



**HAL**  
open science

## **Mycobacterium tuberculosis Resides in Macrophages in Laryngeal Tuberculosis: A Case Report**

Wafaa Achache, Mahmoud A Boualam, Nadim Cassir, Clémence Mimari, Delphine Poitreaud, Soraya Mezouar, Jean Louis Mège, Michel Drancourt, Hubert Lepidi

► **To cite this version:**

Wafaa Achache, Mahmoud A Boualam, Nadim Cassir, Clémence Mimari, Delphine Poitreaud, et al.. Mycobacterium tuberculosis Resides in Macrophages in Laryngeal Tuberculosis: A Case Report. Pathogens, 2023, 12 (12), pp.1413. 10.3390/pathogens12121413 . hal-04509468

**HAL Id: hal-04509468**

**<https://amu.hal.science/hal-04509468>**

Submitted on 18 Mar 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Case Report

# *Mycobacterium tuberculosis* Resides in Macrophages in Laryngeal Tuberculosis: A Case Report

Wafaa Achache <sup>1,2</sup>, Mahmoud A. Boualam <sup>1,2</sup> , Nadim Cassir <sup>1,2</sup> , Clémence Mimari <sup>3</sup>, Delphine Poitrenaud <sup>4</sup>, Soraya Mezouar <sup>1,2</sup> , Jean Louis Mège <sup>1,2</sup>, Michel Drancourt <sup>1</sup> and Hubert Lepidi <sup>1,2,\*</sup>

<sup>1</sup> MEPHI, Aix-Marseille University, IRD, 13005 Marseille, France; cassirnadim@gmail.com (N.C.); soraya.mezouar@univ-amu.fr (S.M.); jean-louis.mege@univ-amu.fr (J.L.M.); michel.drancourt@univ-amu.fr (M.D.)

<sup>2</sup> IHU Méditerranée Infection, 13005 Marseille, France

<sup>3</sup> Service ORL Hôpital de la Conception, 13005 Marseille, France

<sup>4</sup> Service Maladies Infectieuses, CH Ajaccio, 20000 Ajaccio, France; delphine.poitrenaud@ch-ajaccio.fr

\* Correspondence: hubert.lepidi@ap-hm.fr; Tel.: +33-4-13-73-24-01; Fax: +33-4-13-73-24-02

**Abstract:** Laryngeal tuberculosis is a rare form of extrapulmonary tuberculosis that questions the natural history of this infection. We report one such case in which a pathological examination of a laryngeal biopsy revealed granulomatous inflammation with caseous necrosis. Further investigations combining immunofluorescence detection of macrophages and in situ hybridization of *Mycobacterium tuberculosis* indicated the presence of *Mycobacterium tuberculosis* (*M. tuberculosis*) in laryngeal granulomatous inflammatory lesions. This observation suggests that the natural history of laryngeal tuberculosis does not differ from that of other forms, guiding early diagnosis in patients with laryngeal lesions to ensure appropriate check-ups and treatment.

**Keywords:** larynx; tuberculosis; granuloma; *Mycobacterium tuberculosis*; laryngitis; extrapulmonary tuberculosis



**Citation:** Achache, W.; Boualam, M.A.; Cassir, N.; Mimari, C.; Poitrenaud, D.; Mezouar, S.; Mège, J.L.; Drancourt, M.; Lepidi, H. *Mycobacterium tuberculosis* Resides in Macrophages in Laryngeal Tuberculosis: A Case Report. *Pathogens* **2023**, *12*, 1413. <https://doi.org/10.3390/pathogens12121413>

Academic Editor: Hwa-Jung Kim

Received: 31 August 2023

Revised: 1 November 2023

Accepted: 24 November 2023

Published: 30 November 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Tuberculosis is a lethal contagious infectious disease caused by members of the *Mycobacterium tuberculosis* complex, more specifically *M. tuberculosis stricto sensu* [1]. The lungs are the most common site for tuberculosis, reported in 80% of cases, but *M. tuberculosis* complex mycobacteria can reach extrapulmonary organs and tissues, including, among others, lymph nodes, bone, and meninges. Laryngeal localization is a rare form of tuberculosis, reported in approximately 1% of cases [2], in which laryngeal tuberculosis is most often associated with pulmonary tuberculosis [3–5].

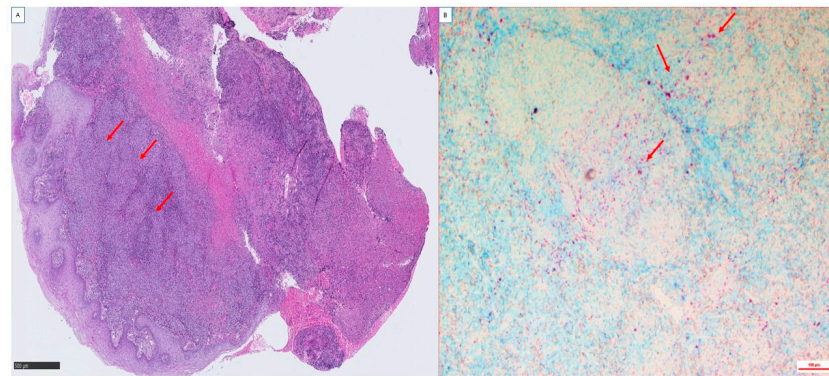
Despite such an association, laryngeal examinations are not usually carried out in patients diagnosed with pulmonary tuberculosis, so the actual incidence of laryngeal tuberculosis is probably underestimated [6]. In fact, laryngeal tuberculosis is the most common granulomatous disease of the larynx [7]. It is highly contagious, and can present very different aspects [8]. Symptoms appear gradually, ranging from hoarseness to difficulty swallowing, and the diagnosis is usually delayed [9]. Its rare appearance explains why it is often overlooked when vocal difficulties are diagnosed [10], challenging the differential diagnosis consisting of non-specific laryngitis, granulomatous diseases and tumors of the larynx [11]. Appropriate management involves tissue biopsy followed by pathological examination for the presence of caseous granulomas and detection of the causative agent, *M. tuberculosis* [12].

We report a rare case of laryngeal tuberculosis in which cellular localization of *M. tuberculosis* was demonstrated in tissue lesions.

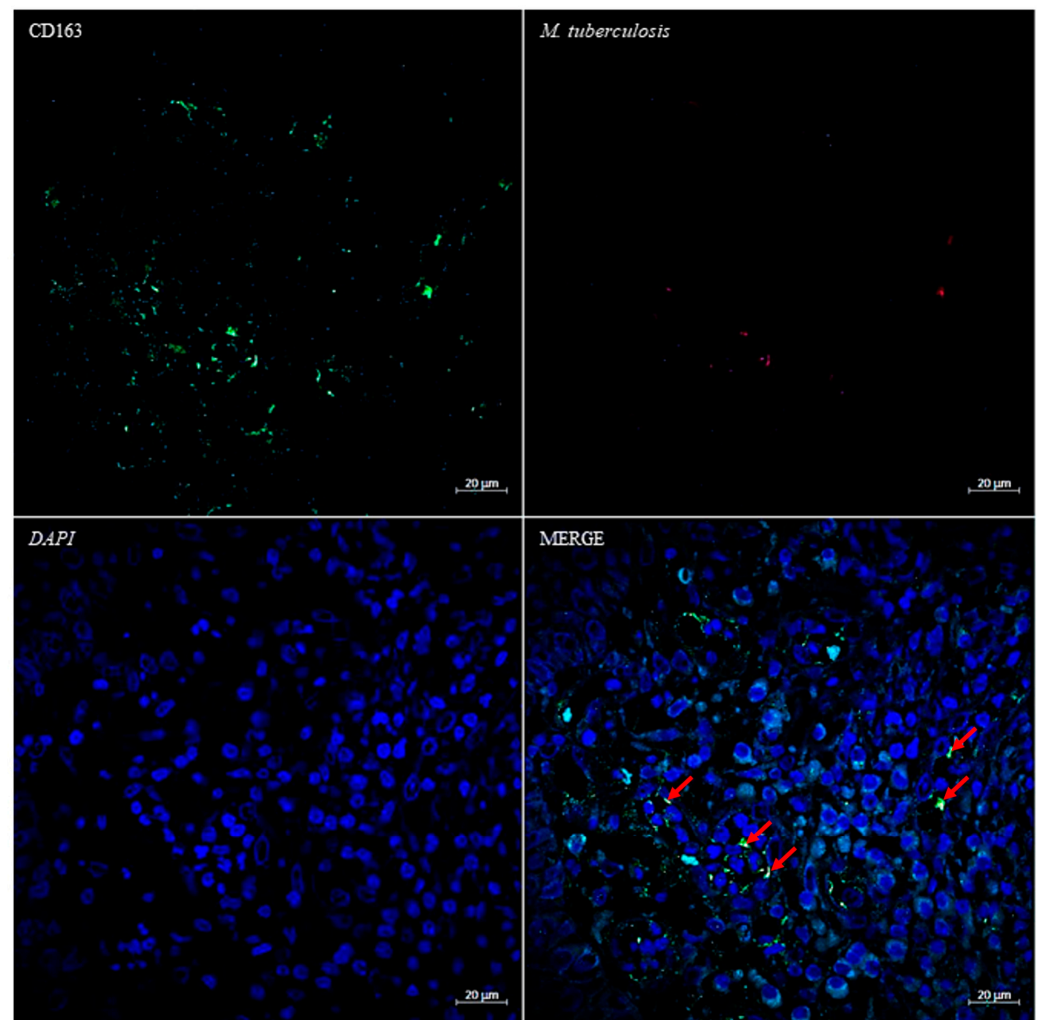
## 2. Case Presentation

A 68-year-old non-smoker born in Algeria and living in Corsica, France, for 30 years was seen for dysphonia. The patient, who gave informed written consent for anonymously reporting the case, had no significant medical history. He had dysphonia for five months, worsening for two months, with progressive dysphagia and odynophagia associated with productive cough. During this period, the patient did not report weight loss, fever, sweating, or chills and denied any recent travel or known exposure to tuberculosis. At the time of admission, the patient was afebrile and physical examination was notable for multiple non-tender mobile lymph nodes of the right posterior cervical chain; no pharyngeal exudate or erythema was noted. White blood cell (WBC) count was 13,500  $\mu\text{L}$ , platelet count was 261,000  $\mu\text{L}$ , and HIV serology was negative. A computed tomography (CT) scan identified mediastinal and hilar adenopathy, including necrotic adenomegaly at the left jugulo-carotid level of sector IIa with infiltration of adjacent fat and bilateral, predominantly right mediastinal–hilar adenomegaly, as well as solid nodules in both lung apices. Microbiological investigations yielded Ziehl–Neelsen-stained mycobacteria identified as *M. tuberculosis* complex by real-time polymerase chain-reaction (GeneXpert, Sunnyvale, CA, USA) in three respiratory tract specimens along with a laryngeal biopsy made by tracheotomy, as previously described [13]. Furthermore, six respiratory tract specimens and one stool specimen cultured colonies identified as *M. tuberculosis* by using matrix-assisted laser desorption/ionization time-of-flight, as previously described [14]. The patient being therefore diagnosed with pulmonary and lymph node tuberculosis, was started on RIPE therapy (Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol) for a period of two months, followed by seven months of therapy including Isoniazid and Rifampicin. The one-year follow up indicated relief of complaints and resolving cough and lymph nodes with complete healing.

Formalin-fixed and paraffin-embedded laryngeal biopsy samples were cut to 3- $\mu\text{m}$  thickness and stained with hematoxylin–phloxine–safran. For each tissue sample, serial sections were also obtained to perform special stains and immunohistological investigations. Special stains that were used for the detection of bacteria and fungi included Giemsa, PAS, Grocott–Gomori methenamine silver, Warthin–Starry, and Ziehl–Neelsen acid-fast stains. Histological examination revealed epithelioid granulomas with giant cells and rare areas of caseous necrosis with polymorphic inflammation. The Ziehl–Neelsen staining revealed the presence of acid-fast bacilli disseminated throughout the laryngeal biopsy (Figure 1). To further investigate tuberculous granulomas, we carried out combined immuno-fluorescence using a specific anti-macrophage antibody and in situ fluorescence hybridization (FISH) using a specific *M. tuberculosis rpoB* DNA probe [15]. In brief, slides were incubated for 15 min with permeabilization buffer consisting of 10% fetal bovine serum and 0.1% Triton X-100 in phosphate-buffered saline (PBS, Thermo Fisher Scientific, Illkirch, France), then were washed three times with PBS, incubated with blocking buffer (1  $\times$  PBS, 10% FBS) for 30 min and rinsed with PBS. Slides were then incubated with an anti-CD163 antibody (Jackson Immuno Research Laboratories, West Grove, PA, USA) diluted at 1:250 in 5% fetal bovine serum-PBS for one hour and washed three times with PBS. FISH was then performed as previously described [15]. After observation and acquisition of fluorescence signal by laser scanning microscopy, a red signal corresponding to *M. tuberculosis*-specific probe (561 nm laser) was observed surrounded by a green fluorescence (488 nm laser) corresponding to a macrophage CD163 signal with a blue (405 nm laser) mark showing large macrophage nuclei (Figure 2).



**Figure 1.** Microscopic observation of a hematoxylin–phloxine–safron-stained laryngeal biopsy from a patient with laryngeal tuberculosis. (A): Observation of very inflamed tissue with areas of necrosis, and the presence of tuberculoid granulomas composed mainly of macrophages, lymphocytes and giant cells (red arrow). (B): Ziehl–Neelsen staining demonstrates the presence of *M. tuberculosis* (red arrow). Scale bars indicate 500  $\mu\text{m}$ . Original images were made by the authors.



**Figure 2.** Fluorescence microscopic images of patient biopsy sections after combined immunofluorescence–fluorescence in situ hybridization techniques: in green, antibodies labeled Alexa 488 anti-CD-163 for macrophage staining. In red, the specific probe of *M. tuberculosis* after FISH. In blue, DAPI for nucleus staining. The collocation of the red and green signals is indicated by the red arrows. Original images made by the authors. Scale bars indicate 20  $\mu\text{m}$ .

### 3. Discussion

While a few previously reported cases have been documented by pathological examination of Ziehl–Neelsen-stained laryngeal tissues allowed for microscopic detection of acid-fast bacilli in tissues [16]; in this patient, *M. tuberculosis* localized in macrophages constituting granulomas in tuberculous laryngitis. Conventional microscopic examination of the laryngeal biopsy specimen by Ziehl–Neelsen staining showed acid-fast bacilli scattered throughout the specimen, and advanced diagnostic combining FISH labeling of *M. tuberculosis* provided more specific detection and immunolabelling of macrophages: indeed, FISH, incorporating probes demonstrated to be specific for *M. tuberculosis*, ensures specificity of the identification (*M. tuberculosis*, in this case) over the Ziehl–Neelsen in staining all the microbes containing mycolic acid in the cell wall, and beyond [15,17]. This observation was carried out using appropriate techniques, including immunofluorescence–fluorescence in situ-hybridization, which allowed the findings to be validated.

This unique observation indicates that the pathology of laryngeal tuberculosis does not differ from that of pulmonary tuberculosis, two related forms of tuberculosis, suggesting a common natural history, relying on the colonization of tissues by the lymphatic pathway [18]. The portal of entry may indeed be the larynx itself [10], as laryngeal tuberculosis clinically appears as primary in 40% of laryngeal tuberculosis cases and is the unique involvement of the larynx in the absence of pulmonary tuberculosis. Alternatively, secondary laryngeal tuberculosis may develop by *M. tuberculosis* spreading from pulmonary tuberculosis [19].

At the end of the 19th century, laryngeal tuberculosis was the most commonly diagnosed laryngeal disease [20]. The advent of efficient anti-tuberculous therapies allowed for the early control of tuberculosis, particularly in its pulmonary form, and a considerable decrease in the frequency of laryngeal tuberculosis was observed [21]. Today, laryngeal tuberculosis is a rare form of extrapulmonary tuberculosis, observed in areas where tuberculosis is highly endemic [22]. The diagnosis of laryngeal tuberculosis is usually delayed due to unremarkable symptoms, while only severe symptoms lead patients to diagnosis following severe odynophagia and dyspnea caused by laryngeal edema and granulations [20]. The vocal cords remain the most frequent location, followed by the arytenoid cartilage and epiglottis [8]. The pathological hallmark of tuberculosis is the formation of caseous granulomas that may result in an infiltrating mass [23,24], with extremely rare cases being located only in the laryngeal organ. A high degree of suspicion is therefore necessary, especially in patients with risk factors for tuberculosis. The gold standard diagnosis relies on the demonstration of *M. tuberculosis* in a laryngeal biopsy. Further diagnostic tests such as culture and imaging studies can be useful in supporting the diagnosis [25]. The diagnosis of laryngeal tuberculosis is usually made based on a biopsy. In some cases, histopathological examination reveals epithelial gigante-cellular inflammation with caseous necrosis. Indeed, the discovery of lymphocytic laryngitis in a clinical context suggestive of tuberculosis should lead to a Ziehl–Nielsen stain and/or a culture of the biopsy fragments [26]. In a further series, the histological lesions were epithelial gigante-cellular granuloma in 100% of cases and caseous necrosis in 20% of cases [11].

### 4. Conclusions

In this patient, the diagnosis of a rare form of laryngeal tuberculosis through histological and microbiological examination showed morphological features similar to those observed in the most commonly infected organs and tissues such as lymph nodes and lung. This observation suggests that the natural history of laryngeal tuberculosis does not differ from that of other forms, being primarily an infection of lymphatic tissues and organs with diffusion to other tissues and organs.



**Author Contributions:** W.A. and N.C.: experimental design, analysis, interpretation, and original draft of the manuscript. N.C., C.M. and D.P.: took care of the patient. M.A.B. and S.M.: contributed to data interpretation and writing of the original draft. M.D., J.L.M. and H.L.: responsible for project design, funding, and critically reviewing the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** Wafaa ACHACHE benefits from a PhD grant from Doctoral College 62, Aix-Marseille-University, Marseille.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Written informed consent has been obtained from the patient to publish this paper.

**Data Availability Statement:** All data deriving from this study are given in the article.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Chakaya, J.; Petersen, E.; Nantanda, R.; Mungai, B.N.; Migliori, G.B.; Amanullah, F.; Lungu, P.; Ntoumi, F.; Kumarasamy, N.; Maeurer, M.; et al. The WHO Global Tuberculosis 2021 Report—Not so Good News and Turning the Tide Back to End TB. *Int. J. Infect. Dis.* **2022**, *124*, S26–S29. [[CrossRef](#)] [[PubMed](#)]
- Agarwal, R.; Gupta, L.; Singh, M.; Yashaswini, N.; Saxena, A.; Khurana, N.; Chaudhary, D. Primary Laryngeal Tuberculosis: A Series of 15 Cases. *Head Neck Pathol.* **2019**, *13*, 339–343. [[CrossRef](#)] [[PubMed](#)]
- Pašvenskaitė, A.; Bajoriūnaitė, D.; Buginytė, R.; Ulozas, V. Laryngeal Tuberculosis: A Case Report. *J. Med. Sci.* **2021**, *9*, 175–180.
- de Santi, P.R. Laryngeal Case for Diagnosis. *Proc. R. Soc. Med.* **1909**, *2*, 140–141. [[CrossRef](#)] [[PubMed](#)]
- Yin, N.; Delord, M.; Giovanni, A.; del Grande, J.; Drancourt, M.; Brouqui, P.; Lagier, J.-C. Laryngeal Tuberculosis Diagnosed by Stool Sample Cultures: A Case Report. *J. Med. Case Rep.* **2015**, *9*, 74. [[CrossRef](#)] [[PubMed](#)]
- Zang, J.; Tian, Y.; Jiang, X.; Lin, X.-Y. Appearance and Morphologic Features of Laryngeal Tuberculosis Using Laryngoscopy. *Medicine* **2020**, *99*, e23770. [[CrossRef](#)]
- Youssef, G.; Mahboub, B.-H.; Azab, S.-N. Laryngeal and Voice Disorders in Patients with Pulmonary Tuberculosis. *Iran. J. Otorhinolaryngol.* **2021**, *33*, 97–102. [[CrossRef](#)]
- Wang, C.-C.; Lin, C.-C.; Wang, C.-P.; Liu, S.-A.; Jiang, R.-S. Laryngeal Tuberculosis: A Review of 26 Cases. *Otolaryngol.—Head Neck Surg.* **2007**, *137*, 582–588. [[CrossRef](#)]
- Lim, J.-Y.; Kim, K.-M.; Choi, E.C.; Kim, Y.-H.; Kim, H.S.; Choi, H.-S. Current Clinical Propensity of Laryngeal Tuberculosis: Review of 60 Cases. *Eur. Arch. Oto-Rhino-Laryngol. Head Neck* **2006**, *263*, 838–842. [[CrossRef](#)]
- Benhoummad, O.; Rochd, S.; Yafi, M.; Rochdi, Y.; Raji, A. Extracranial Localization of Tuberculosis in Otorhinolaryngology: Rare Presentations About 29 Cases. *Med. Res. Arch.* **2022**, *10*. [[CrossRef](#)]
- El Ayoubi, F.; Chariba, I.; El Ayoubi, A.; Chariba, S.; Essakalli, L. La tuberculose primitive du larynx. *Ann. Fr. Oto-Rhino-Laryngol. Pathol. Cervo-Faciale* **2014**, *131*, 347–350. [[CrossRef](#)]
- Cialente, F.; Grasso, M.; Ralli, M.; de Vincentiis, M.; Minni, A.; Agolli, G.; Venti, M.D.S.; Riminucci, M.; Corsi, A.; Greco, A. Laryngeal Tuberculosis in Renal Transplant Recipients: A Case Report and Review of the Literature. *Bosn. J. Basic Med. Sci.* **2020**, *20*, 411–414. [[CrossRef](#)] [[PubMed](#)]
- Fellag, M.; Saad, J.; Barlogis, V.; Michel, G.; Drancourt, M. Shell-Vial Assay in Diagnosis of Disseminated BCG Infection in an Immunodeficient Child. *Pediatr. Infect. Dis. J.* **2020**, *39*, 258–259. [[CrossRef](#)] [[PubMed](#)]
- Robinne, S.; Saad, J.; Morsli, M.; Hamidou, Z.H.; Tazerart, F.; Drancourt, M.; Baron, S.A. Rapid Identification of *Mycobacterium tuberculosis* Complex Using Mass Spectrometry: A Proof of Concept. *Front. Microbiol.* **2022**, *13*, 753969. [[CrossRef](#)] [[PubMed](#)]
- Loukil, A.; Kirtania, P.; Bedotto, M.; Drancourt, M. FISHing *Mycobacterium tuberculosis* Complex by Use of a rpoB DNA Probe Bait. *J. Clin. Microbiol.* **2018**, *56*, e00568-18. [[CrossRef](#)] [[PubMed](#)]
- Guan, L.S.; Jun, T.K.; Azman, M.; Baki, M.M. Primary Laryngeal Tuberculosis Manifesting as Irregular Vocal Fold Lesion. *Turk. Arch. Otorhinolaryngol.* **2022**, *60*, 47–52. [[CrossRef](#)]
- Akbar, A.; Al-Momin, S.; Kishk, M.; Al-Ateeqi, A.; Shajan, A.; Rahmeh, R. Draft Genome Sequence Data of Enterococcus Faecium R9, a Multiple Enterocins-Producing Strain. *Data Brief* **2023**, *48*, 109151. [[CrossRef](#)]
- Behr, M.A.; Waters, W.R. Is Tuberculosis a Lymphatic Disease with a Pulmonary Portal? *Lancet Infect. Dis.* **2014**, *14*, 250–255. [[CrossRef](#)]
- Migliorelli, A.; Mazzocco, T.; Bonsembiante, A.; Bugada, D.; Fantini, M.; Elli, F.; Stacchini, M. Laryngeal Tuberculosis: A Case Report with Focus on Voice Assessment and Review of the Literature. *ACTA Otorhinolaryngol. Ital.* **2022**, *42*, 407–414. [[CrossRef](#)]
- Kurokawa, M.; Nibu, K.; Ichimura, K.; Nishino, H. Laryngeal Tuberculosis: A Report of 17 Cases. *Auris. Nasus. Larynx* **2015**, *42*, 305–310. [[CrossRef](#)]
- Yencha, M.W.; Linfesty, R.; Blackmon, A. Laryngeal Tuberculosis. *Am. J. Otolaryngol.* **2000**, *21*, 122–126. [[CrossRef](#)]

22. Ling, L.; Zhou, S.-H.; Wang, S.-Q. Changing Trends in the Clinical Features of Laryngeal Tuberculosis: A Report of 19 Cases. *Int. J. Infect. Dis.* **2010**, *14*, e230–e235. [[CrossRef](#)]
23. Kulkarni, N.; Gopal, G.S.; Ghaisas, S.G.; Gupte, N. Epidemiological considerations and clinical features of ENT tuberculosis. *J. Laryngol. Otol.* **2001**, *115*, 555–558. [[CrossRef](#)]
24. Hasibi, M.; Yazdani, N.; Asadollahi, M.; Sharafi, M.; Dehghan Manshadi, S.A. Clinical Features of Laryngeal Tuberculosis in Iran. *Acta Med. Iran.* **2013**, *51*, 638–641.
25. Yan, K.; Taxy, J.B.; Paintal, A.; Friedman, A.D. Atypical Laryngeal Infections: Localized Lesions from Unusual Organisms May Simulate Malignancy. *Ann. Otol. Rhinol. Laryngol.* **2020**, *129*, 82–86. [[CrossRef](#)]
26. Thiam, I.; Doh, K.; Gaye, A.; Sonhaye, K.; Ndiaye, M.; Gaye, G.W. La tuberculose laryngée diagnostiquée dans un laboratoire d’anatomie pathologique du Sénégal (2011–2015). *Bull. Soc. Pathol. Exot.* **2018**, *111*, 5–8. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.