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ACTINOMYCETES AND ATYPICAL MYCOBACTERIA: MICROBIOLOGICAL CHARACTERIZATION AND MODERN DIAGNOSTIC METHODS

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Abstract.

This article addresses one of the pressing issues in modern microbiology — the comparative characterization of actinomycosis pathogens and atypical mycobacteria, as well as their laboratory diagnostic methods. The primary objective of the study is to investigate the morphological characteristics of the Actinomyces genus and to differentiate atypical mycobacteria according to the Runyon classification. The article analyzes the role of these microorganisms in human pathology, focusing particularly on the practical significance of mycobacteriosis in immunocompromised patients. In the diagnostic process, special attention is paid to classical microscopic methods, including Gram and Ziehl-Neelsen staining, as well as the specific features of detecting sulfur granules (drusen). The role of biochemical indicators, such as the niacin test and catalase activity, in distinguishing atypical mycobacteria from true tubercle bacilli is highlighted. The results indicate that precise identification of the pathogen at the species level plays a crucial role in determining effective treatment strategies for chronic infections.

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Actinomycosis, Actinomyces israelii, sulfur granules (drusen), atypical mycobacteria, mycobacteriosis, Runyon classification, differential diagnosis, Ziehl-Neelsen staining, opportunistic infections, acid-fastness.

Introduction:

In modern medical microbiology, the increasing prevalence of opportunistic infections, particularly actinomycosis and mycobacteriosis, remains a significant challenge in clinical practice. Although actinomycetes are members of the human body's resident microflora, they can induce severe, chronic pyogenic-granulomatous processes under the influence of certain exogenous and endogenous factors. Simultaneously, atypical mycobacteria, which are widespread in the environment, occupy a distinct position in human pathology. Their clinical presentation often mimics pulmonary tuberculosis; however, their natural resistance to standard antitubercular drugs complicates the differential diagnosis process. Although modern molecular-genetic methods are currently utilized alongside classical techniques in microbiological diagnosis, identification based on the morphological and biochemical characteristics of the pathogen remains predominant in many laboratories. The mechanism of sulfur granule (drusen) formation in actinomycosis and the Runyon classification of atypical mycobacteria serve as the fundamental basis for differentiating these microorganisms. The objective of this work is to analyze the microbiological characteristics of actinomycosis pathogens and atypical mycobacteria, highlight their clinical significance, and systematize modern diagnostic methods used in laboratory settings. This, in turn, will enhance treatment efficacy by ensuring timely and accurate diagnosis for patients.

Main Part:

In the process of studying the microbiological characteristics of actinomycosis and mycobacteriosis, comprehensive laboratory analyses were conducted, focusing primarily on the morphological, cultural, and biochemical differentiation of the pathogens. When smears prepared from selected research objects—such as pus, fistula secretions, and biopsy samples—were Gram-stained, the distinct polymorphism of actinomycetes was observed. Specifically, Actinomyces israelii strains were found to form long, thin, branching filaments, as well as yellowish sulfur granules (drusen) with a diameter of 1–2 mm within the pathological material. The microscopic structure of these granules is characterized by a central cluster of Gram-positive mycelium surrounded by acid-labile, club-shaped peripheral radiations. During the cultivation stage, these microorganisms were

grown under anaerobic conditions in enriched nutrient media, such as blood agar and sugar broth. After 10–14 days of incubation, the formation of rough, whitish colonies with a "molar tooth" appearance on the medium surface served as microbiological confirmation of actinomycosis. Simultaneously, in serological diagnosis, skin-allergy tests conducted with actinolysate yielded positive results, demonstrating the allergenic properties of the pathogen. In the identification of atypical mycobacteria, the Ziehl-Neelsen staining method played a central role; due to the presence of mycolic acid in their cell walls, these bacteria possess acid-fast properties and appear bright red in smears. To differentiate them from Mycobacterium tuberculosis, they were analyzed according to the Runyon classification, which divides them into four groups. Group I (photochromogens, e.g., M. kansasii) were distinguished by their ability to synthesize carotenoid pigments only upon exposure to light, while Group II (scotochromogens) produced pigments even in the dark. Group III, represented by the M. avium-intracellulare (MAC) complex, was characterized by a lack of pigment production and an extremely slow growth rate, primarily causing disseminated mycobacteriosis in immunocompromised patients. Group IV, consisting of rapidly growing mycobacteria, differed fundamentally from other species by forming colonies within 3–7 days. During biochemical analysis, the niacin test yielded negative results for atypical mycobacteria, while the catalase test proved that they maintain enzymatic activity even at a temperature of 68°C. These indicators are of decisive importance in the differential diagnosis of mycobacteriosis in clinical practice, which often presents with a clinical picture similar to tuberculosis but remains resistant to standard therapy. Consequently, in the diagnosis of actinomycosis and mycobacteriosis, the systematic application of not only microscopy but also cultural cultivation and biochemical identification methods allows for the precise determination of the disease type and the development of targeted treatment strategies.

The immunological reactivity of the human body plays a decisive role in the pathogenesis of actinomycosis and mycobacteriosis. In infections caused by actinomycetes, the weakening of cellular immunity allows the pathogen to breach tissue barriers and form specific granulomas. In laboratory studies of this process, the immunofluorescence assay (IFA/RIF) demonstrates high efficacy alongside traditional microscopy. Using this method, the binding of antibodies labeled with luminescent particles to the surface antigens of actinomycetes can be observed in smears; this enables diagnosis even in the early stages before sulfur granules (drusen) have fully formed. Furthermore, in visceral forms of actinomycosis

(pulmonary, abdominal), "radiant" structures identified via X-ray and CT scans, when compared with microbiological analyses, provide more precise data regarding the extent of the disease and destructive changes. The biological diversity of atypical mycobacteria is also explained by their ability to form biofilms in various ecological environments, including water, soil, and medical equipment. Mycobacteria within biofilms exhibit a 100 to 1000-fold higher resistance to disinfectants and many antibiotics. In the process of differential diagnosis, the "Tellurite reduction test" is of particular significance; members of Group III (e.g., M. avium) reduce potassium tellurite to metallic tellurium, turning the medium black. This characteristic facilitates their rapid differentiation from other slow-growing mycobacteria. Furthermore, when the nitrate reductase activity of atypical mycobacteria is examined, M. kansasii yields a positive result, whereas M. avium shows a negative result, serving as an additional marker in defining their metabolic profile. Currently, the drug resistance of atypical mycobacteria is being studied at the genetic level. Specifically, mutations in the rpoB gene determine resistance to rifampicin, while mutations in the embB gene are responsible for resistance to ethambutol. Consequently, this necessitates not only the identification of the pathogen in microbiological diagnosis but also the mandatory determination of its drug sensitivity (antibiogram). In contrast, actinomycetes generally remain sensitive to long-term penicillin therapy; however, due to the presence of accompanying secondary microflora (such as staphylococci and enterobacteria), a complex treatment approach is required. Thus, modern microbiological methods, including mass spectrometry (MALDI-TOF MS) and gene sequencing, reduce the probability of error in identifying actinomycetes and mycobacteria to nearly zero. This provides clinicians with a solid foundation to establish personalized treatment protocols for each specific case.

Conclusion:

Based on the comparative study of microbiological analyses and scientific literature, the following conclusions were reached regarding the diagnosis of actinomycosis and mycobacteriosis. First, the cornerstone of laboratory diagnosis in pathologies caused by actinomycetes is the identification of specific sulfur granules (drusen) within the pathological material. The typical structure of these granules in Gram-stained smears and the growth of "molar tooth" colonies serve as leading criteria for differentiating this pathogen from other pyogenic infections. Second, the role of atypical mycobacteria in human pathology is closely linked to their classification into four Runyon groups and their pigment-production characteristics. To avoid misidentifying these microorganisms as M. tuberculosis, it

is essential to conduct biochemical tests such as the niacin test and catalase activity assays. Research indicates that due to the resistance of atypical mycobacteria to standard antitubercular therapy, precise identification at the species level is the only way to ensure treatment efficacy. In conclusion, it is vital to emphasize that the differential diagnosis of actinomycosis and mycobacteriosis should not be limited to microscopy alone but must involve a comprehensive application of cultural cultivation and biochemical analyses. This approach enables timely and accurate diagnosis of chronic infections, prevents incorrect treatment procedures, and accelerates the patient recovery process.

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