

LAB 15: ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCI

DISCUSSION

Staphylococci (see Fig. 6) are often found in the human nasal cavity (and on other mucous membranes) as well as on the skin. They are **Gram-positive cocci** 0.5-1.0 μm in diameter and occur singly, in pairs, in short chains, and most commonly, in **irregular grape-like clusters**. The staphylococci are strongly catalase positive and generally tolerate relatively high concentrations of sodium chloride (7.5-10%). This ability is often employed in preparing media selective for staphylococci.

Staphylococcal **capsules** play a major role in the ability of the bacteria to **adhere to and colonize biomaterials**.

- [Scanning electron micrograph *Staphylococcus aureus*](#); courtesy of [Dennis Kunkel's Microscopy](#).
- [Scanning electron micrograph of methicillin-resistant *Staphylococcus aureus* \(MRSA\)](#); courtesy of CDC
- [Scanning electron micrograph of methicillin-resistant *Staphylococcus aureus* \(MRSA\) destroying an immune cell](#); courtesy of Frank Deleo, NIAID

There are five species of staphylococci **commonly associated with clinical infections**: *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. saprophyticus*.

A. *Staphylococcus aureus* (coagulase-positive staphylococci)

Staphylococcus aureus is the most pathogenic species and is implicated in a variety of infections. *S. aureus* is with some frequency found as normal human flora in the anterior nares (nostrils). It can also be found in the throat, axillae, and the inguinal and perineal areas. Approximately 30% of adults and most children are healthy periodic **nasopharyngeal carriers** of *S. aureus*. Around 15% of healthy adults are persistent nasopharyngeal carriers. The colonization rates among health care workers, patients on dialysis, and people with diabetes are higher than in the general population.

In the majority of *S. aureus* infections the **source of the organism** is either:

- a **healthy nasal carrier**, or
- **contact with an abscess** from an infected individual.

The portal of entry is usually the skin. *S. aureus* causes pus-filled inflammatory lesions known as **abscesses**. Depending on the location and extent of tissue involvement, the abscess may be called:

1. a **pustule**

A pustule is an **infected hair follicle** where the base of the hair follicle appears red and raised with an accumulation of pus just under the epidermis. Infected hair follicles are also referred to as **folliculitis**.

2. a **furuncle or boil**

Furuncles appear as large, **raised, pus-filled, painful nodules** having an accumulation of dead, necrotic tissue at the base. The bacteria **spread from the hair follicle to adjacent subcutaneous tissue**.

3. a **carbuncle**

Carbuncles occur when **furuncles coalesce and spread into surrounding subcutaneous and deeper connective tissue**. **Superficial skin perforates, sloughs off, and discharges pus.**

S. aureus also causes **impetigo**, a superficial **blister-like infection of the skin** usually occurring on the face and limbs and seen mostly in young children. *S. aureus* may also cause **cellulitis**, a diffuse inflammation of connective tissue with severe inflammation of dermal and subcutaneous layers of the skin. *S. aureus* is also a **frequent cause of accidental wound and postoperative wound infections**.

Less commonly, *S. aureus* may escape from the local lesion and spread through the blood to other body areas, causing a variety of **systemic infections** that may involve every system and organ. Such systemic infections include **septicemia, septic arthritis, endocarditis, meningitis, and osteomyelitis**, as well as **abscesses in the lungs, spleen, liver, and kidneys**. *S. aureus pneumonia* may also be a secondary respiratory complication of viral infections such as measles, and influenza and is a frequent cause of nosocomial pneumonia in patients who are debilitated. Finally, *S. aureus* is frequently introduced into food by way of abscesses or the nasal cavity of food handlers. If it is allowed to grow and produces an **enterotoxin**, it can cause **staphylococcal food poisoning**.

In a 1990-1992 National Nosocomial Infections survey, CDC found *S. aureus* to be the **most common cause of nosocomial pneumonia and operative wound infections**, as well as the **second most**

common cause of nosocomial bloodstream infections. Antibiotic resistant *S. aureus* is a common problem. For example, a survey conducted by CDC reported the proportion of methicillin-resistant isolates *S. aureus* (MRSA) with sensitivity only to vancomycin increased from 22.8% in 1987 to 56.2% in 1997.

Virulence factors for *S. aureus* include exotoxins such as leukocidin (kills leukocytes), alpha and delta toxins (damage tissue membranes), microcapsules (resist phagocytic engulfment and destruction), coagulase and protein A (both help resist phagocytic engulfment). Some strains also produce **TSST-1** (toxic shock syndrome toxin-1) and cause **toxic shock syndrome**, usually associated with wounds. Approximately 25% of all *S. aureus* strains are toxigenic and approximately 6000 cases of toxic shock syndrome occur each year in the U.S. Some strains also produce **exfoliatin**, an exotoxin that causes **scalded skin syndrome**, an infection usually seen in infants and young children.

[Concept map for Lab 15 - Organisms and Infections](#)

For further information on virulence factors associated with *S. aureus*, see the following Learning Objects in your Lecture Guide:

- [Teichoic Acids and Glycopeptides Cell Wall Fragments; Unit 3, Section C1c](#)
- [The Ability to Adhere to Host Cells: Pili and Adhesins; Unit 3, Section B2](#)
- [The Ability to Resist Phagocytic Engulfment; Unit 3, Section B5b](#)
- [The Ability to Resist Phagocytic Destruction and Serum Lysis; Unit 3, Section B5c](#)
- [The Ability to Evade Adaptive Immune Defenses; Unit 3, Section B6](#)
- [The Ability to Produce Harmful Superantigens; Unit 3, Section C2a1](#)
- [A-B Toxins; Unit 3, Section C2b](#)
- [Toxins that Damage Cell Membranes; Unit 3, Section C2c](#)

Since most *S. aureus* strains produce the enzyme coagulase (see the coagulase test described below), they are often referred to as **coagulase-positive staphylococci**.

- Scanning electron micrograph of [Staphylococcus aureus forming a biofilm](#) in an indwelling catheter.
- [Diseases and Organisms in Healthcare Settings; from CDC](#)

B. Coagulase-Negative Staphylococci

Clinically common species of staphylococci other than *S. aureus* are often referred to as **coagulase-negative staphylococci**. These staphylococci are normal flora of the skin and, as such, frequently act as **opportunistic pathogens**, especially in the compromised host. *S. saprophyticus* is a relatively common cause of **urinary tract infections**, especially in healthy young women, but is seldom isolated from other sources. The great **majority of infections caused by other coagulase-negative staphylococci**, including *S. epidermidis*, *S. haemolyticus*, and *S. hominis*, are **associated with intravascular devices** (prosthetic heart valves and intra-arterial or intravenous lines) **and shunts**. Also quite common are **infections of prosthetic joints, wound infections, osteomyelitis** associated with foreign bodies, and **endocarditis**.

- Electron micrograph of [Staphylococcus epidermidis colonizing a vascular catheter](#).
- [Diseases and Organisms in Healthcare Settings; from CDC](#)

[Concept map for Lab 15 - Organisms and Infections](#)

Although certain reactions may vary from strain to strain, a series of biochemical tests will usually differentiate the most common clinically encountered species of staphylococci. Today we will use a number of tests to determine if an unknown is *S. aureus*, *S. epidermidis*, or *S. saprophyticus*.

[Medscape](#) articles on infections associated with organisms mentioned in this Lab Exercise. Registration to access this website is free.

- [Staphylococcus infections](#)

- [Staphylococcus aureus](#)

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCI

1. Blood agar with a novobiocin (NB) disc

To isolate staphylococci, clinical specimens are usually grown on Blood agar (described in Lab 14). Staphylococci produce round, raised, opaque colonies 1-2mm in diameter. The novobiocin disc is used to detect sensitivity or resistance to the antibiotic novobiocin.

Test	Staphylococcus aureus, pigmented strain S. aureus, non-pigmented strain	Staphylococcus epidermidis	Staphylococcus saprophyticus
Hemolysis (*)	Usually beta(1)	Usually gamma(2)	Usually gamma(2)
Pigment	Often creamy gold(1)	Usually white(2)	Usually white(2)
Novobiocin test	Sensitive	Sensitive	Resistant

(*) see Lab 14 for descriptions of hemolysis

(1) some strains do not show hemolysis and/or pigment

(2) some strains do show hemolysis and/or pigment

sensitive = zone of inhibition around disc

resistant = no zone of inhibition around disc

2. Gram stain

All staphylococci appear as Gram-positive cocci, usually in irregular, often grape-like clusters ([see Fig. 6](#)).

3. Mannitol fermentation on Mannitol Salt agar (MSA)

Staphylococci are able to tolerate the high salt concentration found in Mannitol Salt agar and thus grow readily. If mannitol is fermented, the acid produced turns the phenol red pH indicator from red (alkaline) to yellow (acid).

Test	Staphylococcus aureus	Staphylococcus epidermidis	Staphylococcus saprophyticus
Mannitol fermentation	Positive	Negative	Usually positive

positive = acid end products turn the phenol red pH indicator from red to yellow

negative = phenol red remains red

4. Production of deoxyribonuclease (DNase) on DNase agar

DNase agar contains 0.2% DNA. To detect DNase production, the plate is inoculated and incubated. After growth, the plate is flooded with 1N hydrochloric acid (HCl). DNase positive cultures show a distinct clear zone around the streak ([see Fig. 1](#)) where the DNA in the agar was broken down by the bacterial DNase. DNase negative cultures appear cloudy around the growth where the acid caused the DNA in the agar to precipitate out of solution.

Test	Staphylococcus aureus	Staphylococcus epidermidis	Staphylococcus saprophyticus
DNase production	Positive	Negative	Negative

positive = clear zone around growth after adding 1N HCl (no DNA remaining in the agar)

negative = cloudy around growth after adding 1N HCl (DNA remains in the agar forming a precipitate)

5. Production of coagulase

The staphylococcal enzyme coagulase will cause inoculated citrated rabbit plasma to gel or coagulate. The

coagulase converts soluble fibrinogen in the plasma into insoluble fibrin.

Test	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus saprophyticus</i>
Coagulase production	Positive	Negative	Negative

positive = plasma will gel or coagulate
negative = plasma will not gel

6. The Staphyloslide® Latex Test for cell-bound coagulase (clumping factor) and/or Protein A

The Staphyloslide® Latex Test is an agglutination test that detects **cell-bound coagulase (clumping factor) and/or Protein A**. Approximately 97% of human strains of *S. aureus* possess both bound coagulase and extracellular coagulase. Approximately 95% of human strains of *S. aureus* possess Protein A on their cell surface ([see Fig. 7](#)). This test uses blue latex particles coated with human fibrinogen and the human antibody IgG. Mixing of the latex reagent with colonies of the suspected *S. aureus* having coagulase and/or Protein A bound to their surface causes agglutination of the latex particles.

Test	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus saprophyticus</i>
Cell-bound coagulase (clumping factor) and/or Protein A	Positive	Negative	Negative

positive = clumping of latex particles
negative = no clumping of latex particles

[Concept map for Lab 15 - Identification](#)

For further information on coagulase and Protein A associated with *S. aureus*, see the following Learning Objects in your Lecture Guide:

- [The Ability to Resist Phagocytic Engulfment; Unit 3, Section B5b](#)
- [The Ability to Evade Adaptive Immune Defenses; Unit 3, Section B6](#)

Staphylococci are also being identified using chemiluminescent labelled DNA probes complementary to species-specific bacterial ribosomal RNA (rRNA) sequences as well as by other direct DNA techniques.

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SCENERIO FOR TODAY'S LAB

Choose **either unknown #1 or unknown #2** as your unknown for this Case Study.

A 57 year old female who is diabetic, a smoker, and who 30 days ago had hip replacement surgery presents with pain and signs of inflammation at the surgical site. Examination shows she has a fever of 101 °F and an increased total white blood cell count with a left shift. Radiologic examination shows a deep pelvic abscess. A culture of the implant site was taken.

Assume that unknown you are given is the culture from this patient.

MATERIALS

1 plate of blood agar, 1 novobiocin (NB) disc, 1 plate of mannitol salt agar, 1 DNase agar plate, 1 tube of citrated rabbit plasma (coagulase test), materials to perform a Gram stain, inoculating loop

PROCEDURE (to be done in groups of 3)

[Keep in mind that in a real clinical situation other lab tests and cultures for bacteria other than those upon which this lab is based would also be done.]

CAUTION: TREAT EACH UNKNOWN AS A PATHOGEN! Inform your instructor of any spills or accidents. **WASH AND SANITIZE YOUR HANDS WELL** before leaving the lab.

1. Do a **Gram stain** on the unknown (see Lab 6). Make sure you **review the instructions before you do the Gram stain**.

[Instructions for the Gram Stain from Lab 6.](#)

[Instructions for Focusing a Microscope from Lab 1.](#)

2. Using your loop, streak your unknown **for isolation** on a plate of **Blood agar** as described below.

a. Using a sterile inoculating loop, streak your unknown for isolation on a blood agar plate so as to get **single, isolated colonies** ([Fig. 2, step 1](#), [Fig. 2, step 2](#), and [Fig. 2, step 3](#)). Before you streak your plate **draw an "X" on the bottom of the blood agar plate to indicate where you begin the streaking pattern**.

[Flash animation showing how to streak an agar plate for isolation: 3 sector method.](#)

[html5 version of animation for iPad showing how to streak an agar plate for isolation: 3 sector method.](#)

b. Using your inoculating loop, **stab** the agar several times in each of the growth areas in order to detect oxygen-sensitive hemolysins ([Fig. 2, step 4](#)).

c. Place a **novobiocin antibiotic disc in the center of the area of the plate that you streaked first (the area where you drew the "X")** where you expect to see heaviest growth ([Fig. 2, step 5](#)).

d. Incubate **upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

3. Streak your unknown **for isolation** on a plate of **Mannitol Salt agar (MSA)** as shown in [Fig. 3](#). Incubate **upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

[Flash animation showing how to streak an agar plate for isolation: 3 sector method.](#)

[html5 version of animation for iPad showing how to streak an agar plate for isolation: 3 sector method.](#)

4. Streak a **single line** of your unknown down the center of a plate of **DNase agar** as shown in [Fig. 4](#). Incubate **upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

5. Inoculate a tube of **citrated rabbit plasma** with your unknown and incubate your test tube rack at **37°C**.

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RESULTS

Case Study Lab Report for Lab 15: Staphylococci

The concept behind the case studies presented in Lab 15 used to illustrate the genus *Staphylococcus* is for you and your lab partners as a group to:

1. **Come up with a valid diagnosis of the type of infectious disease seen in your case study and identify the bacterium causing that infection.**

2. **Support your group's diagnose** based on:

- a. Any relevant facts in the **patient's history**. (A reliable on-line source will be used to support this.)
- b. The patient's **signs and symptoms**. (A reliable on-line source will be used to support this.)
- c. **Each of the individual lab tests** given in your case study.
- d. All **microbiological lab tests you performed** as part of the project.

The due date for this report can be found on the class calendar. Remember, you are **working as a group to solve a problem**. Your grade for this lab is based on the **completeness of your report and written evidence of the critical thinking process that went into making and supporting your diagnosis**, therefore, it is critical that all members of the group participate, question any conclusions being made by the group, and contribute to the report. Remember, you are trying to convince your instructor that you understand how the diagnosis was made by **supporting that diagnosis with data**. Your group will work together to write the report and **submit one hard copy of that report for your group**. Part of your grade will be based on evaluation of your work by your team members.

Be sure to handle all the bacterial cultures you are using in lab today as if they are pathogens! Be sure to wash and sanitize your hands well at the completion of today's lab.

Also, make sure you **observe the results of someone in your lab who had an unknown different from yours**. The Performance Objectives for Lab 15 tell you what you are expected to be able to do on the practical.

[Rubric used for grading your Case Study Lab Report.](#)

[Team Member Evaluation Form used for grading your Lab 15 Lab Report.](#)

Each member of the group must:

- 1. Print a copy of each of the two rubrics from the links above.***
- 2. Print and fill out a copy of the Team Member Evaluation Form from the link above.***
- 3. Staple them together and hand them in to me the day your Lab 15 Case Study Lab Report is due.***

For more information on writing your Lab Report, see [Course evaluation for Lab \(Core labs, case studies, lab quizzes\)](#) under Course Info in the menu of the BIOL 230 website.

[Click here to print a PDF copy of this lab report.](#)

Your Name:

Others in your group:

Lab section:

Date:

A. Case Study from Lab 15: Unknown #1

Each member of the group must:

- 1. Print a copy of each of the two rubrics from the links above.***
- 2. Print and fill out a copy of the Team Member Evaluation Form from the link above.***
- 3. Staple them together and hand them in to me the day your Lab 15 Case Study Lab Report is due.***

A 57 year old female who is diabetic, a smoker, and who 30 days ago had hip replacement surgery presents with

pain and signs of inflammation at the surgical site. Examination shows she has a fever of 101 °F and an increased total white blood cell count with a left shift. Radiologic examination shows a deep pelvic abscess. A culture of the implant site was taken.

1. Patient's history and predisposing factors

Read the case study. Explain how any relevant parts of the patient's history contributed to your diagnosis as to the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's History section in APA style (<http://www.apastyle.org/>).

The **patient's history should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **Do not look up the bacterium you eventually identify** as the cause of this infectious disease. You don't know the causative bacterium at this point. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's History and Patient's Symptoms sections** of this Lab Report.

2. Patient's signs and symptoms

Read the case study. Explain how the patient's signs and symptoms contributed to your diagnosis as to the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's Symptoms section in APA style (<http://www.apastyle.org/>).

The **patient's signs and symptoms should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **Do not look up the bacterium you eventually identify** as the cause of this infectious disease. You don't know the causative bacterium at this point. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's History and Patient's Symptoms sections** of this Lab Report.

3. Results of laboratory test given in the case study

List each lab test given and explain how the results of that test helps to contribute to your diagnosis. The CBC test is described in [Appendix C](#) of this lab manual.

4. Microbiological lab tests you performed in Lab 15

a. Gram stain

Give the Gram reaction (Gram-positive or Gram negative and how you reached this conclusion) and the shape and arrangement of the unknown you were given. **State how this contributed to your decision as to which microbiological tests and/or media to use next.** The Gram stain is discussed in [Lab 6](#).

b. Blood agar with novobiocin (NB) disc

Give the results of the Blood agar with Taxo NB disc you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for Blood agar and NB disc were discussed in the beginning pages of this lab.

c. Mannitol Salt agar

Give the results of the Mannitol Salt agar you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for Mannitol Salt agar were discussed in the beginning pages of this lab.

d. DNase agar

Flood the surface of your **DNase agar** plate with **1N HCl**. Give the results of the DNase agar you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for DNase agar were discussed in the beginning pages of this lab.

e. Coagulase test

Give the results of the Coagulase test you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for the Coagulase test were discussed in the beginning pages of this lab.

Final Diagnosis

Genus and species of unknown #1 = _____

Infection: _____

B. Case Study from Lab 15: Unknown #2

Each member of the group must:

- 1. Print a copy of each of the two rubrics from the links above.***
- 2. Print and fill out a copy of the Team Member Evaluation Form from the link above.***
- 3. Staple them together and hand them in to me the day your Lab 15 Case Study Lab Report is due.***

A 57 year old female who is diabetic, a smoker, and who 30 days ago had hip replacement surgery presents with pain and signs of inflammation at the surgical site. Examination shows she has a fever of 101 °F and an increased total white blood cell count with a left shift. Radiologic examination shows a deep pelvic abscess. A culture of the implant site was taken.

1. Patient's history and predisposing factors

Read the case study. Explain how any relevant parts of the patient's history contributed to your diagnosis as to the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's History section in APA style (<http://www.apastyle.org/>).

The **patient's history should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **Do not look up the bacterium you eventually identify** as the cause of this infectious disease. You don't know the causative bacterium at this point. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's History and Patient's Symptoms sections** of this Lab Report.

2. Patient's signs and symptoms

Read the case study. Explain how the patient's symptoms contributed to your diagnosis as to the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's Symptoms section in APA style (<http://www.apastyle.org/>).

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c. Mannitol Salt agar

Give the results of the Mannitol Salt agar you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for Mannitol Salt agar were discussed in the beginning pages of this lab.

d. DNase agar

Flood the surface of your **DNase agar** plate with **1N HCl**. Give the results of the DNase agar you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for DNase agar were discussed in the beginning pages of this lab.

e. Coagulase test

Give the results of the Coagulase test you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your your decision as to what bacterium is causing the infection.** The possible results for the Coagulase test were discussed in the beginning pages of this lab.

Final Diagnosis

Genus and species of unknown #2 = _____

Infection: _____

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PERFORMANCE OBJECTIVES FOR LAB 15

After completing this lab, the student will be able to perform the following objectives:

DISCUSSION

1. Name three common clinically important species of *Staphylococcus* and state which species is most pathogenic.
2. State the sources and the portal of entry for most *Staphylococcus aureus* infections.
3. Name and describe three types of abscesses caused by *Staphylococcus aureus*.
4. Name four systemic *Staphylococcus aureus* infections.
5. State the significance of *Staphylococcus aureus* enterotoxin, the exotoxin TSST-1, and the exotoxin exfoliatin.
6. Name the infection normally caused by *Staphylococcus saprophyticus*.
7. Name the types of infections most commonly caused by coagulase-negative staphylococci other than *Staphylococcus saprophyticus*.

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCI

1. State the Gram reaction and morphology of all staphylococci.
2. Describe the typical reactions of *S. aureus*, *S. epidermidis*, and *S. saprophyticus* on each of the following media:
 - a. Blood agar (pigment, hemolysis, novobiocin resistance)
 - b. Mannitol Salt agar (for mannitol fermentation)
 - c. DNase agar (for the enzyme DNase)
 - d. coagulase test with citrated rabbit plasma
 - e. Staphyloslide® test for bound coagulase and/or Protein A

RESULTS

1. Recognize staphylococci in a Gram stain preparation.
2. Recognize an organism as *Staphylococcus aureus* and state the reasons why after seeing the results of the following:
 - a. a Blood agar plate with a novobiocin disc
 - b. a Mannitol Salt agar plate
 - c. a DNase agar plate
 - d. a tube of citrated rabbit plasma
 - e. a Staphyloslide® test

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SELF-QUIZ

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