

RESPIRATORY SYSTEM

MICROBIOLOGY



Title: Sheet 2 – Group B strep.

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-The respiratory tract is the most common site of body acquired infection by pathogens and opportunistic pathogens.
-This site becomes infected frequently because it comes into direct contact with physical environment and is exposed continuously to many microorganisms in the air.
-The human respiratory tract is exposed to many potential pathogens via close contact with human healthy carriers via air droplets, hand & mouth contact, smoke and dust particles.
-It has been calculated that the average individual inhaled & ingests at least to 8 microbial cells per minute or 10,000 per day.

Bacterial infections of the Respiratory Tract 1

Streptococcus

🌸 Important general properties:

- > Gram (+) cocci, oval in shape and arranged in chains or pairs. They're more ovoid, and smaller than the coccus of staphylococci. Another important thing that they divide on a single axis plane, that's why they appear in chains (in contrast to the cocci of staph. coccus; they divide on several planes so they appear grapes-like)
- > **Remember:** Streptococci are **catalase negative**, while staphylococci are catalase (+).
- > They can tolerate oxygen reactive species as they contain superoxide dismutase.
- > Some types are part of the normal microbiota, while others are associated with direct human infections or immunologic response to them.

🌸 The classification of streptococci into major categories has been based on a series of observations over many years:

- Hemolysis pattern on blood agar.
- Serologic specificity of the cell wall's group-specific substance (Lancefield antigens) and other cell wall or capsular antigens.
- Biochemical reactions and resistance to physical and chemical factors.

Let's discuss each classification:

1. According to hemolytic reactions:



Beta Hemolysis

Alpha Hemolysis

Gamma Hemolysis

- β-Hemolytic** streptococci form a **clear zone** around their colonies because of **complete lysis** of RBCs. β-Hemolysis is due to the production of enzymes (hemolysins) called **streptolysin O** and **streptolysin S**.
- α-Hemolytic streptococci form a **green zone** around their colonies as a result of incomplete (partial) lysis of RBCs in the agar. The green color is formed when hydrogen peroxide produced by the bacteria oxidizes **hemoglobin** (red color) to **biliverdin** (green color).
- Some streptococci are nonhemolytic (γ-hemolysis).



2. Lancefield Grouping: lancefield is a name of a female bacteriologist

- Polysaccharide and teichoic acid antigens of streptococci are **carbohydrates** contained in the peptidoglycan of the cell wall that form the basis of serologic grouping into Lancefield groups A – H and K – U.
- Nowadays latex agglutination is used.
- Only those that are Catalase (-) and coagulase (-) are grouped.
- Groups A, B, C, F, and G are human pathogens.

Species	Lancefield Group	Typical Hemolysis	Diagnostic Features ¹
<i>S. pyogenes</i>	A	β	Bacitracin-sensitive
<i>S. agalactiae</i>	B	β	Bacitracin-resistant; hippurate hydrolyzed
<i>E. faecalis</i>	D	α or β or none	Growth in 6.5% NaCl ² Resistant to penicillins
<i>S. bovis</i> ³	D	α or none	No growth in 6.5% NaCl sensitive to penicillins
<i>S. pneumoniae</i>	NA ⁴	α	Bile-soluble; inhibited by optochin
Viridans group ⁵	NA	α	Not bile-soluble; not inhibited by optochin

- ↪ GBS (strep agalactiae) is the most common cause of neonatal sepsis and meningitis
- ↪ Lancefield grouping is not applicable to the alpha hemolytic bacteria (they've no unique antigen)

3. Biochemical reactions and resistance to physical and chemical factors:

The most important reaction here is the bacitracin sensitivity reaction. Bacitracin is an antibiotic that kills GAS when cultured on blood agar. **So, Strep. Pyogenes is Bacitracin sensitive.** Therefore, the bacitracin test is very specific for GAS.

Enough talking about classifications and let's dive into the real deal:

Group A Beta hemolytic streptococci (GAS) (aka strep. Pyogenes)

 GAS are among the **most prevalent of human bacterial pathogen**, and are exclusively human pathogens.

- They cause a wide range of suppurative (pus forming) infections:
 - **The respiratory tract (Pharyngitis)**, which can cause local and distant complications)
 - Skin infections
 - Life-threatening soft tissue infections, and certain types of toxin-associated reactions.
 - They have a serious hallmark of POST infective immunological disorders like RF.
 - A similar spectrum of infections may be caused by group C and group G streptococci.



Pathogenesis

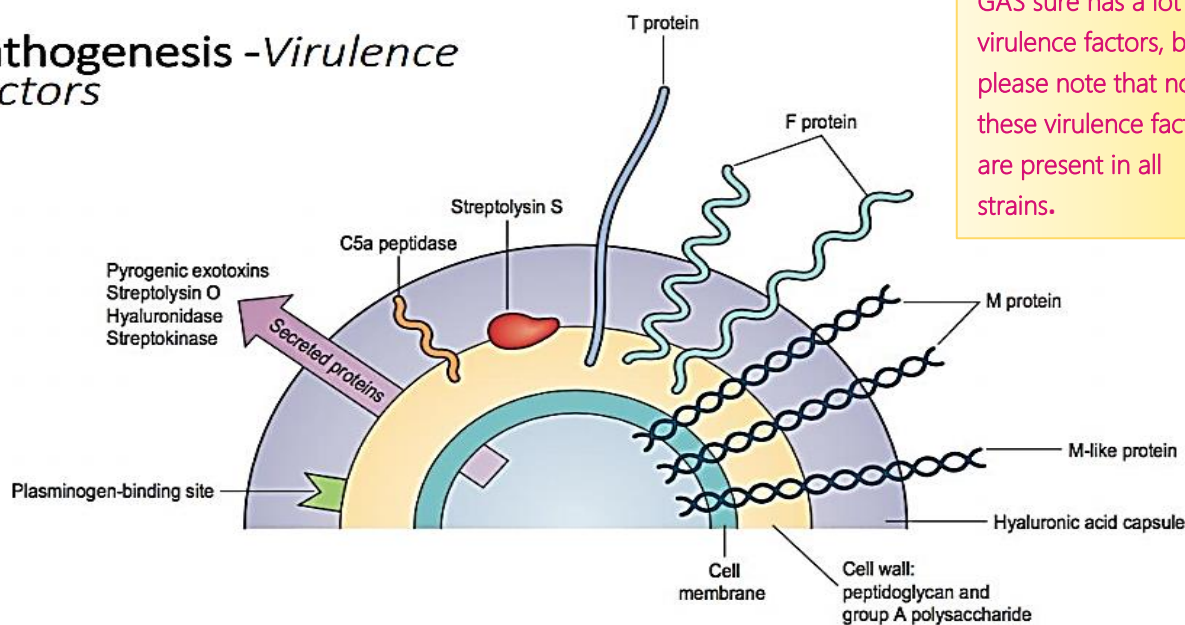
GAS cause disease by three **mechanisms**:

- Pyogenic inflammation** (pus forming) induced locally at the site of the organisms in the tissue (whether in the pharynx or skin).

- ii. **Exotoxin production**, which can cause widespread systemic symptoms in areas of the body where there are no organisms (e.g. scarlet fever).
- iii. **Immunologic**, which occurs when antibodies formed against a component of the organism cross-react with normal tissue or form immune complexes that damage normal tissue (post strep. diseases).

Virulence factors

Pathogenesis - Virulence factors



GAS sure has a lot of virulence factors, but please note that not all these virulence factors are present in all strains.

a. Adhesion (F protein)

- › The F protein initiates infection and internalization of bacteria by binding to Bronectin, which is a matrix protein that's present on cells of the skin and pharynx.
- › Adhesion is also aided by surface-exposed lipoteichoic acid and M proteins.

b. M proteins (THE most important: GAS without M protein is NOT virulent)

- › M proteins are immunogenic; they stimulate an immune reaction which may later cause the post-strep. diseases.
- › M proteins provide GAS with the ability to resist phagocytosis by host neutrophils.
- › They also prevent opsonisation of **C3b** and even some regulatory factors from the complement system.
- › Some of these proteins bind host proteins and cover the bacterium with them (Imposters' action 🧑🏻).
- › Resistance to GAS is formed when antibodies are formed against the M protein. However, the distal part of the protein shows hypervariability and thus recurrent infections.

c. Capsule

- › Their capsule (if present) is identical to the hyaluronic acid of the connective tissue of the host and is **not immunogenic**. In this way the bacteria can disguise themselves with an immunological 'self' substance. i.e. the body won't attack them.

- › Although rarely seen in uncomplicated cases, severe and difficult cases are often found to be caused by capsulated organisms.
- › Capsules are anti-phagocytic factors, with a different mechanism than M proteins. This combined anti-phagocytic effect of M protein and capsule make these strains much harder to clear.

d. C5a peptidase

- › C5a peptidase is present on the surface of all strains.
- › It specifically cleaves, and thereby inactivates, human C5a, one of the principal chemo-attractants of phagocytic cells. Consequently, immune cells are not attracted to the site of inflammation.

e. Streptolysins (hemolysins)

- › GAS produces two distinct hemolysins (heme-lysing enzymes lyse RBCs and some PMNs), **Streptolysin O (oxygen labile) and S (serum soluble).**
- › To diagnose previous GAS infection, we use **Streptolysin O as it's immunogenic** (we'll find antibodies against this hemolysin - ASO test) unlike streptolysin S.

f. Other virulence factors

- › SPE-A, SPE-B and SPE-C are pyrogenic (fever inducing) and erythrogenic (rash inducing) exotoxins.
- › These function as super antigens and cause an exaggerated immune response (rash). The cytokines induced by these erythrogenic toxins are thought to be responsible for the hypotensive shock and organ failure in severe GAS infections.
- › These exotoxins are implicated **in scarlet fever and toxic shock.**

g. Invasion/escape factor

1. **Hyaluronidase:** Degrades hyaluronic acid, the ground substance of host connective tissue. Usually pathogenic bacteria harbor this enzyme which facilitates tissue invasion.
2. **Streptokinase:** To stop host buildup of serum barriers, thus facilitating spread. (they convert plasminogen to plasmin)
3. **DNAase (immunogenic):** Enzymes to break the DNA net that is released from phagocytes (neutrophil extracellular traps).



Clinical features

Transmission

- › The most common route of entry of GAS is the **upper respiratory tract** (mainly through direct contact with respiratory secretions; oropharyngeal saliva and nasal secretion), and is usually the primary site of infection.
- › Spread from person to person is by **respiratory droplets** or by **direct contact** with infected wounds or sores on the skin. Or, by indirect contact through infected surfaces.
- › Only a few **healthy adults** carry Str. pyogenes in their respiratory tractas normal flora, but the carriage rate in young school children is just over 10%
- › **Age population of Strep. pyogenes is mainly 5-15 year old.**

Pharyngitis

This is the most common infection caused by *Str. pyogenes*.

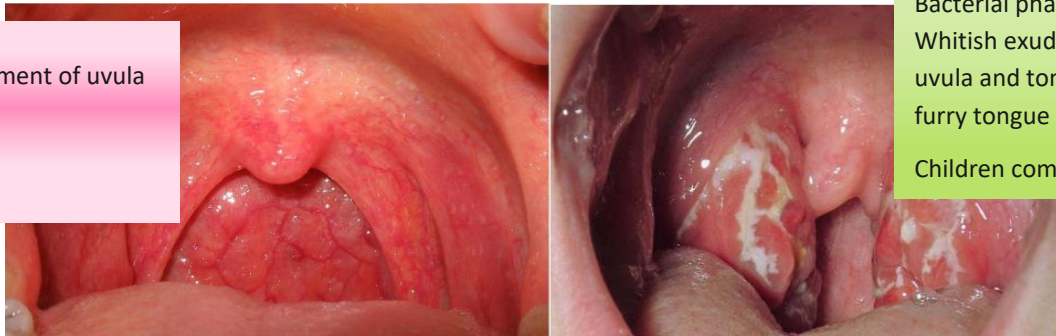
Clinical signs and symptoms:

- › Abrupt onset of sore throat.
- › Fever, malaise and headache generally develop 2–4 days after exposure to the pathogen.
- › Redness of the posterior palate.
- › **Odynophagia** (pain in the throat upon swallowing) and enlarged tonsils that may show patches of **grey–white exudate** on their surface.
- › Inflammation causes swelling of cervical lymph nodes.
- › Untreated pharyngitis may develop tonsillar abscesses; these are very painful and potentially dangerous as the pathogen may spread to neighboring regions, e.g. the middle ear (otitis media), the sinuses (sinusitis), the mastoids (mastoiditis), or the meninges (meningitis) or even to the bloodstream. Continuing inability to swallow may indicate a peritonsillar or retropharyngeal abscess.
- › If untreated, spontaneous recovery often occurs in 10 days, but rheumatic fever may occur. Viral pharyngitis: Start with Abrupt onset of fever then sore throat

Most common cause of pharyngitis: viral infection

Most common cause of BACTERIAL pharyngitis: GAS

Viral pharyngitis:
- without enlargement of uvula
- oral ulcers
- cough
- conjunctivitis
- hoarseness



Bacterial pharyngitis:
Whitish exudate, enlarged uvula and tonsils, gray furry tongue
Children come with GI symptoms

- › Despite the significant symptoms and clinical signs, differentiating streptococcal pharyngitis ('strep throat') from viral pharyngitis is impossible without microbiological or serological examination.
- › Culture studies show that only 20–30% of cases of pharyngitis are associated with *Str. pyogenes* and *Str. Equisimilis* [not as much as we thought]. This means we sometimes give antibacterial agents for viral infections! Thus, we are causing positive selection of the more virulent bacteria and aiding in the increase of antimicrobial resistance.

Scarlet fever

- › If the GAS causing pharyngitis produces exotoxins (SPE-A, SPE-B, SPE-C), the upper respiratory tract infection will be associated with a diffuse **erythematous rash** of the skin and mucous membranes; a condition called **scarlet fever**.
- › The pathognomonic rash (**sandpaper-like**) develops within 1–2 days after the first symptoms of pharyngitis. They are first seen on the upper chest then extremities. characterized by maculopapular rash that starts over the torso, then it extends to the limbs

- After an initial phase with a yellowish-white coating, the tongue becomes red and denuded ('strawberry tongue'); a characteristic lesion seen in scarlet fever.



Others

- Streptococcal toxic shock syndrome:** toxin-mediated.
- Skin and soft tissue infections** such as cellulitis, erysipelas, necrotizing fasciitis (streptococcal gangrene), and impetigo.
- Retropharyngeal Abscesses**, which are urgent.
- Meningitis, mastoiditis & lymphangitis**, especially on the forearm associated with an infection on the hand.

Streptococcal toxic shock syndrome has clinical findings similar to those of staphylococcal toxic shock syndrome. The difference is that streptococcal toxic shock syndrome usually has a recognizable site of pyogenic inflammation and blood cultures are often positive, whereas staphylococcal toxic shock syndrome typically has neither a site of pyogenic inflammation nor positive blood cultures.

Poststreptococcal (Nonsuppurative) Diseases

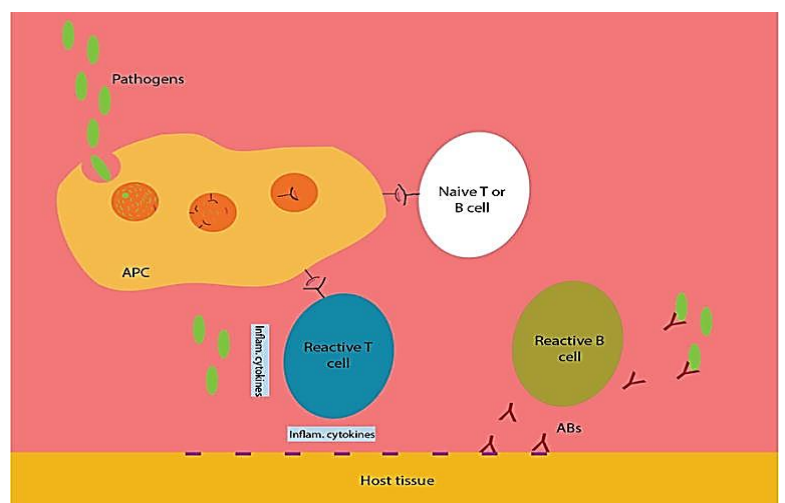
- Weeks later, after a local infection in the tissue (pharyngitis, skin infection) with GAS, an inflammation in a distant organ that was not infected can occur.

Molecular mimicry:

Antibodies raised against M proteins of GAS can cross-react with normal host tissues causing immune-mediated inflammation.

(it takes several weeks for enough cross-reacting antibodies to be produced, remember adaptive immunity takes time to develop).

- Some strains of *S. pyogenes* bearing certain M proteins are nephrogenic and cause AGN, while other strains bearing different M proteins are rheumatogenic and cause acute rheumatic fever. (remember there are 80+ types of this protein).



Acute Glomerulonephritis (AGN)

- Typically occurs 2 to 3 weeks following infection by certain group A streptococcal types in children (e.g., **M protein type 49 causes AGN most frequently**).
- Type 3 hypersensitivity reaction**: The formation of antigen-antibody aggregates called immune complexes that precipitate in the glomerular basement membrane.
- AGN occurs more often following skin infections rather than after pharyngitis**.
- The most striking clinical features are:
 - Hypertension (almost always a very odd finding in children)
 - Edema of the face (especially periorbital edema) and ankles due to loss of protein with urine.
 - “smoky” urine (due to red cells in the urine).
- Most patients recover completely, but some can proceed to renal failure. However, they are still NOT prone to develop this again if reinfection with streptococci happens.
- It can be prevented by early eradication of nephritogenic streptococci from skin colonization sites by administration of penicillin, but can't be treated with it after the onset of symptoms. Remember, it's immune-mediated and the organism itself may not even be present [**we actually use immunologic tests like anti DNAase for diagnosis**]

Acute Rheumatic Fever (RF)

Both AGN & RF are systemic diseases, but there's no problem in the organs that are involved (the microorganism is not present there) it's an immunological response to a previous infection by group A beta-hemolytic streptococci there are 2 strains: nephrogenic strain -> AGN / rheumatogenic -> RF

- Similar to post strep AGN, rheumatic fever is due to an immunologic reaction, but RF is a **type 2 hypersensitivity reaction** between cross-reacting antibodies to certain streptococcal M proteins and antigens of joints (migratory polyarthritis), the heart especially myosin (endocarditis), and brain tissue (chorea). (NOT ag-ab complexes)
- Occurs approximately 2 weeks after a GAS infection, **usually** pharyngitis.

- RF is characterized by:

- Fever
- Migratory polyarthritis
- Endocarditis

Remember J♥NES:

Joints, cardiac, nodules, erythema marginatum, Sydenham's chorea

The carditis (inflammation of heart muscle tissue) is the most serious, as damage to the myocardial and endocardial tissue, especially the mitral and aortic valves, can result in vegetations on the valves.

- Uncontrollable, spasmodic movements of the limbs or face (chorea) may also occur (indicates brain damage).

prognosis is much worse than AGN

- Anti-streptolysin O (ASO) titers are elevated. [**Important in diagnosis**]
- Unlike post strep AGN, RF is GREATLY exacerbated by recurrence of streptococcal infections. So, **reinfection must be prevented by long-term prophylaxis**.
- If streptococcal infections are treated within 8 days of onset (onset of pharyngitis in this case), rheumatic fever is usually prevented.

Arbitrary clues claim that skin infection by group A beta-hemolytic strep. cocci, the patient will develop AGN,

Patient who develop acute pharyngitis -> develops rheumatic fever (This is true in most cases) —

People who Suffer from Acute RF need prophylaxis (single dose of penicillin / 4 weeks for several year)

- > Most cases of pharyngitis caused by GAS occur in children 5-15-year-olds, and hence rheumatic fever occurs in that age group.
- > In the United States, fewer than 0.5% of group A streptococcal infections lead to rheumatic fever, but in developing tropical countries, the rate is higher than 5%.

Summary of Clinical Findings

S. pyogenes causes three types of diseases:

- Pyogenic diseases such as pharyngitis and cellulitis.
- Toxicogenic diseases such as scarlet fever and toxic shock syndrome.
- Immunologic diseases such as rheumatic fever and acute glomerulonephritis.

Laboratory Diagnosis

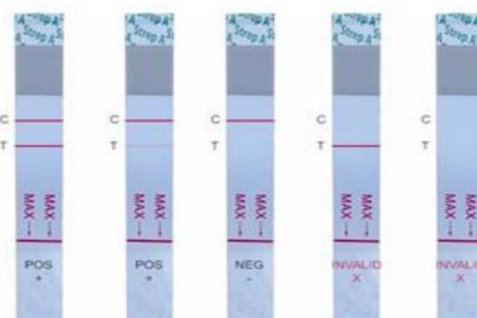
Microbiologic

- Gram-stained smears** are **useless** in streptococcal pharyngitis because viridans streptococci are members of the normal flora and cannot be visually distinguished from the pathogenic *S. pyogenes*.
- Cultures** of swabs from the pharynx on blood agar plates show small, translucent β -hemolytic colonies in 18 to 48 hours. If **inhibited by bacitracin** disk (sensitive to bacitracin), they are likely to be GAS.
- Stained smears from skin** lesions or wounds that reveal streptococci are also diagnostic.
- Rapid tests** that provide a diagnosis in approximately 10 minutes were developed. The rapid test detects bacterial antigens in a throat swab specimen. The specificity of these tests is high, but the sensitivity is low (i.e., false-negative results can occur). If the test result is negative but the clinical suspicion of streptococcal pharyngitis is high, a culture should be done.



Although cultures remain the gold standard for the diagnosis of streptococcal pharyngitis, a problem exists because the results of culturing are not available for at least 18 hours, and it is beneficial to know while the patient is in the office whether antibiotics should be prescribed. So we use rapid tests.

In this test, specific antigens from GAS are extracted from the throat swab by certain chemicals, then reacted with antibodies specific to these antigens. Agglutination of the colored latex particles occurs if group A streptococci are present in the throat swab.



The C line is the control line, it means the test is working properly. (must be present)

The T line is the test line indicating a positive or negative result

Serologic

- › **ASO titers** are high soon after group A streptococcal infections. They are especially used in diagnosis of RF.
- › Titers of **anti-DNase B** are high in group A streptococcal **skin infections** and serve as an indicator of previous streptococcal infection in patients suspected of having AGN. However, these tests do not imply active infection. They only indicate previous infection.

Treatment

- › Group A streptococcal infections can be treated with either **penicillin G or amoxicillin**.
- › In mild group A streptococcal infections, oral penicillin V can be used.
- › Patients of RF and AGN do not benefit from penicillin treatment.
- › In penicillin-allergic patients, erythromycin or one of its derivatives (e.g., azithromycin) can be used. However, erythromycin resistant strains of *S. pyogenes* have emerged that may limit the effectiveness of the macrolide class of drugs in the treatment of streptococcal pharyngitis.
- › Clindamycin can also be used in penicillin-allergic patients.

Prevention

- › Rheumatic fever can be prevented by prompt treatment of group A streptococcal pharyngitis with penicillin.
- › In RF susceptible people (previous infection), prevention of streptococcal infections is needed, usually with benzathine penicillin once each month for **5 years**.
- › There is no evidence that patients who have had AGN require similar penicillin prophylaxis.
- › There are **no vaccines** available against any of the streptococci **except *S. pneumoniae* (its capsule is the basis of the vaccine)**.

healthy carriers do not need treatment unless the occupation is in crowded places like prisons or places where there's over crowdeness especially for children population who are susceptible to group A. beta hemolytic streptococci

Good luck