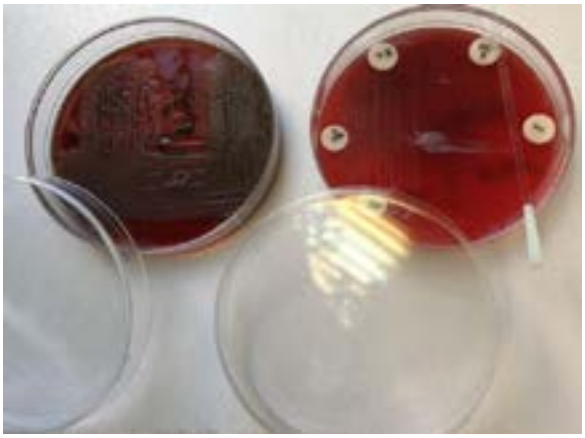

**Figure 2.** The result of subculture from LJ to blood agar in aerobic [left] and anaerobic [right] conditions

The growth of *Pseudomonas aeruginosa* is visible in aerobic condition

**Figure 3.** De-colorization of LJ medium at the beginning MTB growth in compare with not inoculated medium [left]

*aeruginosa* was isolated (Figure 2). No anaerobe organism was isolated. The results, including the susceptibility test, were reported. The ceased antibiotic therapy was re-started with cefixime 400 mg per day for 21 days. At the time of checking up, about 2 months after surgery, although her ESR was still relatively high, the x-ray from the ankle showed improvement. The patient was doing well with no wound discharge or swelling.

## Discussion

Regarding the negative result of TB culture, the discussion is concentrated on talus osteomyelitis of children due to Pseudomonas and the resistance of this organism to MG. Although the rich blood supply of talus provides an anatomical framework for acute or sub-acute osteomyelitis, hematogenous infection of this bone is a rare cause of ankle swelling [9, 10].

Non-nosocomial Pseudomonas infection of the talus is usually caused by penetration injury to the sole through shoe gear [10]. In a Clinical Case Reports and Reviews (CCRR), among a relatively large series (N=49) of children with osteomyelitis, only two cases of Pseudomonas osteomyelitis of the talus was described [10]. In 2018, Abdulmuhsen et al. reported subacute osteomyelitis of talus in a child as a rare case in Saudi Arabia. The result of the material culture of the present case with the conventional method was reported as negative like the Saudi Arabia case, in that no bacterium was isolated from pus swab and curetted material [1].

Pseudomonas was isolated from Lowenstein Jensen (LJ) medium after around 30 days (including 15 days gap of New year holiday). The main selectivity substance in LJ is MG. This dye inactivates the respiratory chain enzymes of microorganisms with irreversible oxidation. The operating form of MG is its oxidized form [8, 11]. To overcome the MG anti-microbial potency, Mycobacterium spp. reduce and de-colorize it (Figure 3). Some cell membrane lipoproteins of *Pseudomonas spp* have a very similar influence on azo and diaminotriphenylmethane dyes, including MG [11-13]. This enzymatic Pseudomonas potency is a valuable feature for textile waste fluid biodegradation [6].

The case described in this article provides a real insight into the unusual or unexpected isolation of an organism in a non-relative or selective medium. A blind smear preparation from atypical tiny colonies on the LJ slop revealed the L-form of MTB. It was isolated from the blood sample of a pet [12]. In our lab, a blind smear that was prepared from a tiny-bloody accumulation of bronchoalveolar lavage in the bottom of the LJ medium, exposed the growth of MTB, beneath the tiny accumulate.

## Conclusion

Tuberculosis of talus should be considered before any other ankle inflammatory disease. In this study, isolation of pseudomonas as an uncommon species was the result of our previous experiment. In clinical applied microbiology, the basic principle of diagnosis of infectious diseases depends on strong experiment and practitioner skill. Since the selectivity of almost all microbiology media are not 100%, the isolation of non-specific organisms should be always considered.

## Ethical Considerations

### Compliance with ethical guidelines

Written informed consent was obtained from the patient's mother for publication of this case. She is an experienced and educated nurse.

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### Conflict of interest

The authors declared no conflict of interest.

## References


[1] Alshammari AN, Alotaibi MH, Abouelnaga MA, Almohammadi AM. Primary subacute osteomyelitis of the talus in a child. Journal of Musculoskeletal Surgery and Research. 2018; 2:34-7. [DOI:10.4103/jmsr.jmsr_36_17]

[2] Holland TS, Sangster MJ, Paton RW, Ormerod LP. Bone and joint tuberculosis in children in the Blackburn area since 2006: A case series. Journal of Children's Orthopaedics. 2010; 4(1):67-71. [DOI:10.1007/s11832-009-0232-3] [PMID] [PMCID]

[3] Teklali Y, Alami ZFE, Madhi TE , Gourinda H, Miri A. Tuberculosis of the talus in the child. European Journal of Orthopaedic Surgery and Traumatology. 2003; 13:52-4. [DOI:10.1007/s00590-003-0061-x]

[4] Kassaza K, Orikiriza P, Llosa A, Bazira J, Nyehangane D, Page AL, et al. Lowenstein-Jensen selective medium for reducing contamination in mycobacterium tuberculosis culture. Journal of Clinical Microbiolog. 2014; 52(7):2671-3. [DOI:10.1128/JCM.00749-14] [PMID] [PMCID]

[5] Tille PM. Mycobacteria. Baily & Scott's Diagnostic Microbiology. 14th ed. Missouri USA: Elsevier; 2017.

[6] Du LN, Wang S, Li G, Wang B, Jia XM , Zhao YH , et al. Biodegradation of malachite green by Pseudomonas sp. strain DY1 under aerobic condition: Characteristics, degradation products, enzyme analysis and phytotoxicity. Ecotoxicology. 2011; 20:438-46. [DOI:10.1007/s10646-011-0595-3] [PMID]

[7] Forbes BA, Hall GS, Miller MB, Novak SM, Rowlinson MC, Salfinger M, et al. Practice guidelines for clinical microbiology laboratories: Mycobacteria. Clinical Microbiology Reviews. 2018; 31(2):e00038-17. [DOI:10.1128/CMR.00038-17] [PMID] [PMCID]

[8] Banaei N, Kincaid EZ, Lin SYG, Desmond E, Jacobs WR, Joel D, et al. Lipoprotein processing is essential for resistance of mycobacterium tuberculosis to malachite green. Antimicrobial agents and chemotherapy 2009; 53(9):3799-802. [DOI:10.1128/AAC.00647-09] [PMID] [PMCID]

[9] Natsis K, Grammatikopoulou D, Kokkinos P, Fouka E, Totlis T. Isolated tuberculosis arthritis of the ankle: A case report and review of the literature. Hippokratia. 2017; 21(2):97-100. [PMID] [PMCID]

[10] Narang S. Pseudomonas osteomyelitis of the talus: Review of the pathophysiology and report of a rare case. The Journal Of Foot & Ankle Surgery. 2009; 48(6):672-6. [DOI:10.1053/j.jfas.2009.07.012] [PMID]

[11] Godlewska EZ, Przystaś W, Sota EG. Dcolourisation of different dyes by two pseudomonas strains under various growth conditions. Water, Air, Soil, & Pollution. 2014; 225:1846. [DOI:10.1007/s11270-013-1846-0] [PMID] [PMCID]

[12] Mattman LH. Cell wall deficient forms: Stealth pathogens. 3rd edition. Boca Raton, London, New York, Washington DC: CRC Press. 2001. P.196- 197. [DOI:10.1201/b16928] [PMID]

[13] Mcclean M, Stanley T, Stanley SH, Maeda Y, Goldsmith CE, Shepherd R, et al. Identification and characterization of breakthrough contaminants associated with the conventional isolation of Mycobacterium tuberculosis. Journal of Medical Microbiology. 2011; 60:1292-8. [DOI:10.1099/jmm.0.030619-0] [PMID]