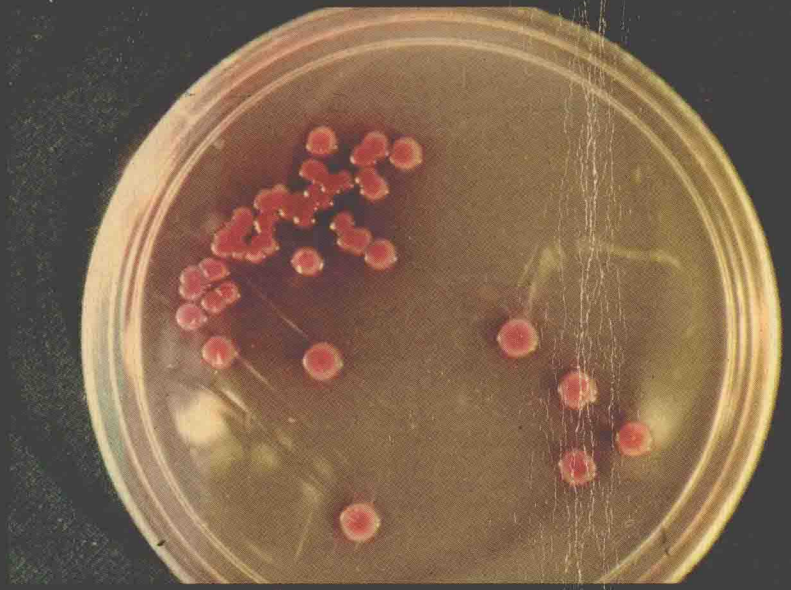
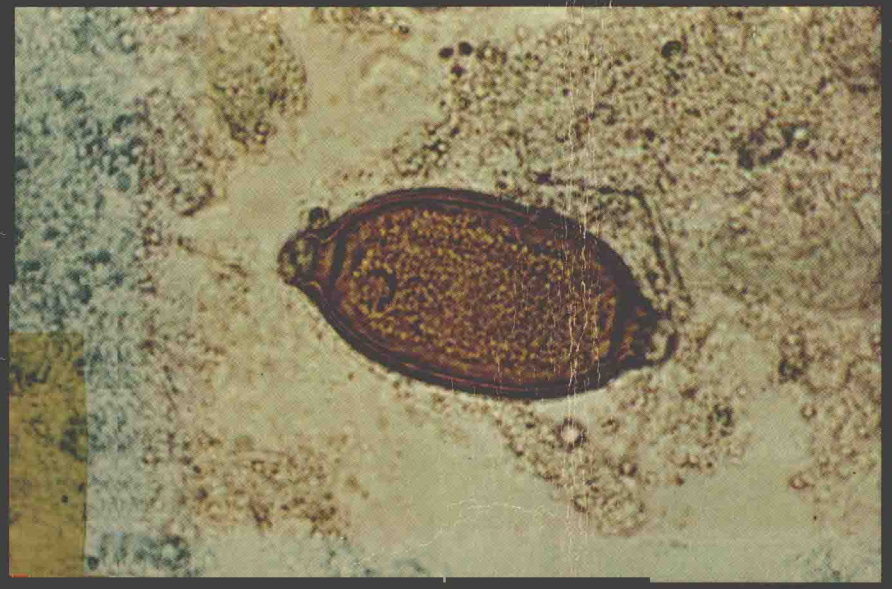
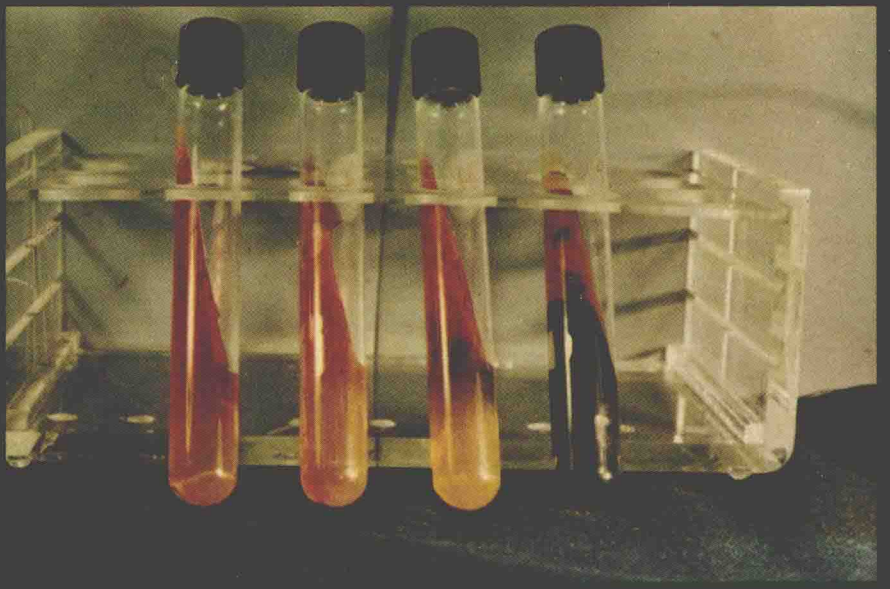


ATLAS OF DIAGNOSTIC MICROBIOLOGY

by S. Stanley Schneierson, M.D.





Photographs by S. Stanley Schneierson, M.D.

Text by Alan F. Sewell

ATLAS OF DIAGNOSTIC MICROBIOLOGY by S. Stanley Schneierson, M.D.

DIRECTOR OF MICROBIOLOGY, THE MOUNT SINAI HOSPITAL, NEW YORK

Published as a service to the medical profession by Abbott Laboratories, North Chicago, Illinois

TABLE OF CONTENTS

BACTERIOLOGY

MYCOLOGY

PARASITOLOGY

SPECIAL TESTS

Photographs by S. Stanley Schneierson, M.D.

Text by Alan F. Sewell

ATLAS OF DIAGNOSTIC MICROBIOLOGY by S. Stanley Schneierson, M.D.

DIRECTOR OF MICROBIOLOGY, THE MOUNT SINAI HOSPITAL, NEW YORK

Published as a service to the medical profession by Abbott Laboratories, North Chicago, Illinois

TABLE OF CONTENTS

BACTERIOLOGY

MYCOLOGY

PARASITOLOGY

SPECIAL TESTS

INTRODUCTION

Reviewing the various textbooks of microbiology, and the definitive reference texts as well, we have been impressed by the fact that none is a truly effective laboratory guide. There seemed to us to exist a need for something more highly visual than any of the existing texts. In short, there was a need for an atlas of diagnostic microbiology.

An atlas is, in fact, a visual guide. And it is in the spirit of atlas-making that the present volume has been created. Perhaps more than most sciences, microbiology is a visual science; microbiologists judge things by their appearances. Color and shape are among the most important of the diagnostic clues to the microbiologist. And, therefore, it seems inadequate merely to present simple line-drawings or, at best, black-and-white photographs.

Instead, here the illustration is king. Cultures were not specifically prepared, but the most exacting of photographic techniques were applied in order to depict ordinary cultures exactly as they appear to the experienced microbiologist. The illustrations in this volume have been culled from thousands, most of which were rejected because they failed in one way or another to present the subject exactly. In this way we hope to have established a guide of immediate practicality and of usefulness to novice, expert, and instructor alike.

In selecting photographs we chose to concentrate on the two extremes of the microbiological spectrum: those species which are most commonly encountered in pathology, and those species which are so uncommonly encountered that for the most part they remain as obscure entries in well-thumbed texts. It is certainly true that the species of bacteria, fungi, and parasites included here account for an overwhelming majority of the infections and infestations seen in the American hospital laboratory; and for this reason the volume should serve handily in the day-to-day work of the laboratory technician, pathologist, and student. At the same time, other species were deliberately included because of their relative rarity: to the microbiologist this adds the lure of the uncommon; but to the worker engaged in the more routine laboratory tasks it provides a guide to those microorganisms which do occur just often enough to cause serious problems of identification.

It should be noted, however, that no standard magnification or enlargement has been applied throughout. The sizes of organisms, therefore, are not proportionate from one illustration to another; magnifications and enlargements have been chosen as indicated for purposes of greatest clarity.

Bearing in mind that this work should find a major use in the training and teaching of laboratory workers and by others who must deal with microorganisms, we have also included a section depicting a few of the more important and a few of the more unusual laboratory tests and diagnostic aids. This section is by no means complete; still its practicality should be obvious to those responsible for the training of the next generation of microbiologists, physicians, and technical workers.

It would be insufficient, however, for us simply to have provided a volume of illustrations cursorily identified. Therefore, we have included a text discussion of the most important facts concerning each of the illustrated species. For all species, the text provides information on pathogenicity and morphology. For the bacteria and fungi, additional information on culture characteristics is provided.

It should be emphasized, however, that the text material is intended to supplement the illustrations, which are, after all, primary in an atlas. The text, limited as it is by practical considerations, is intended to be accurate but not complete. Further information on the species illustrated here can be obtained from most of the standard references.

This *Atlas of Diagnostic Microbiology* is the product of many, many months of concentrated effort—and of many, many years of effortfully developed experience. But all of this effort will have proved worthwhile if the Atlas finds its place on the laboratory workbench, in the library, and in the life of the user.

BACTERIOLOGY

GRAM-POSITIVE BACTERIA

The many thousands of known bacterial species have traditionally been classified into two very large groups on the basis of their reactions to Gram staining. Species which retain a violet coloration despite washing with alcohol are said to stain *Gram-positive:* species which lose the violet coloration with alcohol washing and may be counterstained with a contrasting dye, such as safranin, are said to stain *Gram-negative*. Gram staining characteristics appear to be related to structural or metabolic properties of the species, and this characteristic serves as a rapid and very basic step in the identification of the species.

The Gram-positive species are generally susceptible to basic dyes; their isoelectric point is about pH 2; and, most important, they are generally more susceptible to antimicrobial agents than are Gram-negative species.

Bacillus

Many species of aerobic, sporogenous bacilli have been identified and classified; most stain Gram-positive, although Gram-variable and Gram-negative strains are not uncommon. Of these species, the anthrax bacillus, *B. anthracis*, is the only significant pathogen. Other species, however, frequently occur as laboratory contaminants.

Bacillus anthracis

Pathogenicity. *B. anthracis* is the cause of anthrax, primarily a disease of herbivorous animals, and relatively uncommon in man in the United States. The usual source of infection in man is cutaneous inoculation through cuts or scratches upon the skin of men who occupationally handle livestock or hides. Pulmonary and alimentary infection are also possible. Neither exotoxins nor endotoxins have been demonstrated.

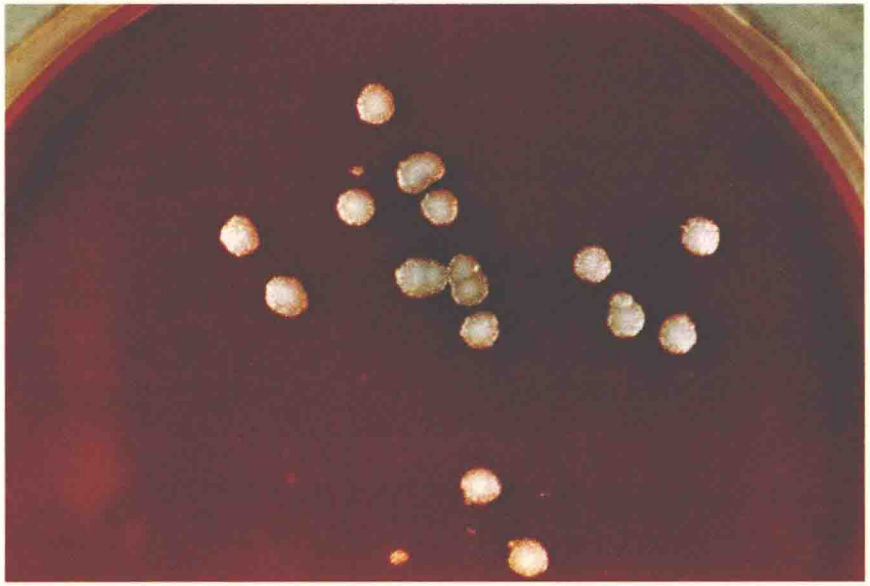
Morphology. *B. anthracis* is a very large bacterium, a straight rod 5 to 10 microns in length and 1 to 3 microns in width, with square or concave ends. Individuals may occur singly, in pairs, or in long chains. They are encapsulated, non-motile, and stain Gram-positive. Spores are ellipsoidal or cylindrical, located centrally or paracentrally, and 0.8 to 1.0 micron by 1.3 to 1.5 microns. Germination is polar.

Culture characteristics. The species is aerobic but facultatively anaerobic. Optimal temperature is 37°C, but the species will continue to grow at considerably higher or lower temperatures. Colonies will grow profusely on most common laboratory media. Agar colonies are large, dense, and irregular, composed of parallel chains of cells giving a curled or combed appearance. Broth cultures show a thick pellicle, but little or no turbidity. Blood hemolysis is variable. Lactose is not fermented, but acid without gas is produced from glucose. Neither morphological nor cultural characteristics will absolutely differentiate *B. anthracis* from similar but non-pathogenic species; testing for pathogenicity is essential.

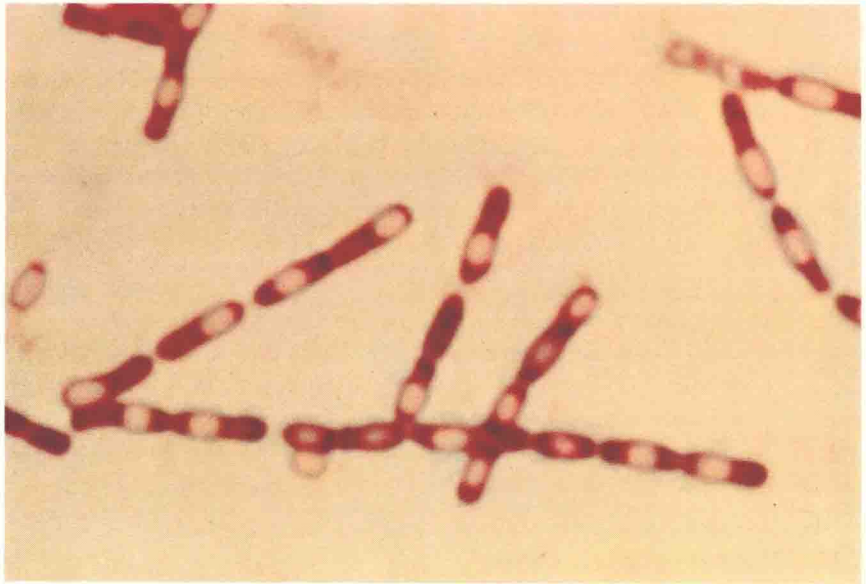
Bacillus subtilis

This species is a common laboratory contaminant. The Gram-positive rods are 2.0 to 8.0 microns long and 0.7 to 0.8 wide, unencapsulated, and occurring as short chains or singly. Spores are ellipsoidal or cylindrical, located centrally or paracentrally. Spore germination is equatorial. Agar colonies are rough, opaque, dull, spreading, and off-white, but considerable variations occur. The species is considered non-pathogenic. Another species in this genus is *Bacillus cereus* which closely resembles *Bacillus anthracis* in morphology and cultural characteristics.

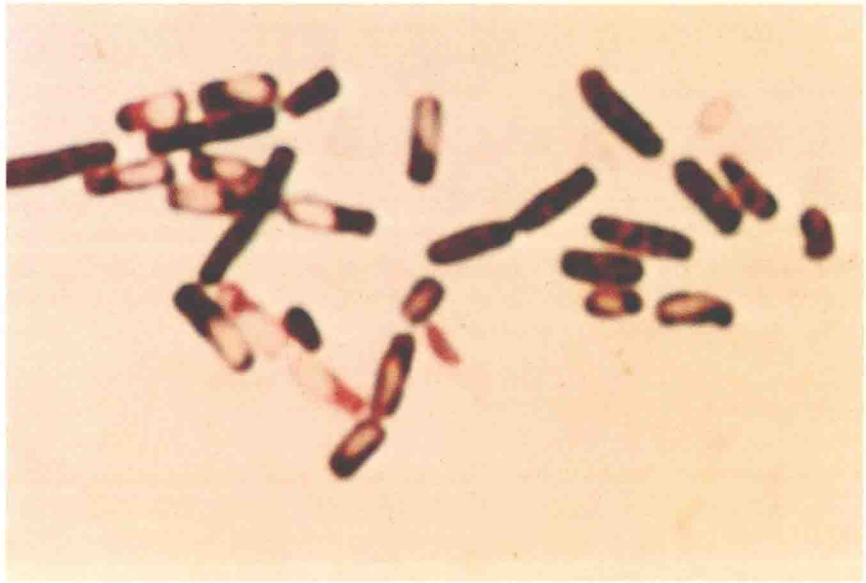
Bacillus anthracis. Culture on blood agar.



Bacillus anthracis. Gram stain of culture smear.



Bacillus subtilis. Blood agar culture. The large, abundant, irregular, spreading, rough and waxy colonies are usually surrounded by a large zone of beta hemolysis.



Bacillus subtilis. Gram stain of culture smear.

Clostridium

The Clostridia include many saprophytic and pathogenic species. The bacilli are rod-shaped, but the rods are often swollen and distorted at sporulation. Most of the species are motile via flagella. The Clostridia are strictly anaerobic and stain Gram-positive. Many species are saccharolytic and fermentative, while other species are proteolytic. Organisms of this genus are commonly found in soil and in the intestinal tracts of man and other animals.

The pathogenic Clostridia are characterized by their ability to produce powerful exotoxins. They may be divided into three groups: the gas gangrene organisms, such as *C. perfringens*, which infect only traumatized and devitalized tissues; *C. tetani*, which causes insignificant local infection but general intoxication; and the botulism organisms, such as *C. botulinum* and *C. parabotulinum*, which do not normally invade the body but synthesize exotoxins in food products which may be ingested.

Clostridium perfringens

Pathogenicity. Wound infections are characterized by a mixed flora, within which may be found both pathogenic and non-pathogenic organisms. *C. perfringens* is the organism most commonly responsible for gas gangrene, but *C. sporogenes*, a species generally not considered pathogenic, is frequently also present in such infections; plating studies are necessary for isolation of the causative species. In wound infections, *C. perfringens* ferments muscle sugar, producing gas within the tissues.

Morphology. *C. perfringens* is a short, plump, spore-bearing bacillus, occurring singly and in pairs. The species is capsulated and non-motile. The rods are from 1.0 to 1.5 microns wide and from 4.0 to 8.0 microns long. The spores are ovoid, central to eccentric in location, and do not swell the cells. The species stains Gram-positive.

Culture characteristics. The species is strictly anaerobic. Optimum temperature is between 35°C and 37°C, but growth does occur at 50°C. Anaerobic agar surface colonies are circular, moist, slightly raised, and entire with an opaque center. Broth cultures are turbid and peptolytic, clearing with a viscid sediment. *C. perfringens* actively ferments sugars with the production of large amounts of gas. Gelatin is liquefied and blackened, but coagulated serum is neither liquefied nor blackened. The majority of strains do not form spores readily; an alkaline, sugar-free, protein-rich medium, such as alkaline egg, is required to demonstrate spore formation. An exotoxin is produced, for which an antitoxin can be prepared.

C. sporogenes, on the other hand, ranges in size from 0.6 to 0.8 micron by 3.0 to 7.0 microns. The rods have rounded ends and occur singly, in pairs, or sometimes in short to long chains and filaments. Spores are ovoid, eccentric to subterminal in position, and cause the cell to swell. *C. sporogenes* is motile. Gelatin is liquefied and blackened, as is blood serum. Anaerobic agar surface colonies are small, irregular, transparent becoming opaque, yellowish white, and fimbriate. Broth cultures are turbid, and gas with a putrid odor is produced. Blood agar is hemolyzed. The species is intensely proteolytic. *C. sporogenes* is usually responsible for the foul odor of wounds in which the actual destruction of tissue or toxic effects may be produced by *C. perfringens*.

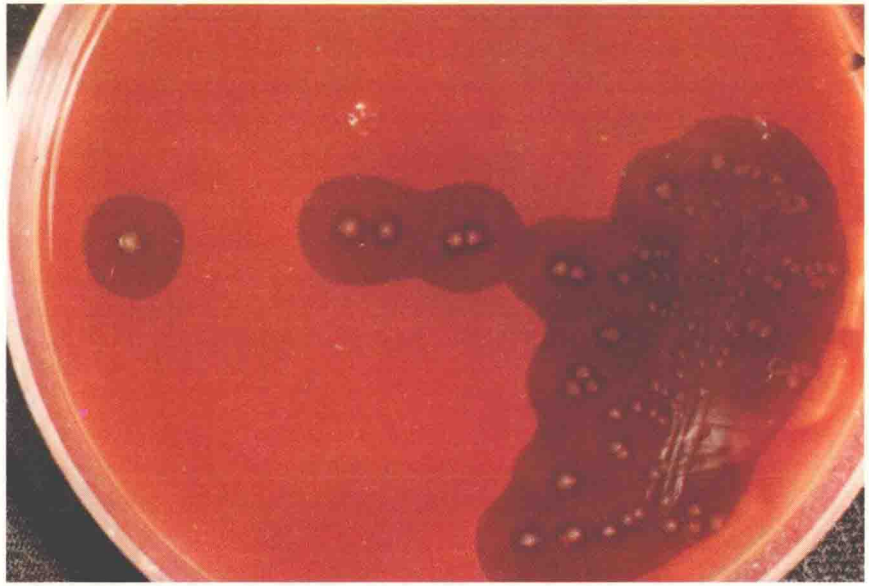
Clostridium tetani

Pathogenicity. *C. tetani* is the etiological agent of tetanus. The species is considered both pathogenic and toxic. A powerful exotoxin, which acts as a neurotoxin, causes generalized muscle spasm in man. The incubation period in man ranges from five days to five weeks, with the longer incubation period indicating a more favorable prognosis. An antitoxin can be prepared.

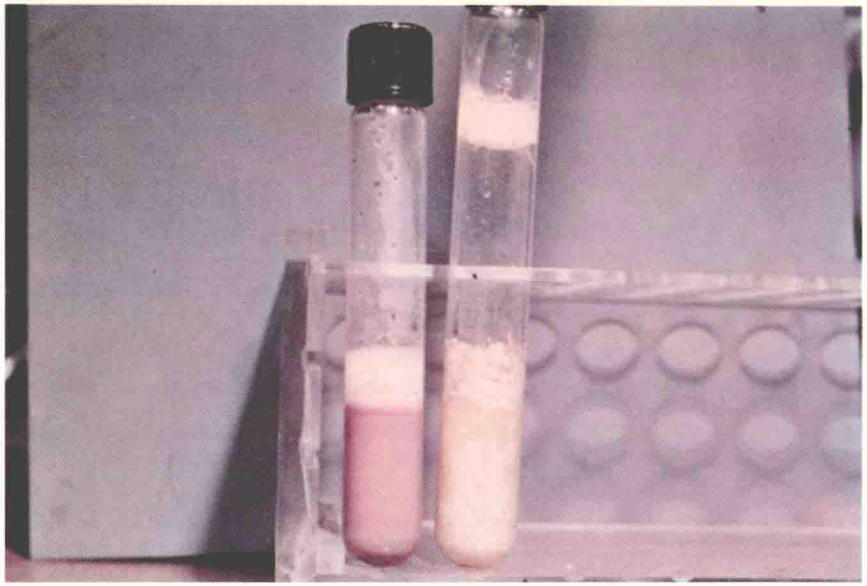
Morphology. *C. tetani* is found as rods from 0.4 to 0.6 micron in width and from 4.0 to 8.0 microns in length, occurring singly, in pairs, and often in long chains and filaments. Spores are spherical and terminally located, producing the characteristic "drumstick" appearance of the cell. The species is motile and stains Gram-positive.

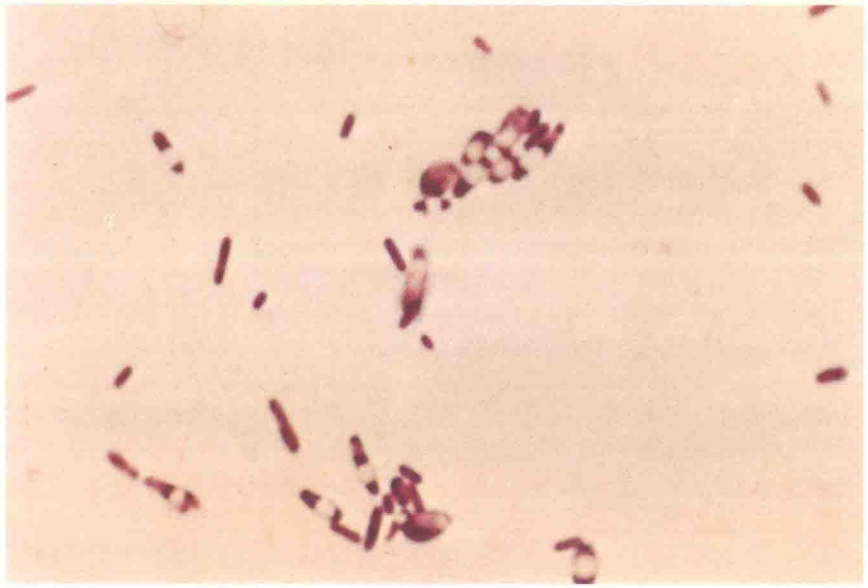
Culture characteristics. *C. tetani* is strictly anaerobic. Optimum temperature is 37°C. Anaerobic surface colonies on serum agar are small and transparent, with a villous to fimbriate margin. Broth cultures are slightly turbid, and gas is produced. The species does not ferment carbohydrates, but does cause hemolysis of blood agar. Gelatin is slowly liquefied and blackened. Blood serum is slowly softened and only feebly digested.

Clostridium perfringens. Blood agar culture from gas gangrene lesion. Round, grayish-white colonies, 2.0 to 5.0 mm. in diameter, with opaque raised center and surrounded by a double zone of beta hemolysis.

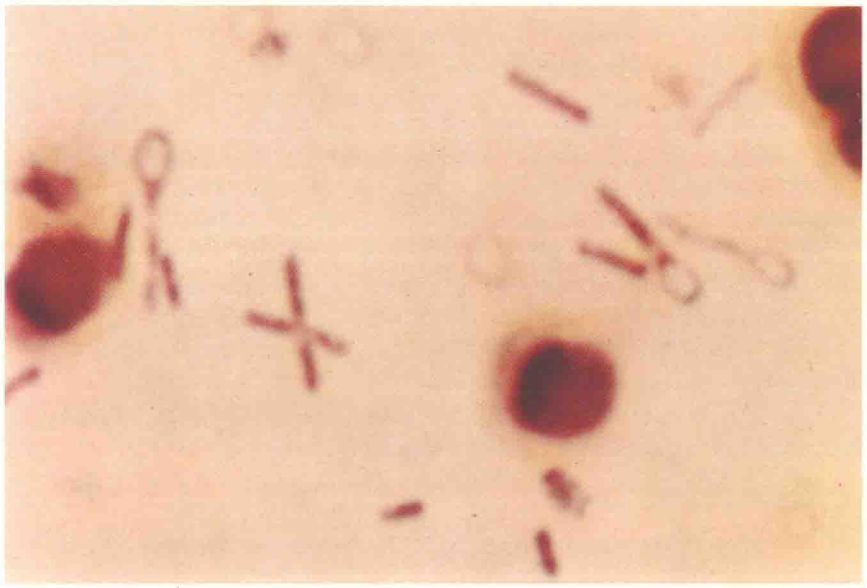


Clostridium perfringens. Characteristic stormy fermentation in vaseline-sealed litmus milk. Left: uninoculated tube; right: marked acid coagulation of milk and marked gas production lifting off vaseline seal, clot undigested.





Clostridium sporogenes. Gram stain of culture.



Clostridium tetani. Gram stain of culture. A number of the terminal spores are free from the bacterial cell.

Corynebacterium

Pathogenicity. *C. diphtheriae* is the pathogen responsible for diphtheria, classically regarded as a disease of children; but in recent years adult incidence has increased, although total incidence of diphtheria in the United States has shown a very marked reduction within the past fifty years. In man, infection is marked by a local lesion, followed by systemic intoxication, usually accompanied by a grayish pseudomembrane in the nose or throat. The bacterial toxin affects the heart and nerves, death resulting from heart failure or respiratory paralysis. Susceptibility to the toxin is indicated by a positive Schick Test.

Morphology. The bacteria occur as straight or slightly curved rods, frequently swollen at one or both ends, and varying greatly in dimensions: from 1.0 to 8.0 microns in length and from 0.3 to 0.8 micron in width. They are non-motile and may occur in "V", "L", or "Y" arrangements ("Chinese letters"). *C. diphtheriae* stains Gram-positive. Darkly stained metachromatic granules, located at the poles, may be observed.

Culture characteristics. *C. diphtheriae* is aerobic, but facultatively anaerobic. The organism grows readily on meat infusion agar. Optimum temperature is between 34°C and 37°C, but growth will occur at 15°C and 40°C. Smooth, rough, intermediate, and dwarf colony forms have been observed. The smooth form is round and umbonate or convex with an even margin and smooth surface, usually 1.0 to 3.0 mm. in diameter. The rough form is flat, with an irregular margin and an uneven surface, averaging 1.0 to 5.0 mm. in diameter. Dwarf colonies resemble smooth colonies, except that they are 0.2 mm. or less in diameter.

Three serological types of *C. diphtheriae* are distinguishable by colony form on McLeod blood-tellurite medium. Type *gravis* grows with dark gray, daisy-head colonies; type *mitis* grows in convex, black, shiny, entire colonies; type *intermedius* produces a small, flat, umbonate colony with a black center and slightly crenated edge.

Definitive identification is based upon guinea pig pathogenicity; the powerful exotoxin of *C. diphtheriae* produces a zone of hemorrhagic necrosis on intradermal injection or adrenal hemorrhage on subcutaneous injection. Both reactions may be prevented by diphtheria antitoxin.

C. diphtheriae must be distinguished from *C. pseudodiphtheriticum*, which may be cultured from throat smears of healthy persons. The rods of *C. pseudodiphtheriticum* are shorter and thicker, with rounded ends, and are found in parallel rows or irregular groups. They usually stain darker with Gram stain; metachromatic granules are absent or rare. *C. pseudodiphtheriticum* does not ferment glucose or maltose and is not pathogenic to guinea pigs.

Diplococcus

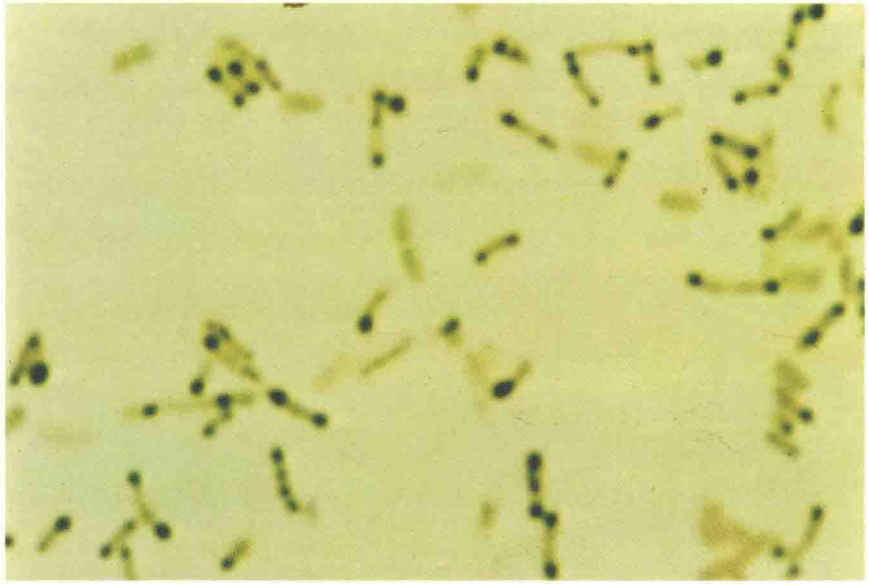
Pathogenicity. *D. pneumoniae*, commonly known as pneumococcus, is the primary etiological agent in pneumonia of all types. The organism is particularly responsible for lobar pneumonia. Sinus infections, otitis media, osteomyelitis, arthritis, peritonitis, corneal ulceration, and meningitis are also caused by *D. pneumoniae*. Septicemia, empyema, endocarditis, pericarditis, meningitis, and arthritis are among the common complications of pneumococcal pneumonia. Secondary pneumococcal pneumonia may also follow viral infections such as measles and influenza.

Morphology. *D. pneumoniae* typically occurs as oval or spherical bacteria in pairs, singly, or as short chains. Individuals are 0.5 to 1.25 microns in diameter. The distal ends of paired organisms tend to be pointed or lancet-shaped. *D. pneumoniae* is encapsulated and non-motile. Young cells stain Gram-positive, but Gram-negative forms may be found in older cultures.

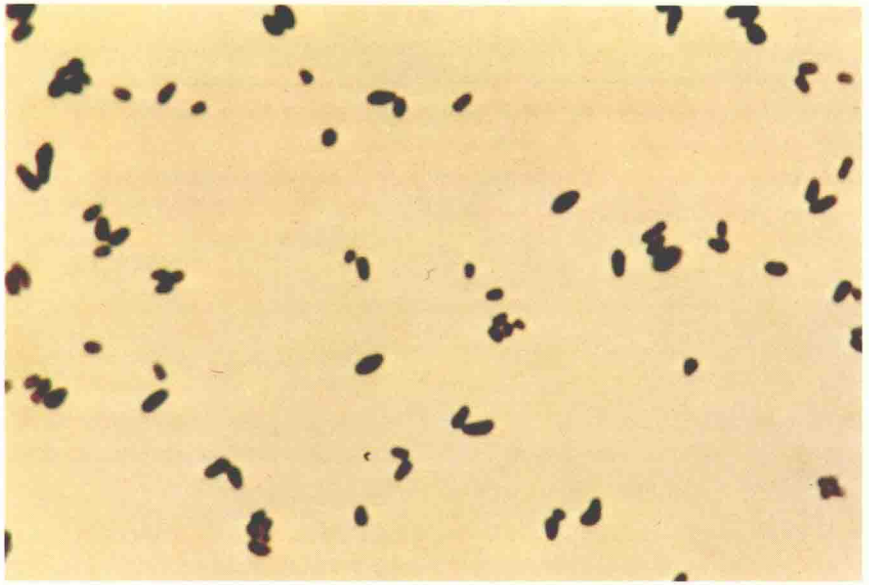
Culture characteristics. The Diplococci are bile soluble, and this characteristic serves as the most reliable means of differentiating the genus from other coccal forms. In addition, the cocci may be differentiated from other alpha hemolytic cocci by their inhibition by optochin (ethylhydrocupreine hydrochloride), by their ability to ferment inulin, their pathogenicity for mice upon intraperitoneal injection, and their specific serological reactivity.

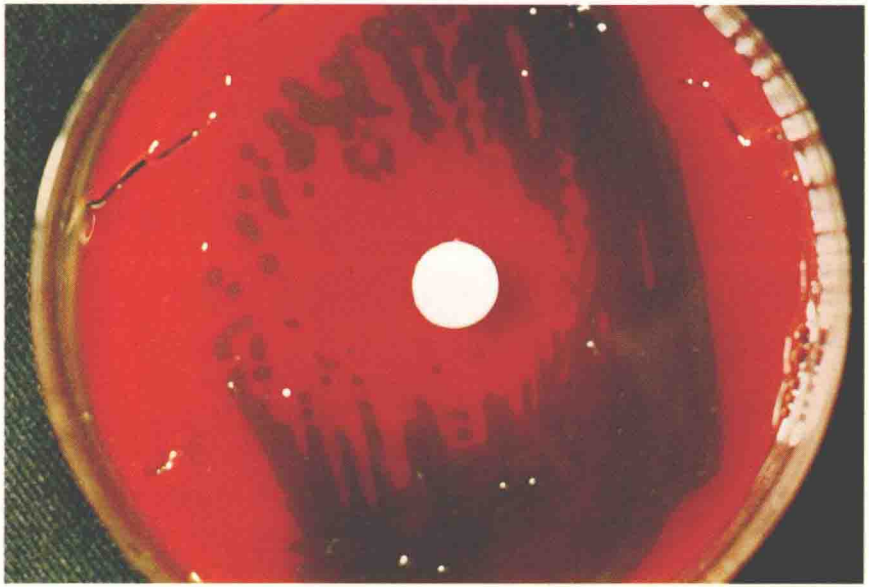
D. pneumoniae is aerobic but facultatively anaerobic. On blood agar the colonies are elevated at the center with concentric elevations and depressions. Growth on meat extract media is usually poor. Growth in beef heart infusion broth produces a uniform turbidity with a variable amount of sediment; the addition of glucose, serum, whole blood, or ascitic fluid enhances growth. Inulin serum water is usually marked by acidity with coagulation. The optimum temperature is 37°C, with usually no growth between 18°C and 22°C. The optimum pH is 7.8; any marked acidity will promptly kill the culture.

Corynebacterium diphtheriae. Smear of throat culture on Loeffler medium, stained with Epstein methylene blue—Gram iodine stain.



Corynebacterium pseudodiphtheriticum. Gram stain of throat culture smear.





Diplococcus pneumoniae. Culture on blood agar with zone of inhibition around optochin disk.

Diplococcus pneumoniae. Culture on blood agar of a mucoid strain.

Immunological types. Currently some 75 or more types of *D. pneumoniae* are recognized and classified on the basis of their antigenic structure; the essential antigen of each type is believed to be a capsular polysaccharide which determines both type and virulence. Typing reactions may take the form of agglutination of the whole organism, precipitation of specific capsular polysaccharides, or capsular swelling when the organisms are mixed with the appropriate type-specific rabbit antiserum (Neufeld-Quellung Reaction).

Listeria

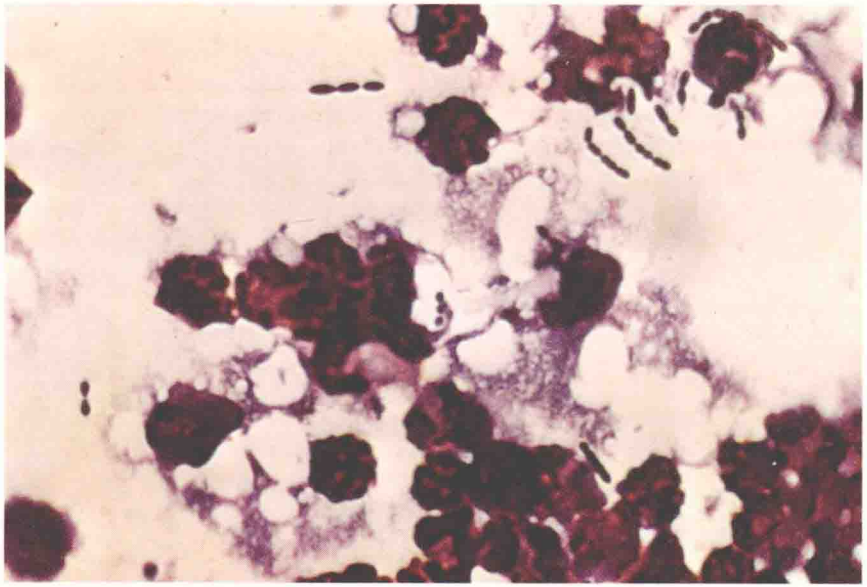
Pathogenicity. *L. monocytogenes* may be the causative organism in meningitis, and it has been shown to be a frequent cause of abortions and stillbirths.

Morphology. *L. monocytogenes* is highly pleomorphic, occurring generally as small rods with rounded ends, 0.5 to 2.0 microns long and 0.4 to 0.5 micron wide, singly, and in V-shaped or parallel pairs. Short coccal forms may also be seen. In some culture media the rods are slightly curved. The species is motile via flagella. Gram staining may give variable results; young cultures usually stain uniformly Gram-positive, but in older cultures many elements may stain Gram-negative.

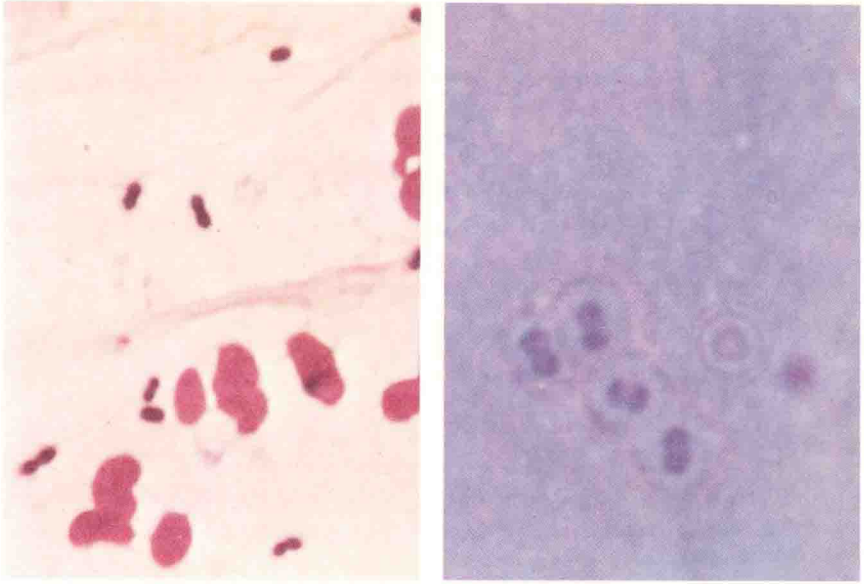
Culture characteristics. The organism is aerobic but facultatively anaerobic. Optimum temperature is 37°C but growth may be observed at temperatures as low as 2.5°C. Blood agar colonies show good growth with a zone of hemolysis varying with the blood species. In gelatin, growth is confined to the needle track; liquefaction is absent.

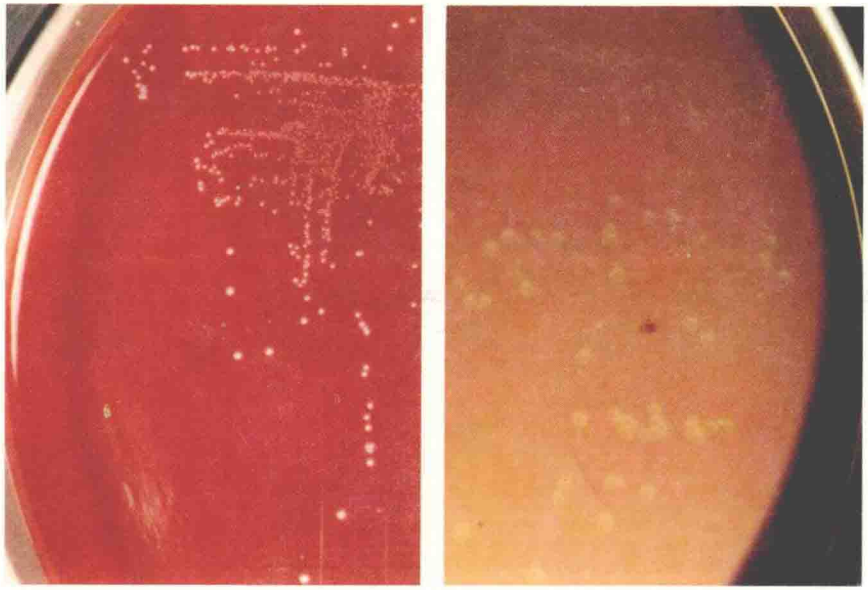
L. monocytogenes may be differentiated from *Erysipelothrix insidiosa*, which it resembles, by the weak beta hemolysis of the former as contrasted to the alpha hemolysis of the latter; by its tumbling motility, especially at 22°C; by its ability to grow at very low temperatures; by its acid fermentation of salicin, its positive methyl red and Voges-Proskauer reactions; and by its pathogenicity for guinea pig. In addition, the rods of *E. insidiosa* are generally more slender than those of *L. monocytogenes*.

Diplococcus pneumoniae. Gram stain of blood culture smear.

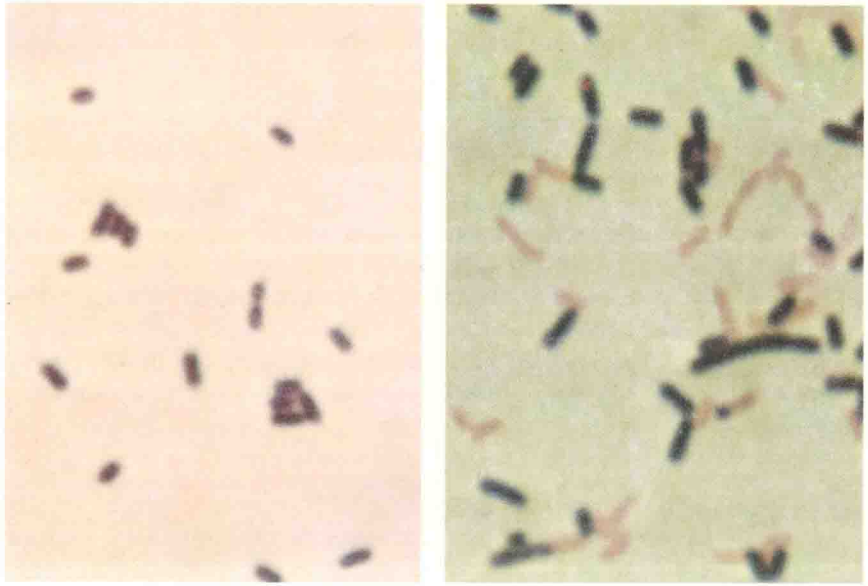


Diplococcus pneumoniae. Left: Gram stain of sputum smear. Right: typing of organism in spinal fluid by positive Neufeld-Quellung reaction.





Listeria monocytogenes. Culture of spinal fluid on blood agar. Left: by reflected light; right: by transmitted light. Small, translucent colonies, 0.2 to 0.4 mm. in diameter, surrounded by characteristic faint "ground glass" or opalescent beta hemolysis. Colonies appear white in reflected light.



Listeria monocytogenes. Gram stain of spinal fluid culture. Left: young culture; right: after 2 days incubation at 37°C.

Staphylococcus

The Staphylococci are spherical, non-motile, non-sporogenous bacteria, occurring singly, in pairs, in tetrads, and in irregular clusters (from which they derive their genus name). They are aerobic but facultatively anaerobic. Many strains produce orange or yellow pigments which have traditionally been associated with but are not definitive of the genus or species. The coagulase-positive strains produce a variety of toxins, and the genus as a whole is potentially pathogenic.

Staphylococcus aureus

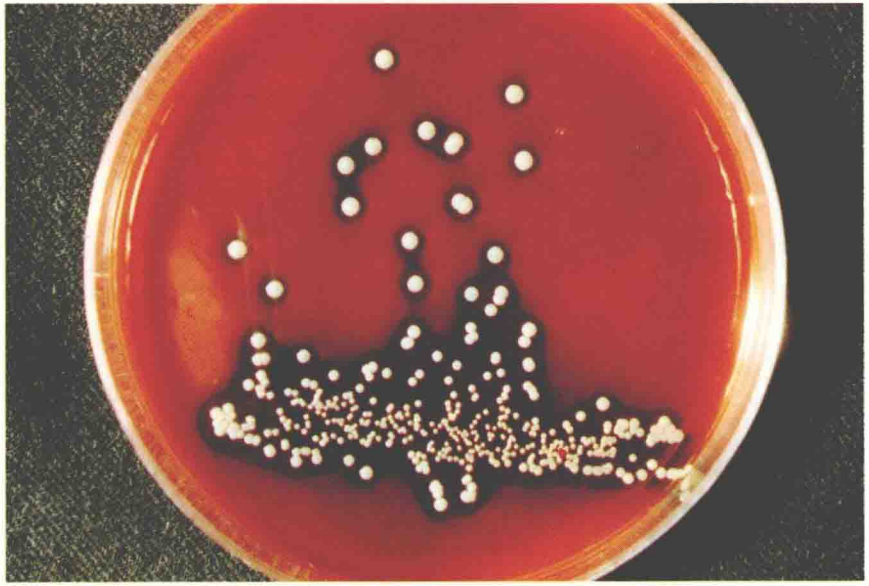
Pathogenicity. Clinical forms of staphylococcal infection depend upon the size of the dose, route of invasion, presence or absence of antibodies from previous infections, susceptibility of the host, and characteristics of the invading strain. The most common infections caused by *S. aureus* include pimples, boils, carbuncles, paronychiae, cystitis, pyelitis, and impetigo. More severe infections include septicemia, endocarditis, meningitis, brain abscess, puerperal sepsis, orbital and cavernous sinus thromboses, osteomyelitis, and pneumonia. Secondary infections following primary pulmonary infections include empyema, pulmonary abscess, and bronchiectasis. Recently staphylococcal enteritis has increased in incidence.

Morphology. *S. aureus* is found as spheres generally from 0.8 to 1.0 micron in diameter, occurring singly, in pairs, in short chains, or in irregular clusters. The organism stains Gram-positive.

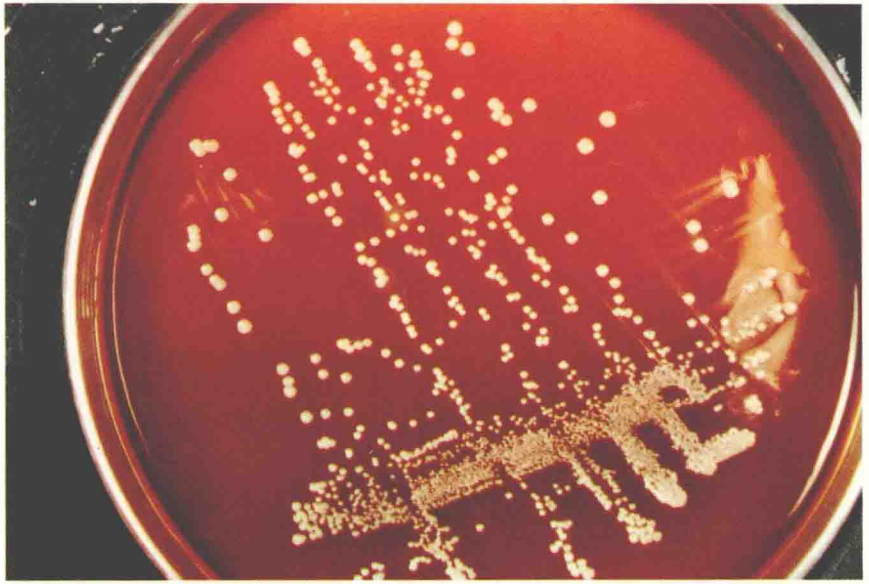
Culture characteristics. Optimum temperature for the species is 37°C, but growth may be observed at 10°C and at 45°C. Agar colonies are 1.0 to 2.0 mm. in diameter, circular, smooth, glistening, and with a butter-like consistency. Young colonies are colorless, but older colonies may range in color from orange to white. Blood agar colonies normally produce beta hemolysis. Broth cultures are turbid, becoming clear and characterized by a yellowish ring and sediment. The species is normally coagulase-positive, and ferments both glucose and mannitol anaerobically.

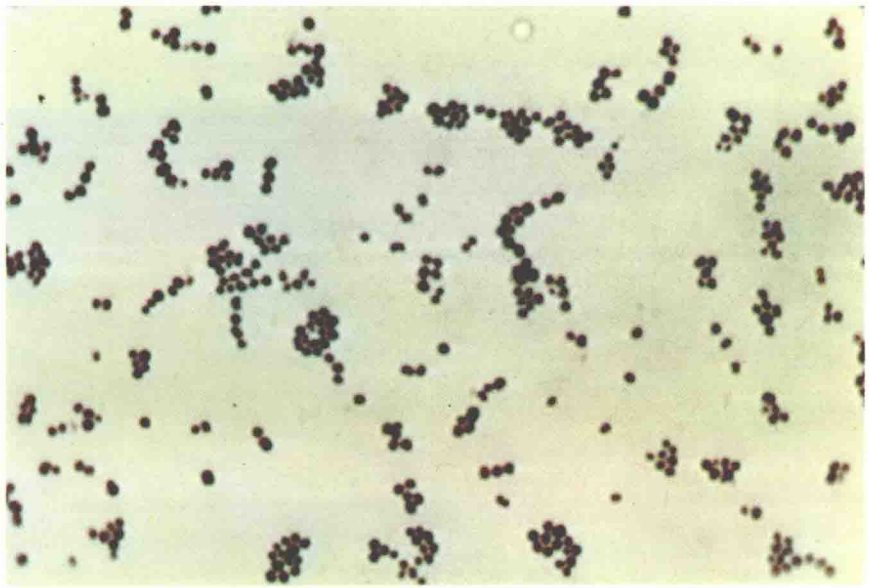
Toxins and resistance. Certain strains of *S. aureus* produce various exotoxins (hemotoxin, dermotoxin, lethal toxin, leukocidin), and, in addition, a potent enterotoxin which is a significant cause of food poisoning. Currently a large percentage of hospital-isolated strains are penicillin-resistant. Many strains can and do produce penicillinase, which inactivates penicillin. The predominance of resistant strains is probably due to the progressive elimination of sensitive strains. Resistance to other antibiotics has become a problem of major medical concern.

Staphylococcus aureus. Culture of abscess exudate on blood agar. Pigment production is enhanced on potato agar or 33% milk agar.

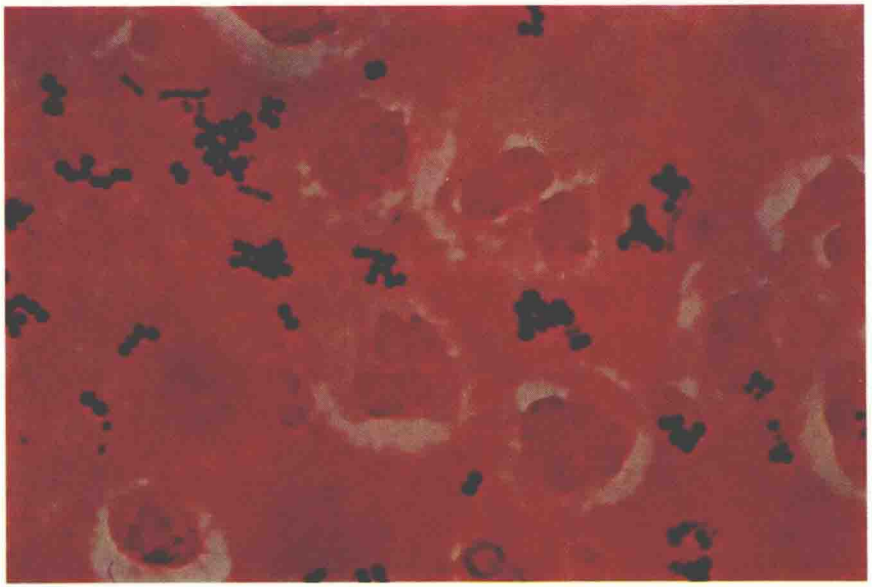


Staphylococcus epidermidis. Culture on blood agar.





Staphylococcus aureus. Gram stain of smear from culture.



Staphylococcus aureus. Gram stain of smear of sputum.

Staphylococcus epidermidis

Pathogenicity. As opposed to *S. aureus*, *S. epidermidis* is primarily parasitic rather than pathogenic. It is commonly found on the skin and mucous membranes of man and other animals. The species does cause minor lesions, especially stitch abscesses, and may cause subacute bacterial endocarditis.

Morphology. Microscopically, *S. aureus* and *S. epidermidis* are superficially similar. *S. epidermidis* is found as spheres averaging 0.5 to 0.6 micron in diameter, occurring singly, in pairs, or in irregular clusters. The species is non-motile and stains Gram-positive.

Culture characteristics. Agar colonies of *S. epidermidis* are circular, smooth, and generally a pale, translucent white. In broth containing a fermentable carbohydrate, *S. epidermidis* produces a heavy, uniform turbidity and a ring pellicle. Distinctively, the species is coagulase-negative; it ferments glucose but not mannitol anaerobically. Blood agar colonies may show no hemolysis, and the species is not considered pathogenic to mice.