Lecture Objectives

Staphylococcus aureus is a leading pathogen and will be reviewed in detail.

Points of emphasis for this organism will include:

- Its varied disease manifestations and their link to specific virulence factors
- Concepts of community v. hospital acquired (nocomial) infection
- Challenges of treatment due to the problem of antibiotic resistance
- Epidemiology of the on-going 'epidemic' of community-acquired MRSA

Staphylococci **Physiology and Structure**

Staphylococcus is derived from the Greek *saphyle*, meaning a bunch of grapes. Staph is **gram positive, aerobic, and catalase positive** (remember that *Strep* is catalase negative).

They are nonmotile and are facultative anaerobes (i.e. can grow aerobically as well as anaerobically).

Differentiating Staphylococcal species.

Staph aureus is the only Staphylococcal species (medically important, that is) that is coagulase positive. This is a simple test that is typically run, so the term *coagulase-negative staph* means that it is something other than S. aureus. Coagulase: found bound to the cell wall, this is also referred to as 'clumping factor'. It binds and cleaves fibrinogen, converting it to insoluble fibrin, which can cause S. aureus to clump as a result.

There are many species of Staph, with S. aureus and S. epidermidis being the two that you will hear the most about (and see clinically most often). These have very different features and it is important not to confuse them. Other Staph species that cause disease include S. haemolyticus but generally isolates that are not S. aureus are referred to as coagulase-negative (or coag-negative) Staph.

Key Points: Physiology and Structure

- Staph are gram positive catalase positive cocci
- The coagulase test distinguishes S. aureus from other species
- Coagulase-positive (S. aureus) and –negative (S. epidermidis) species are very different clinically

Pathogenesis and Immunity.

Like Group A Streptococcus, S. aureus expresses many virulence factors with overlapping functions allowing for attachment, evasion of host defenses, and tissue penetration. These are often highly variable among strains. No single factor appears to account for its behavior in a host.

Capsule. Only some clinically relevant strains of S. aureus express a capsule. Eleven different capsular serotypes have been described, with serotypes 5 and 7 being most commonly associated with infection. The capsule helps prevent phagocytosis of the bacterium by leukocytes, and also plays a role in adherence of bacteria to catheters and other synthetic materials - this is important, particularly for the less virulent coagulase-negative staph strains that frequently colonize in-dwelling catheters.

Protein A (Spa): this is on the surface of most S. aureus strains, but not coagulase-negative staph. Protein A binds very tightly to the Fc region of antibodies, and so prevents antibodies from binding to its antigens. Thus, it plays a role in immune evasion.

S. aureus produces many toxins that play a significant role in its virulence. Different toxins contribute to tissue destruction that is commonly associated with invasive S. aureus infection, and also with skin exfoliation (scalded skin syndrome), food poisoning, and toxic shock syndrome. Most of these toxins are encoded by plasmids, and some are more common than others amongst S. aureus strains. Production of antibodies to the toxins can confer protection from later disease. The major toxins are:

Alpha toxin: produced by most strains; it integrates into cell membranes and forms pores – Na^{+} and Ca^{++} flow into the cell, water follows, and the cell undergoes osmotic lysis. This toxin plays an important role in tissue damage.

Beta toxin: this is a sphingomyelinase enzyme and is present in most S. aureus strains. By cleaving sphingomeylin in the membranes of cells, it damages the membrane and can lead to cell lysis. Along with alpha toxin, it plays a role in the tissue destruction so commonly seen in S. aureus infections.

Exfoliative toxins: Staphylococcal scalded skin syndrome (SSSS) will be covered later. It is associated with two S. aureus exfoliative toxins (ETA and ETB) that are present in less than 10% of S. aureus strains. These are proteases, and are thought to digest proteins involved in cell-cell contacts, leading to skin exfoliation.

Enterotoxins: these are associated with food poisoning, and are found in 30 to 50% of staph strains. There are a number of different enterotoxins; they are heat stable and resistant to hydrolysis by stomach and intestinal enzymes. So, if a food product is contaminated, there is nothing that can really be done except avoid it. The mechanisms by which these work are not well understood, but they do function as 'superantigens', and so nonspecifically activate T cells and cytokine release. Stimulation of mast cell degranulation is thought to lead to the emesis (vomiting) that is characteristic of staph food poisoning.

Toxic shock syndrome toxin: a very stable toxin; virtually all strains responsible for staph associated toxic shock syndrome have this toxin. The toxin is a superantigen and induces

nonspecific and massive release of cytokines that lead to vascular permeability and falling blood pressure.

In addition to toxins, Staph can produce a number of enzymes that help it penetrate tissues and spread. These include lipases, hemolysins, fibrinolysin, and hyaluronidase. The coagulase helps it bind fibrin and form walled off abscesses, a major feature of S. aureus infection. In contrast, S. epidermidis expresses different factors and the tissue response does not cause abscess formation.

Immunity:

Adaptive immunity ineffective and so recurrent infection is common.

As a consequence there is no vaccine and clinical trials to test candidates have been disappointing.

Key Points: Pathogenesis and Immunity

- S. aureus expresses multiple virulence factors that allow it to attach to tissues, evade clearance, and invade and damage its host. Coagulase-negative Staph are much less virulent.
- Expression of specific toxins by some S. aureus strains results in distinct clinical syndromes.
- S. aureus infection often leads to abscess formation.
- Infection may be recurrent as immunity from prior exposure is not protective.

Epidemiology.

Staph are really common - everyone has S. epidermidis colonizing their skin, and S. aureus is common on skin as well. Colonization is by S. aureus is most often detected in the external nares on squamous epithelium. Also found on skin, especially where in contact with the nose, and in the GI and urogenital tracts. About 20% of normal adults are persistent carriers.- an issue for health care providers. Some individuals seldom are colonized by S. aureus (non-carriers). Since it is on skin, shedding and autoinoculation and person to person spread are common. This accounts for why staph is a leading common cause of hospital acquired infections. Decolonization protocols have emphasized hygiene and treatment of external nares with topical antibiotics (mupirocin).

Molecular typing technologies have shown that S. aureus infections are largely due to the success and expansion of genetically-related 'clones'. Methicillin-resistant S. aureus (MRSA) clones have arisen out of several distinct lineages. An example is USA300, a MRSA clone that has widely disseminated in the community and is now commonly associated with disease. Why these clones are more successful remains unclear.

The success of these more virulent clones has driven increased rates of infection due to S. aureus and the higher proportion of MRSA over sensitive MSSA strains.

Key Points: Epidemiology

- Staph colonizes human skin. For S. aureus, the external nares are the best single site to find the organism.
- There are persistent carriers, intermittent carriers and non-carriers.
- Outbreaks of S. aureus often associated with the success and widespread dissemination of individual clones, including the currently circulating MRSA clone USA300.

Clinical diseases caused by Staph aureus

Staph aureus can cause a number of diseases at different host sites; some are mostly due to toxins, some are mostly due to proliferation of staph and tissue destruction and abscess formation. The major categories include:

- Wide variety of skin, soft tissue, and deep seated, life-threatening diseases. Some depend on production of specific microbial products.
- Food intoxication-heat stable enterotoxins consumed resulting in rapid (2-6 hours) onset of symptoms such as emesis and diarrhea
- Toxic Shock Syndrome- toxin shock syndrome toxin 1 or exotoxin B or C
- Staphylococcal scalded skin syndrome -blistering disease caused by exfoliative toxins A or B associated with epidemics in nurseries
- More invasive and disseminated disease (bacteremia, endocarditis, intraveneous catheter infection, septic arthritis, seeding of bone to cause osteomyelitis) result when tissue barriers are interrupted
- An important cause of wound infection

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Staphylococcal Scalded Skin Syndrome. Primarily affects neonates and young children. Results from infection of skin by an S. aureus strain that expresses exfoliative toxins (ETA or ETB), which are serine proteases that cause separation of epidermal layers. Abrupt onset; localized erythema that often starts around the mouth and eventually can cover the entire body within 2 days. The skin can slough off if pressure is applied, and large blisters (bullae) form, after which the skin desquamates. Fortunately, only the epithelium is involved, so scarring does not occur, mortality rates are low (and are due to secondary infections), and the epithelium becomes intact by 10 days and is correlated with the production of antibodies to the toxin.

Impetigo. Like Group A Strep causes a superficial crusting infection impetigo. A localized form of Staphylococcal Scalded Skin Syndrome called **bullous impetigo**. Mostly infants and young children; localized blisters; highly communicable; self resolving.

Cutaneous infections. S. aureus causes a number of pyogenic (i.e. **pus producing**) skin infections. If pus is present in an abscess, think staph!

Folliculitis. Infection of hair follicles. Called a stye if it is at base of the eyelid. Furuncles (boils) are an extension of folliculitis - large, painful red nodules with dean tissue underneath.

Carbuncles. Term used with there are multiple furuncles that coalesce; associated with fever and chills indicating a more severe and systemic infection.

Staph aureus is particularly problematic where skin surface is damaged and the organism gains access to deeper tissues.

Bacteremia and endocarditis. S. aureus is a common cause of bacteremia, often arising from an innocuous skin infection. A serious complication, however, is endocarditis, and this carries with it a high mortality rate due to rapid destruction of heart values. S. aureus also causes catheter infections.

Pneumonia and empyema (pus in the space between the lung and chest wall). Can occur from aspiration (remember, S. aureus frequently colonizes oropharynx) or from hematogenous spread. Presents like other bacterial pneumonias, but abscesses and tissue destruction are more common due to the array of enzymes produced by staph.

Osteomyelitis and septic arthritis. As you might expect, an organism that is a common cause of bacteremia will no doubt also cause more localized infections as a result of bacterial seeding. S. aureus is the most common cause of bone infection, septic arthritis in young children, and intraarticular infections in those with abnormal or artificial joints.

Staphylococcal food poisoning. A common form of food poisoning, is due to an intoxication (ingesting the enterotoxin) rather than an infection per se. Most commonly affected foods are processed meats, potato salad, ice cream. Due to contamination of food by a human carrier, most often an asymptomatic carrier. Once contaminated, the food needs to be at room temperature (or higher) long enough for the bacteria to grow and release the enterotoxin. As the toxin is stable, even if the food is properly prepared it won't help. Onset is abrupt and rapid; mean incubation period of 4 hours. Symptoms usually last less than 24 hours since it is due to toxin ingestion rather than infection that leads to toxin production over time. Severe vomiting, diarrhea, abdominal pain and nausea.

Toxic shock syndrome. About 6000 cases a year. Due to localized growth of toxin-producing S. aureus (producing toxic shock syndrome toxin described above), with release of toxin into blood stream. Symptoms start abruptly: fever, hypotension, diffuse macular rash. Multiple organ systems fail due to hypotension. Skin can be affected and slough off late in the course of the disease. Need to rapidly treat with antibiotics. Mortality rate is about 5%. Patients who survive often have antibodies to the toxin, and this protects them from subsequent disease. Historically, this came to public attention when the disease afflicted a large number of women: it was found that TSST-1 producing S. aureus could multiply quickly in certain hyperabsorbant tampons.

Clinical disease caused by S. epidermidis and other coagulase-negative staph

S. epidermidis, as a very common colonizer of human skin, is frequently responsible for infections involving in-dwelling medical devices. Major considerations include:

Endocarditis. Staph epi is a major cause of endocarditis of artificial heart valves. More commonly, the infection takes root at the sites where the valve is sewn into the heart. This can lead to separation of the valve at the annulus. S. epidermidis and other coag-negative staph can also infect native heart valves, but this is much less common.

Catheter and shunt infections. The presence of an exopolysaccharide or 'slime layer' on staph enables efficient attachment to artificial surfaces. Thus, from 20 to 65% of all

infections of indwelling catheters and shunts are due to coag-neg staph - this is a major problem because indwelling catheters are used extensively especially in debilitated patients. Such colonization leads to persistent bacteremia, and the organisms can be seeded most anywhere else in the body, leading to other localized infections.

Prosthetic joint infections. Again, an important problem that is commonly associated with coag-negative staph. Importantly, patients usually have localized pain and joint failure, but lack more systemic symptoms.

Urinary tract infections. S. saprophyticus is a significant cause of UTIs in sexually active women.

Key Points: Clinical diseases

- S. causes a large variety of localized, invasive infections and toxin-mediated infections.
- S. aureus is a particularly common cause of skin and soft tissue infection.
- Staph is a common culprit in contamination of clinical cultures.
- Staph, including coag-negative Staph, is a common cause of foreign body infections.

Laboratory Diagnosis

Not difficult as Staph is easily grown on plates supplemented with sheep blood (blood agar) and has a characteristic appearance as colonies or on gram stain. The real challenge is in determining whether it is a contaminant (especially with Staph. epi) or cause of infection when isolated. If there is a mix of organisms, high salt and lipids can be included as staph will grow just fine under these conditions (This is why Staph persists on skin). Can also add mannitol, which is fermented by S. aureus but inhibits growth of most other staph. Colonies of S. aureus creamy and often golden in color. Serology is not that useful. Biochemical tests are also employed (coagulase, catalase).

Treatment, prevention, control.

Resistance is a major and increasing problem.

Almost all staph are resistant to penicillin due to acquisition of penicillinases, which hydrolyzes the beta-lactam ring of penicillin.

Thus, anti-staphylococcal semisynthetic penicillins (the methicillins) are used, but resistance to these is increasing. MRSA refers to methicillin-resistant Staph aureus. These acquired an altered penicillin-binding protein introduced by a phage rendering them resistant to all β-lactam antibiotics. Now >50% of all S. aureus and most coag-negative staph are methicillin resistant. Additionally, most hospital acquired MRSA are multi-drug resistant. Community-acquired MRSA is increasing in prevalence but still mostly sensitive to some other non-β-lactams such as trimethoprim-sulfamethoxazole (Bactrim). These infections are often more severe than MSSA, although the virulence determinant(s) accounting for more severe infections in these clones remains unclear. Vancomycin remains effective, but is only useful given IV and resistance to this has also been reported several times. Increasing reliance on vancomycin drives resistance in other microbes (Vancomycin-resistant enterococcus –VRE).

Since treatment is becoming increasingly challenging, prevention of spread (i.e. hygiene) and infection control (hand washing, gowning and gloving) have taken on greater importance, particularly in hospitals.

Treatment of S. epidermidis often depends on removal of the foreign body. Vancomycin is generally the drug of choice if antibiotics are necessary.

Key Points: Treatment and Prevention

- Antibiotic resistance, especially the spread of MRSA, has complicated treatment.
- MRSA isolates encode altered penicillin-binding proteins resulting in resistance to all β-lactam agents.
- Different patterns of resistance in community-acquired and hospital-acquired strains.
- MRSA has driven the use of vancomycin in hospitals, which has contributed to the spread of vancomycin-resistance among some other bacteria.