EDUCATIONAL COMMENTARY – CAUSES OF PHARYNGITIS – HOW TO DIFFERENTIATE THEM

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Learning Outcomes

On completion of this exercise, the participant should be able to:

- define pharyngitis and its clinical presentations;
- discuss pathogenesis and etiologic agents associated with pharyngitis;
- discuss microbiologic testing to differentiate causative organisms of pharyngitis; and
- discuss traditional culture differentiation of bacterial pharyngitis.

Introduction

Pharyngitis is the medical term used to describe inflammation and infection of the pharynx, more commonly called a sore throat. Eleven million cases of pharyngitis are diagnosed in the United States each year.\(^1\) Pharyngitis can be observed in persons of all ages at any time of year; however, the prevalence is higher in children.\(^1\) Increased incidence of pharyngitis is observed during respiratory illness seasons (winter through spring). Acute pharyngitis is an illness that typically causes people to seek medical attention.\(^1\) Symptoms begin with pain in the back of the throat and may include fever, swollen cervical lymph nodes, white patches or streaks of pus in back of the throat, headache, or nausea.\(^2\) Based on the clinical presentation, the clinician will need to decide whether to prescribe antimicrobial treatment, which should be managed with laboratory testing when possible.

Pathogenesis and Etiologic Agents of Pharyngitis

Depending on the causative agent of the pharyngeal infection, irritated and red tissue, pus, tiny blister-like vesicles, mucosal ulcerations, or swollen lymph nodes may be present upon examination. Pathogenic mechanisms differ depending on the organism causing the infection. Some organisms directly invade the pharyngeal mucosa, others release toxins at the site of the infection, and yet some organisms both invade the pharyngeal tissue and release toxins with other complex virulence factors simultaneously.\(^3\)
The cast of microbial characters responsible for the symptoms related to pharyngitis can vary, underscoring the importance of proper identification. Most cases of pharyngitis occur during the respiratory illness seasons and accompany other infections, primarily those caused by viruses. Patients with respiratory infections caused by rhinoviruses, coronaviruses, influenza type A or B, or parainfluenza viruses commonly experience pharyngitis during their illness. Cytomegalovirus and Epstein-Barr virus infections are often associated with ulcerative pharyngitis. Less commonly, adenovirus and herpes simplex virus infections cause severe pharyngitis with painful blister-like vesicles causing extensive destruction to the mucosal lining of the pharynx. Because viral pharyngitis is not treatable with antibiotics, it is important to differentiate it from bacterial pharyngitis.

Although viruses cause most cases of pharyngitis, bacterial pharyngitis accounts for 15% to 30% of the infections. Pyogenic, or pus-forming, infections of the pharynx and tonsils are associated with streptococcal bacterial pharyngitis. Different bacteria can cause pharyngitis; however, the common cause of bacterial pharyngitis is Streptococcus pyogenes, also known as group A β-strep. Large colony groups C and G β-hemolytic streptococci have been found to exhibit similar virulence factors to S pyogenes and may also be associated with pyogenic pharyngitis; however, these organisms occur less frequently. Another organism sometimes encountered in bacterial pharyngitis is Arcanobacterium haemolyticum, which is often associated with adolescent patients. Other microorganisms rarely but occasionally associated with pharyngitis are Neisseria gonorrhoeae, Corynebacterium diphtheriae, Mycoplasma pneumoniae and Candida species.

Microbiologic Testing to Differentiate Causative Organisms of Pharyngitis

Identifying the etiologic agent causing pharyngitis is necessary to determine the appropriate treatment. Collecting an adequate specimen is crucial. It is important that clinical laboratories provide education and an easy-to-follow procedure regarding specimen collection and transport. To properly collect a throat specimen, the clinician must use a sterile flocked swab with a plastic shaft, depress the patient’s tongue with a tongue depressor, and avoid touching the swab to the tongue, teeth, or anterior buccal cavity. The clinician must sample the posterior pharynx, tonsils, and inflamed areas with the swab, rubbing gently to absorb exudate and fluid. Being careful not to contaminate with flora in the mouth, the swab should be removed and placed into a commercially available transport device. A sterile transport container with the appropriate transport medium is necessary to maintain viability and to prevent overgrowth of contaminating organisms. Depending on the methodology of identification, the laboratory will need to define the appropriate collection devices required per the testing procedure.
Pharyngitis caused by \textit{S} \textit{pyogenes} and other bacteria is treatable with antibiotics. Treatment of bacterial pharyngitis is important because it is highly contagious. Untreated infections caused by \textit{S} \textit{pyogenes} can lead to complications such as glomerulonephritis, rheumatic fever, and, in worst-case scenarios, sepsis and death.\(^6\) Transmission of \textit{S} \textit{pyogenes} pharyngitis is a community health concern because transmission can occur easily when people are in close proximity, which can result in widespread illness. Today, many testing methods are used to detect and differentiate causes of acute pharyngitis. Methods include antigen detection, molecular detection, and traditional culture. Owing to the serious health complications associated with bacterial pharyngitis, antigen and molecular detection tests have been developed to rapidly detect \textit{S} \textit{pyogenes}. These rapid testing methods are favored in emergency departments and urgent care clinics because of their ability to obtain a result in 15 to 60 minutes.\(^7\) A limitation to the highly specific antigen and molecular tests for group A \(\beta\)-hemolytic streptococci is that not all bacterial organisms responsible for pharyngitis are included. Other organisms are typically not considered until a patient returns with ongoing symptoms, after receiving negative strep A antigen or molecular test results. Traditional throat culture testing is being challenged by the heightened performance of antigen and molecular assays. Some antigen and molecular assays for strep A no longer require culture confirmation for negative results.

Throat culture has been considered the criterion standard for the diagnosis of group A \(\beta\)-hemolytic streptococci for many years and some direct antigen detection assays still require throat culture confirmation of negative results.\(^3\) A limitation to culture is the turn-around time, which results in a 24-to-48 hour delay in treatment. On the other hand, it is currently only with traditional throat culture that all of the bacterial organisms can be identified. Advances in technology will likely lead to development of a pharyngitis panel to detect the most common organisms associated with acute pharyngitis in a single test.

**Traditional Culture Differentiation**

Clinical laboratories offering traditional culture typically refer to the order for bacterial pharyngitis testing as a “strep screen.” Throat culture procedures have been tailored to maximize recovery of group A \(\beta\)-hemolytic streptococci because \textit{S} \textit{pyogenes} accounts for most bacterial pharyngitis. It also has the potential for further health complications when left untreated. The traditional method used to recover bacteria that cause pharyngitis must include the growth medium, the atmospheric environment, and the duration of incubation.

In general, most laboratories use 5\% sheep blood agar plates incubated in ambient air at 35\(^\circ\)C for a minimum of 24 hours before initial examination; incubation under anaerobic conditions or CO2 is also
used.3 In addition, selective growth medium agars are available to suppress the growth of non-pathogenic oral microbiota to assist with isolation of group A β-hemolytic streptococci. An example of selective growth agar is 5% sheep blood agar supplemented with trimethoprim-sulfamethoxazole (SXT).3 Although selective media are helpful for isolation of group A β-hemolytic streptococci, they will suppress the growth of groups C and G β-hemolytic streptococci, as well as other organisms potentially responsible for bacterial pharyngitis. Laboratories using selective growth media should consider plating non-supplemented blood agar at the same time. Regardless of the growth medium and atmospheric environment, culture plates should be incubated for 48 hours and examined before the culture is reported as negative. As stated above, a limitation to traditional culture is the incubation time required to allow appropriate growth of the organisms.

Inoculation of the agar plates employs the 4-quadrant streaking method customarily used in microbiology. An identifiable characteristic of the most common bacteria related to bacterial pharyngitis is β-hemolysis on blood agar plates. The colony appearance of groups A, C, and G β-hemolytic streptococci are similar. They are all grayish white, transparent to translucent, matte or glossy, with large zones of β-hemolysis.3 Visualization of β-hemolysis is enhanced by anaerobic growth conditions in Streptococci species, which is the reason why the atmospheric environment is important. During inoculation, the agar may be stabbed with the loop to enhance beta hemolysis produced by the bacteria in an anaerobic environment. This technique results in the organisms growing in the stabs of the agar which will display enhanced β-hemolysis.3 This technique is shown in Figure 1.

β-hemolysis, visualized on culture, is key to targeting the organisms of interest. A catalase test should be performed on all β-hemolytic colonies to avoid confusion with Staphylococcus aureus, which is sometimes recovered as part of the oral microbiota. Streptococcus species are catalase negative, whereas Staphylococcus species are catalase positive. Less than 1% of S pyogenes are non-hemolytic and could be overlooked when relying on traditional culture alone.3
EDUCATIONAL COMMENTARY – CAUSES OF PHARYNGITIS – HOW TO DIFFERENTIATE THEM (cont.)

If mixed or rare β-hemolytic Streptococcus colonies are observed, it will be necessary to sub-culture the β-hemolytic streptococci colony (or colonies) to a new blood agar plate for further incubation. At the time of sub-culturing, a commercially available bacitracin disk may be placed directly on the first quadrant to aid in presumptive identification of S pyogenes. This is shown in Figure 2.

After 18-to-24 hours of incubation, a zone of inhibition around the bacitracin disk of 10 mm or greater must be observed to be considered susceptible and for a presumptive identification of S pyogenes to be made. Laboratories may use the bacitracin disk as a screening method to differentiate group A β-hemolytic streptococci from other β-hemolytic organisms.

If sufficient isolated β-hemolytic streptococcus colonies are recovered at the initial incubation of the culture, commercially available direct antigen latex agglutination testing may be used to rapidly identify groups A, C, and G β-hemolytic streptococci. The latex agglutination tests are commercially developed specifically for β-hemolytic streptococci and are commonly referred to as Lancefield typing. This is shown in Figure 3.

Finally, A haemolyticum is a gram-positive rod with growth characteristics in culture that closely resemble the growth characteristics of group A β-hemolytic streptococci. A Gram stain should be performed after the catalase test of β-hemolytic colonies, which will result in quick differentiation of this bacterial organism. The clinical picture of A haemolyticum is indistinguishable from that of streptococcal pharyngitis so proper identification and treatment is important. Commercially available identification systems are used for the confirmation of organisms other than S pyogenes. After ruling out S pyogenes, groups C and G large colony β-hemolytic streptococci, and A haemolyticum, patients with continued and recurring symptoms of pharyngitis will require additional testing to identify the etiologic agent. Additional growth media and methods will need to be considered for proper diagnosis.
EDUCATIONAL COMMENTARY – CAUSES OF PHARYNGITIS – HOW TO DIFFERENTIATE THEM (cont.)

Summary

Although the most common causes of pharyngitis are viruses that cannot be treated with antibiotics, there are clinically important bacterial causes that require identification and antibiotic treatment. Without laboratory diagnostics, treatment of patients with acute pharyngitis is a challenge, and improper identification can contribute to inappropriate use of antibiotics.

References


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