Laboratory Diagnosis of Bacterial Skin and Soft Tissue Infections

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Skin Microbiota

- The usual flora of the skin consists of those microbes able to adapt to its high salt concentration, acidic environment and relative lack of moisture.

- Coagulase-negative staphyloccoci are permanent skin residents.
Skin Microbiota

- *Staphylococcus aureus* is typically a transient colonizer.

- Diphtheroids such as *Propionibacterium acnes* and *Corynebacterium xerosis*
<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
<th>Possible Etiologic Agents (Infections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macule</td>
<td>A circumscribed (limited), flat discoloration of the skin</td>
<td>Dermatophytes, Treponema pallidum (secondary syphilis), Viruses such as enteroviruses (exanthems rashes)</td>
</tr>
<tr>
<td>Papule</td>
<td>An elevated, solid lesion $\leq 5$ mm in diameter</td>
<td>Human papillomavirus types 3 and 10 (flat warts), Pox virus (Molluscum contagiosum), Sarcoptes scabiei (scabies), S. aureus, P. aeruginosa, etc. (folliculitis)</td>
</tr>
<tr>
<td>Nodule</td>
<td>A raised, solid lesion $&gt;5$ mm in diameter</td>
<td>Corynebacterium diphtheriae, Sporothrix schenckii, Miscellaneous fungi (subcutaneous mycoses), Mycobacterium marinum, Nocardia spp., S. aureus (furuncle)</td>
</tr>
<tr>
<td>Pustule</td>
<td>A circumscribed, raised, pus-filled (leukocytes and fluid) lesion</td>
<td>Candida spp., Dermatophytes, Herpes simplex virus, Neisseria gonorrhoeae (gonorrhea), S. aureus (folliculitis), S. aureus or group A streptococci (impetigo), Varicella-zoster virus (chickenpox)</td>
</tr>
<tr>
<td>Vesicle</td>
<td>A circumscribed, raised, fluid-filled (blister-like) lesion $\leq 5$ mm in diameter</td>
<td>Herpes simplex virus, Varicella-zoster virus (chickenpox and shingles)</td>
</tr>
<tr>
<td>Bulla</td>
<td>A circumscribed, raised, fluid-filled lesion $&gt;5$ mm in diameter</td>
<td>Clostridial species (necrotizing gas gangrene), Herpes simplex virus, Other gram-negative rods, S. aureus (bullous impetigo and scalded skin syndrome), Vibrio vulnificus and other vibrios</td>
</tr>
<tr>
<td>Scales</td>
<td>Dry, horny, platelike lesions</td>
<td>Dermatophytes (tinea)</td>
</tr>
<tr>
<td>Ulcer</td>
<td>A lesion with loss of epidermis and dermis</td>
<td>Bacillus anthracis (cutaneous anthrax), Bowel flora (decubiti), Haemophilus ducreyi (chancroid), T. pallidum (chancre of primary syphilis)</td>
</tr>
</tbody>
</table>
In the management of skin infections, the surface of broken or ulcerated skin is often swabbed for purposes of Gram staining and culture.
This technique provides little clinically useful information.

Swabs of surface wounds or skin are likely to yield colonizing or contaminating bacteria, and there is a lack of correlation between surface colonization and below-the-surface infection.
جدول ۱-۴

<table>
<thead>
<tr>
<th>عامل آماری</th>
<th>نوع ارگانیسم</th>
<th>پروتئیناسیون در محل</th>
<th>عامل آماری</th>
<th>آسیب نسجی</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination</td>
<td>کم</td>
<td>mixed</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>Colonization</td>
<td>متوسط</td>
<td>Commensal (low Pathogen)</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>Infection</td>
<td>زیاد</td>
<td>Potent Pathogen</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>
کتاب راهنمای آزمایشگاهی تشخیص عفونت‌های بیمارستانی - مرکز مدیریت بیماری‌ها

- کتاب راهنمای آزمایشگاهی تشخیص عفونت‌های بیمارستانی - مرکز مدیریت بیماری‌ها
- کتاب الکترونیکی
فصل چهارم

عفونت‌های زخم‌های جراحی

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در کتاب‌های آموزشی، به‌طور کلی، عوامل زخم‌های جراحی را جایگزین یا جایگزینی از بروز عفونت‌های زخم‌های جراحی از 25 میلی‌متر جراحی انجام می‌دهد. در ماه جولای 1380، 890 مورد عفونت زخم به کارگاه شناخته شدند.

میزان ابتلا به عفونت‌های زخم جراحی به موارد زیر بستگی دارد:

1. عوامل مرتبط با بیمار: ماتن، استریپ و بلوکتیک مرتبط با بیمار.
2. عوامل مرتبط با زخم کار: مانند استفاده از وسایل در محل با خرابی به بیمار می‌رساند دریچه انجام جراحی.
3. عوامل مرتبط با ارگانیسم بیماری: مانند تلاش ارگانیسم به بافت بیمار تهیه متریک در مقاله تشخیص عفونت‌های مبتلا از صحت می‌رساند.

برای تشخیص عفونت‌های زخم‌های جراحی، نیازمند نمودار و امکانات اختصاصی بیمارستان است.
Laboratory Diagnosis of Bacterial Skin and Soft Tissue Infections

- Communication between the clinician and microbiology laboratory is essential for optimizing the information obtained from a clinical specimen
Label specimen and requisition

SPECIMEN COLLECTION

- Describe the type of specimen (deep tissue, superficial tissue, decubitus, catheter site, boil, abscess, cellulitis, aspirate, pus, drainage, surgical incision site, etc.)

- State anatomic location (arm, leg, etc.)
Label specimen and requisition

**SPECIMEN COLLECTION**

- Record collection time and date
- List antimicrobial therapy prior to specimen collection
- Deliver aspirates and tissues to the laboratory within **30 min** for best recovery
Cleanse skin or mucosal surfaces

SPECIMEN COLLECTION

- For **closed wounds** and aspirates, disinfect as for a blood culture collection with 70% alcohol

- Followed by a 10% solution of povidone-iodine

- Remove iodine with alcohol prior to specimen collection
Cleanse skin or mucosal surfaces

**SPECIMEN COLLECTION**

- For open wounds, remove all superficial exudates with scalpel and swabs

- Thoroughly rinse with non bacteriostatic sterile saline prior to collection

- Collect biopsy or curette sample from base or advancing margin of lesion
SPECIMEN COLLECTION

- **Deep aspirates** of fluid / pus from the depths of wounds and abscesses or **Biopsies** of involved tissue are generally much more informative.
Closed abscesses
SPECIMEN COLLECTION

- Aspirate infected material with needle and syringe

- If the initial aspiration fails to obtain material, inject sterile, nonbacteriostatic saline subcutaneously
SPECIMEN COLLECTION

- In the past the aspirating syringe was often used as the transport container, provided the needle was capped.

- This procedure is discouraged now because of the risk that a blood borne virus will be transmitted by a needle stick.
SPECIMEN COLLECTION

- The needle can be removed safely and replaced with a nonsharp syringe cap, using implements designed for this purpose.

- In any event, **if a delay of more than 30 minutes** is anticipated before processing, the specimen should be transferred to a transport container.
Swabs - SPECIMEN COLLECTION

- Collect swabs only when tissue or aspirate cannot be obtained

- Swabs (ideally, submit two, one for Gram stain and one for culture)

- If wound is relatively dry, collect with two cotton-tipped (Dacron) swabs moistened with sterile saline
Swabs - SPECIMEN COLLECTION

- It may be necessary either to separate the wound margins with the thumb and forefinger of one hand (wearing a sterile glove).

- Gently roll swab over the wound approximately five times.

- Focusing on area where there is evidence of pus or inflamed tissue.
Swabs may also lead to false-negative Gram stains and cultures because they do not contain sufficient amounts of material for culture, and bacteria do not survive as well on a swab as they do within fluid or a tissue sample.
Illustration of sterile-scalpel method of homogenization of tissue.

Illustration of mortar-and-pestle method of homogenization of tissue.
Illustration of tissue-grinding kit method of homogenization of tissue.
MICROSCOPIC EXAMINATION OF SPECIMENS

- **Gram's stain** should be performed on all specimens

- Morphologic clues to the etiology may suggest additional diagnostic procedures beyond those requested by the clinician.
CULTURE

- Both selective and enriched nonselective media should be used to recover both eugonic and fastidious bacterial species.

- Anaerobic culture is appropriate if tissue or aspirated material has been obtained.
Media

- BAP
- CHOC
- MAC
- MSA : 72 h & SC
- TSB with 0.1% agar with or without yeast extract
Media

- **THIO**: least desirable broth medium to grow low numbers of aerobic organisms and yeasts and it is excellent for anaerobic organism recovery.

- Anaerobic culture media, if appropriate for site of collection and transport conditions.
Incubate

- Incubate BAP and CHOC in humidified incubator at 35 to 37°C with 5% CO2.

- Incubate for a minimum of 48 - 72 h for open wound cultures and for 3 to 5 days for invasively collected specimens with no initial growth.
Incubate

- For negative cultures from critical sources or, possibly, for specimens with PMNs and no organisms isolated in culture, extend incubation time longer, even up to 7 days.

- Incubate MAC plates in ambient air at 35 to 37°C for 24 - 48 h.

- Incubate broth in ambient air at 35 to 37°C for 3 to 4 days.
CULTURE / Quantitative

- Quantitative microbial cultures:
- Culture of wounds to determine the significance of isolates
- Predict the likelihood of burn wound sepsis
Quantitative culture of biopsied tissue is complex, expensive, and time-consuming.

Tissue must be weighed on an analytic balance, homogenized in a measured amount of broth, diluted serially and inoculated onto multiple agar plates.

These maneuvers are difficult for most laboratories to accomplish.
Semiquantitative cultures (as performed routinely by serial streaking of quadrants on agar plates – heavy, moderate growth) have provided information that is equivalent to the more difficult quantitative approaches.
Identification

- Generally identify up to three microorganisms, if any of the following is true.

- **PMNs were present on direct smear.**

- The specimen was collected from a normally sterile site.

- The specimen was of good quality (e.g., **no or few epithelial cells present**).

- The organism was seen on the direct smear.
Examples of observations that guide the provision of relevant information

<table>
<thead>
<tr>
<th>Clinical and microbiological observation</th>
<th>Examples of specimen submissions&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Example 1</td>
</tr>
<tr>
<td>Clinical signs of infection reported</td>
<td>None</td>
</tr>
<tr>
<td>Leukocytes in Gram stain</td>
<td>-</td>
</tr>
<tr>
<td>Wound malodor</td>
<td>1+</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1+</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td>-</td>
</tr>
<tr>
<td>Coliform bacteria</td>
<td>3+</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em> spp.</td>
<td>-</td>
</tr>
<tr>
<td><em>Clostridium</em> spp.</td>
<td>1+</td>
</tr>
<tr>
<td>Pigmented gram-negative anaerobes</td>
<td>-</td>
</tr>
<tr>
<td>Nonpigmented gram-negative anaerobes</td>
<td>1+</td>
</tr>
<tr>
<td>Information provided on microbiology report</td>
<td>Moderate growth of mixed aerobes and anaerobes, including <em>S. aureus</em>; no antibiogram provided unless requested</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1+, light growth/minimal malodor or leukocytes; 4+, heavy growth/offensive odor or numerous leukocytes.
<table>
<thead>
<tr>
<th>Infection</th>
<th>Major Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical-wound infection</td>
<td></td>
</tr>
<tr>
<td>Clean</td>
<td><em>Staphylococcus aureus</em>, gram-negative bacilli</td>
</tr>
<tr>
<td>Contaminated, such as colon</td>
<td>Plus anaerobes, streptococci</td>
</tr>
<tr>
<td>Intravenous infusion sites</td>
<td><em>S. aureus</em>, coagulase-negative staphylococci</td>
</tr>
<tr>
<td>Trauma</td>
<td></td>
</tr>
<tr>
<td>Soil contamination</td>
<td><em>Pseudomonas aeruginosa</em>, <em>clostridia</em></td>
</tr>
<tr>
<td>Freshwater contamination</td>
<td><em>Aeromonas</em>, <em>Plesiomonas</em></td>
</tr>
<tr>
<td>Saltwater contamination</td>
<td><em>Vibrio vulnificus</em></td>
</tr>
<tr>
<td>Bites</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Oral aerobes and anaerobes, <em>S. aureus</em></td>
</tr>
<tr>
<td>Dog, cat</td>
<td><em>Pasteurella multocida</em>, <em>S. aureus</em>, anaerobes</td>
</tr>
<tr>
<td>Rat</td>
<td><em>Streptobacillus moniliformis</em>, <em>Spirillum minus</em> (minor)</td>
</tr>
<tr>
<td>Decubitus ulcer</td>
<td><em>Streptococci</em>, <em>S. aureus</em>, coliforms, <em>Pseudomonas</em>, anaerobes including <em>Bacteroides fragilis</em></td>
</tr>
<tr>
<td>Foot ulcer in diabetic patients</td>
<td><em>S. aureus</em>, streptococci, coliforms, <em>P. aeruginosa</em>, anaerobes</td>
</tr>
<tr>
<td>Hidradenitis suppurativa</td>
<td><em>S. aureus</em>, streptococci, coliforms, <em>Pseudomonas</em>, anaerobes</td>
</tr>
<tr>
<td>Burns</td>
<td><em>S. aureus</em>, <em>Candida</em>, <em>P. aeruginosa</em></td>
</tr>
</tbody>
</table>
Pyodermas are a group of inflammatory skin disorders caused by bacteria that produce pus.

### Common Primary Pyodermas

<table>
<thead>
<tr>
<th>Infection</th>
<th>Organism</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impetigo</td>
<td><em>Streptococcus pyogenes</em>, occasionally; <em>Staphylococcus aureus</em>, if bullous</td>
<td>Children affected most; communicable; no fever</td>
</tr>
<tr>
<td>Erysipelas</td>
<td><em>S. pyogenes</em>, occasionally; other β-streptococci or <em>S. aureus</em></td>
<td>Distinct raised borders; fever common</td>
</tr>
<tr>
<td>Cellulitis</td>
<td><em>S. pyogenes</em>, <em>S. aureus</em>; <em>Haemophilus influenzae</em> in children</td>
<td>Erythema, tenderness, pain, edema, warmth; fever common</td>
</tr>
<tr>
<td>Folliculitis</td>
<td><em>S. aureus</em>; gram-negative bacilli or <em>Candida</em> if predisposing conditions</td>
<td>Papules around hair follicles; areas exposed to whirlpool bath (Pseudomonas aeruginosa)</td>
</tr>
<tr>
<td>Furuncle</td>
<td><em>S. aureus</em></td>
<td>Fluctuant, painful nodules often in intertriginous areas</td>
</tr>
<tr>
<td>Carbuncle</td>
<td><em>S. aureus</em></td>
<td>Multiple abscesses</td>
</tr>
<tr>
<td>Paronychia</td>
<td><em>S. aureus</em>, gram-negative bacilli, <em>Candida</em></td>
<td>Periungal swelling</td>
</tr>
</tbody>
</table>
Impetigo

- Impetigo is a common pyoderma that is most often seen in children
- *Streptococcus pyogenes*
  - small vesicles and pustules
- *Staphylococcus aureus*
  - bullous form
- *Group B Streptococcus* occasionally causes impetigo in newborns
Impetigo
SPECIMEN COLLECTION

- **Pustules or vesicles**
  - The roof or crust should be removed with a **sterile blade**, and any pus or exudate should be Gram stained and cultured.
Group A streptococci

- Infections by Streptococcus pyogenes are clinically important, no matter what the quantity of bacteria present.

- Group A streptococci have been uniformly susceptible to penicillin, so antibiotic testing for this organism is typically presumed unnecessary.
Erysipelas

- Erysipelas is a type of superficial cellulitis
- Diagnosis is based on the clinical presentation
- It is difficult to isolate GAS from the skin lesions.
- Aspiration of the advancing edge of a lesion and culture has been successful in identifying the organism.
Cellulitis

- Cellulitis is a diffuse inflammation and infection of both the superficial skin layers and subcutaneous tissues
- Blood cultures are usually negative
- Diabetic patients with foot ulcers, may develop surrounding cellulitis because of a mixture of gram-positive and gram-negative organisms and anaerobes
• **Cellulitis**: injecting a small amount (about 3 mL) of preservative-free, physiologic saline into the advancing margin of the affected skin, aspirating back, and then culturing the fluid that is withdrawn.
**Cellulitis**

**SPECIMEN COLLECTION**

- **Cellulitis**: Alternatively, a punch biopsy of the skin can be performed and submitted for Gram stain and culture.
Folliculitis

- Folliculitis is an inflammation and infection of hair follicles.
- **Sycosis barbae** is a form of folliculitis occurring in bearded men.
- **Staphylococcus aureus** is the most common etiologic agent.
- **Pseudomonas aeruginosa** in cases acquired from contaminated swimming pools or whirlpool / jacuzzi bath.
Furuncles and Carbuncles

- **Furuncle**: folliculitis can sometimes progress to form deeper inflammatory nodules called furuncle; *S. aureus*

- **Carbuncle** is a more serious lesion that extends into the subcutaneous fat and consists of multiple coalescing abscesses that can drain at several adjacent sites along hair follicles; *S. aureus*
  - nape of the neck and on back of the thighs
Paronychia

- Paronychia is an infection of the cuticle surrounding the nail bed

- *Staphylococcus aureus* or *Candida* are usually the causative organisms
**Intertrigo**

- Intertrigo is an inflammatory cutaneous condition that occurs in body areas subjected to heat, *moisture*, and *friction* which work together to cause maceration.

- Skin folds of *infants* and *obese* adults and often can be found in the *axillae, perineum*, beneath the *breasts*.

- Candida, *S. aureus* and coliforms
  - *Surface culture*
Hidradenitis Suppurativa

- Hidradenitis suppurativa is recurrent infection of the apocrine sweat glands, particularly in the axillae and groin.

- Staphylococci, Streptococci, gram-negative bacteria including *E. coli* and *Pseudomonas*, and anaerobic bacteria are commonly involved.
Diabetic Foot Infections
Soft Tissue Infections

- Ulcerative lesions and gangrene maybe the result of *staphylococci* and *streptococci* but often are *mixed infections* and include *gram-positives*, *Enterobacteriaceae*, *Pseudomonas*, and anaerobic bacteria.

- Low virulence organisms such as *coagulase-negative staphylococci* and *diphtheroids* to be pathogens in the skin.
Burns

- Cultures should be performed on any purulent wound exudates
- Blood cultures should also be collected
- The most prevalent organism in a polymicrobial infection
Burns

- Organisms may not be distributed evenly in a burn wound, so sampling different areas of the burn is recommended.
Semiquantitative culture

- This type of culture is reported in colony-forming units (CFUs) per gram of tissue, with a result of $\geq 10^5$ CFUs/g indicative of a potentially serious infection.
Semiquantitative culture

Semiquantitative Bacteriologic Culture of Tissue

Principle
The degree or extent of bacterial wound contamination is directly related to the risk of wound sepsis. Because of this relationship, physicians use the results of a quantitative culture (the number of colony-forming units [CFUs] per gram of the eschar biopsy) in their management of severely burned patients.

Method
1. Cut a piece of tissue, measuring several cubic millimeters, aseptically onto a small, preweighed, sterile urine cup.
2. Determine the weight of the tissue by subtracting the weight of the aluminum foil from the total weight.
3. Place the specimen and 2 mL of sterile nutrient broth in a sterile tissue grinder; macerate the specimen.
4. Inoculate 0.1 mL of sample to a blood agar plate, in duplicate, and an anaerobic blood agar plate (if indicated), in duplicate. In addition, inoculate 0.01 mL of sample using a calibrated loop to a blood agar plate, in duplicate. Spread the inoculum on the plates with a sterile glass spreading rod or a loop.
5. Incubate plates in 5% to 10% carbon dioxide overnight, and count the colonies of bacteria on the plate that contain 30 to 300 CFUs. If more than 300 colonies are obtained on both plated dilutions, the factor 300 is used as N for calculations and the result is considered greater than the value.
6. Calculate the number of CFUs per gram of tissue with the following formula:

\[
\frac{68 \times 10^2 \times 2}{0.002} = \frac{136 \times 10^2}{2 \times 10^{-3}} = 6.8 \times 10^6 \text{ CFU/g}
\]

For example, for a tissue that weighed 0.002 g, 68 CFUs were observed on the plate that received the 10\(^{-2}\) dilution of suspension.

\[
\frac{68 \times 10^2 \times 2}{0.002} = \frac{136 \times 10^2}{2 \times 10^{-3}} = 6.8 \times 10^6 \text{ CFU/g}
\]

Surgical site infections - SSI

- Surgical site infections are among the most common nosocomial infections and occur after nearly **3-5 %** of all surgical procedures.

- The most common organism involved in postoperative infections is **S. aureus**.
<table>
<thead>
<tr>
<th>Organisms Encountered in Postoperative Wound Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
</tr>
<tr>
<td><strong>Streptococcus pyogenes</strong></td>
</tr>
<tr>
<td><strong>Streptococcus anginosus</strong> group streptococci (S. anginosus, S. constellatus, S. intermedius)</td>
</tr>
<tr>
<td>Microaerophilic streptococci</td>
</tr>
<tr>
<td>Enterococci</td>
</tr>
<tr>
<td>Proteus, Morganella, Providencia</td>
</tr>
<tr>
<td>Other Enterobacteriaceae</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
</tr>
<tr>
<td><strong>Pseudomonas</strong> spp.</td>
</tr>
<tr>
<td><strong>Candida</strong> spp.</td>
</tr>
<tr>
<td><strong>Bacteroides</strong> spp.</td>
</tr>
<tr>
<td><strong>Prevotella and Porphyromonas</strong> spp.</td>
</tr>
<tr>
<td><strong>Fusobacterium</strong> spp.</td>
</tr>
<tr>
<td><strong>Clostridium</strong> spp.</td>
</tr>
<tr>
<td><strong>Peptostreptococcus</strong> spp.</td>
</tr>
<tr>
<td>Non-spore-forming, anaerobic, gram-positive rods</td>
</tr>
</tbody>
</table>
**Pseudomonas aeruginosa**

- *Pseudomonas aeruginosa* is a common pathogen when a penetrating injury of the foot occurs in patients who are wearing sneakers.

- Ecthyma gangrenosum is the result of bacterial invasion of dermal veins leading to hemorrhage and necrosis.
  - Biopsy of the lesions will reveal bacteria on Gram stain.
Aeromonas

- **Aeromonas spp.**, which are present in fresh or brackish water

- Medicinal leech & Snakebites

- Cellulitis & hemorrhagic bullae formation and skin necrosis

- Osteomyelitis and myonecrosis

- **Aeromonas spp. can be cultured on MAC** and are oxidase-positive
Vibrio vulnificus

- Vibrio vulnificus is part of the ocean flora

- Hemorrhagic bullae & ulcers with skin necrosis.

- V. vulnificus grows well on MacConkey agar (MAC) but can be overlooked among other gram-negatives because it is a lactose fermenter
Human bite

- Most commonly isolated are viridans streptococci (particularly S. anginosus), S. aureus, and *Eikenella corrodens*.

- These infections are usually **polymicrobial** and contain both aerobic and anaerobic organisms.
**Eikenella corrodens**

- **Human bites or fights** (i.e., clenched fist wounds, or after the skin has been broken by human teeth).

- *Eikenella corrodens* isolates are fastidious, gram-negative coccobacilli that grow best under conditions of increased CO2 with hemin.
**Eikenella corroden**

- They are nonmotile, oxidase positive, and asaccharolytic

- About 45% of the isolates of *Eikenella corroden* "pit" or corrode the surface of the agar
**Eikenella corrodens**

- A chlorine bleach like odor from the agar surface may be obvious.

- Isolates do not usually grow on MAC or eosin-methylene agar (EMB) media.

- In broth medium, E. corrodens may adhere to the sides of the tube and produce granules.
Eikenella corrodens

Growth of *Eikenella corrodens* on chocolate
Eikenella corrodens

Gram-stain morphology of *Eikenella corrodens* (1000×).
Pasteurella / Animal bites

- The most common manifestation of human pasteurellosis is cellulitis, primarily from the Animal bites or scratches of dogs and cats.

- Pasteurella spp. will grow on SBA and CHOC agar, producing grayish colonies.
**Pasteurella**

- *Pasteurella spp.* are gram-negative, nonmotile, facultative, anaerobic coccobacilli appearing ovoid, filamentous, or as bacilli.

- **Bipolar** staining (safety pin appearance)

- Bacteria are catalase and oxidase positive (most isolates)
Erysipeloid

- Erysipeloid is a superficial soft tissue infection caused by *Erysipelothrix rhusiopathiae*.

- It is an occupational hazard of handlers of animals, meat, poultry, hides, and saltwater fish.

- Typically introduced by trauma, the *fingers* and *dorsum* of the hand are the most frequent sites of infection.
Erysipeloid
**Erysipelothrix rhusiopathiae**

- It is a gram-positive, catalase negative, non-sporeforming, pleomorphic rod that has a tendency to form long filaments.

- *Erysipelothrix rhusiopathiae* decolorizes easily, so it may appear gram-variable.

- Usually, a Gram stain or culture of superficial wound drainage is negative.

- **H2S from TSI Agar**
Erysipelothrix rhusiopathiae

A, Gram stain of Erysipelothrix rhusiopathiae at 24 hours. B, Gram stain of E. rhusiopathiae at 72 hours showing the tendency to form long filaments, which are easily decolorized. (Courtesy Cathy Bissonette.)
Francisella tularensis

- Francisella tularensis a gram-negative coccobacillus, causes several forms of tularemia

- The disease is transmitted to humans by **direct inoculation of skin** through handling of infected **rabbits** and other wild or, less commonly, domestic animals
Francisella tularensis

- The **ulceroglandular** type of tularemia is most common. It presents as an indolent ulcer, often on the hand, accompanied by painful swelling of the regional lymph nodes.

- Some patients can have nodular lymphangitis
Francisella tularensis

- Examination of ulcer exudates, lymph node aspirates, and other clinical specimen.

- Organism is not often seen on Gram stain
Francisella tularensis

- F. tularensis is a highly infectious agent with **as few as 50 organisms** causing an infection through the cutaneous (ulceroglandular form) or inhalational (pneumonia) routes and has been the cause of **many laboratory acquired infections**.

- **Biosafety level 3**
Francisella tularensis

FIGURE 18-26  *Francisella tularensis* colonies grown on chocolate agar. Gray-white, raised colonies with a smooth appearance are visible following 72 hours of incubation.
Mycobacteria
Nontuberculous Mycobacteria - NTM

- **NTM** can cause localized cutaneous infection, generally as the result of skin inoculation via *trauma* with resulting contained disease of the *skin* and adjacent *soft tissues*, sometimes extending to bone and joints.
Mycobacteria
Nontuberculous Mycobacteria - NTM

- *Mycobacterium chelonae*
  - most often in immunocompromised patients

- *Mycobacterium fortuitum*

- *Mycobacterium abscessus*
  - single, pruritic ulcer with undermined edges, also known as a **Buruli ulcer**
Outbreak of *Mycobacterium chelonae* Infection Associated with Tattoo Ink

*NEJM*, August 22, 2012
NTM cultured from a wound or skin biopsy

19 confirmed *Mycobacterium chelonae*

3 confirmed *Mycobacterium abscessus*
**Mycobacterium marinum**  
Nontuberculous Mycobacteria - **NTM**

- Nodular lymphangitis

- **Swimming pool granuloma** develops in individuals with a history of cleaning fish tanks or exposure to saltwater or nonchlorinated swimming pools.

- The microorganism enters through an open wound or through traumatic inoculation of skin.
Mycobacterium marinum
Nontuberculous Mycobacteria - NTM

• The diagnosis is made by biopsy and culture of a skin lesion or drainage material (Lowenstein-Jensen or Middlebrook) in association with obtaining a history suggestive of exposure.
  ◦ Ziehl-Neelsen acid-fast stain

• Mycobacterium marinum grows best at 25° to 32° C.
Nocardia

- In the **skin and soft tissue** form of nocardiosis, which results from direct cutaneous inoculation, manifestations range from **lymphocutaneous syndrome** to **subcutaneous abscesses**, **cellulitis**, and **mycetoma**.
Nocardia

- Direct smears from nocardial skin lesions will show **gram positive, beaded, branching filaments**.

- The organisms are acid-fast negative but can be seen with a **modified acid-fast stain**

- Nocardia spp. may take as long as **2** weeks to grow on blood culture media.

- **Aerobic growth**
Anthrax

- *Bacillus anthracis*, the agent of anthrax, is a **gram-positive rod** that can cause ulcerative skin lesions
  - The most common form of anthrax (95% of cases)

- Skin lesion begin as **painless** papules that vesiculate and are surrounded by significant erythema and a gelatinous type of edema. The vesicle evolves into a hemorrhagic and then necrotic lesion.
Anthrax

- Bacillus stain gram-positive or gram variable large, square-ended rod
- Gray, and flat
- Non hemolytic
- Non motile
- Gelatin hydrolysis: Neg.
Abscesses
Anaerobic bacteria

- Many wounds and abscesses are polymicrobial, particularly those that result from fecal spillage, bedsores, and infections in diabetic patients.

- Anaerobic bacteria are commonly a problem when the infectious site is adjacent to the intestine.
Abscesses
Anaerobic bacteria

- Problems of sampling make it difficult to ensure that all pathogenic species have been recovered, particularly in abscesses:
  - II of 37 total anaerobic strains were not recovered in the initial plating.
Gangrene
Soft Tissue Infections

- Infections that produce tissue necrosis and soft tissue gas

- These soft tissue infections can be classified according to their level of soft tissue involvement (e.g., superficial, epidermal, or dermal structures, fascia, or muscle) and according to the causative microbiologic agent or agents
Gangrene
Soft Tissue Infections

• Type 1: polymicrobial **necrotizing fasciitis**, typically caused by **Enterobacteriaceae** and **anaerobes**

• Type 2: Streptococcal gangrene caused by **Streptococcus pyogenes**

• Gas gangrene: Clostridial myonecrosis caused by Clostridia species, usually C. perfringens