

Feel the Burn:

Biochemical Testing and the Integumentary System

by

Holly A. Basta, Department of Biology, Rocky Mountain College, Billings, MT

Sheela Vemu, Department of Science and Mathematics, Waubonsee Community College, Sugar Grove, IL



Burned

Alison, a 22-year-old female, was admitted to the emergency room with a burn from a campfire accident on her foot (Figure 1). She insisted that it was not very painful and had therefore waited several days before coming to the hospital.



Figure 1. Ankle burn upon patient admission to the hospital.

1. What degree of burn is this?
2. What layers of skin have been damaged?
3. Why was it not very painful?
4. What treatment might the doctor recommend?

Graft

The doctor recommended Alison receive a skin graft. Although the burn was severe, it was relatively small, so the graft was taken from another location on Alison's body (her leg) and transplanted to her ankle.

5. Would this be considered an "autograft," an "allograft," or a "xenograft"?

6. List one benefit and one drawback to this type of graft.

Inflammation

The graft was successful and Alison was sent home. After a few days, the area around the graft became red and inflamed. Alison otherwise felt fine, so she did not return to the hospital. After a couple more days, the inflammation had spread and her entire foot was red, hot and painful. She began to feel feverish and returned to the ER.

7. Why might inflammation of the grafted area occur?
8. What tests might be performed to determine if the cause of the inflammation is a bacterium?
9. What would the first line of treatment be?

Bacterium

Alison received her lab report (see Diagnostic Laboratory 1).

10. Which bacterium do you think is responsible for the inflammation? What lab results support this conclusion?

Antibiotics

The doctor prescribed IV antibiotics, specifically methicillin, a common antibiotic to treat *Staphylococcus* infections. After 24 hours on antibiotics, Alison's infection had not improved; in fact, the inflammation and fever had worsened. The infection was found to have spread to her blood stream, causing bacteremia.

11. What factors likely caused Alison to be at increased susceptibility to this infection?
12. Are antibiotics appropriate to prescribe for bacterial infections?
13. Form a hypothesis addressing why the antibiotics were not immediately effective.

Abscess

As the bacteremia grew worse, Alison's graft began to develop an abscess below it, which was filled with pus. The doctor ordered tests for antibiotic resistance (see Diagnostic Laboratory 2 and the MRSA information sheet).

MRSA

In addition to PML necrosis, MRSA can cause more systemic illness. Most strains of MRSA encode enterotoxins, which can cause gastroenteric and toxic shock syndromes. MRSA infections are common in hospitals, prisons, military barracks and child care facilities.

14. What do you think the cause of the pus is?
15. Describe three reasons why MRSA is so dangerous.
16. Draw a picture of what is happening molecularly with the latex agglutination test.

17. Why, of all the proteins, does the latex agglutination test assess presence of PBP2a?
18. Where do you think Alison acquired this infection? What is an infection acquired in this manner called?
19. Describe three reasons why MRSA is so difficult to treat.

Extreme Measures

Alison's doctor first ordered complete sanitation of Alison's room and enforced strict handwashing rules with all medical staff. She also instructed Alison and her parents that they needed to be very diligent with their handwashing. Next, the doctor drained the pus from the abscess. The doctor then bathed the area with chlorhexidine, a collagenase and mupirocin. Topical vancomycin was then applied and the wound was wrapped. Alison was worried about disrupting the graft, but her doctor asserted that IV medication was not very effective on burns.

Treatment

20. Why are extreme measures taken to rid the hospital of MRSA?
21. Why do you think IV medication is not very effective at burn sites?
22. Why does chlorhexidine not damage Alison's tissue?
23. Why would the collagen protein be targeted to break down necrotic tissue?
24. Why do you think vancomycin is effective on most strains of MRSA when nearly no other antibiotics are?

Healing

As Alison's inflammation subsided, she slowly began to heal. Her doctor told her she was very lucky, as most graft patients infected with MRSA lose their graft. She told Alison that she expected a full recovery with some minor scarring.

25. Draw a concept map of integument repair using the following terms:
 - a. Mast cells
 - b. Collagen fibers
 - c. Macrophage
 - d. Fibroblasts
 - e. Inflammatory response
 - f. Scab
 - g. Scar tissue
 - h. Phagocytosis
26. Based on your knowledge of MRSA and the immune system, why might the healing process proceed slower than usual in Alison's case?

DIAGNOSTIC LABORATORY 1

Patient: Alison

DOB: 01/02/1995

SEX: F

****FINAL REPORT****

GRAM STAIN: gram-positive cocci

MSA PLATES: yellow colonies

CATALASE TEST: positive

COAGULASE TEST: positive

DESCRIPTION OF TESTS

GRAM STAIN: Gram staining is a technique of staining bacteria that allows visualization of morphology (cocci, rods, etc.) and characterization of the cell wall. Gram-positive bacteria have a thick layer of peptidoglycan that absorbs ample crystal violet dye and therefore appear purple (Figure 2). Gram-negative bacteria have a very thin peptidoglycan layer that is dissolved in the ethanol wash step and therefore do not absorb crystal violet. They do, however, absorb the counter stain (safranin), which makes them appear pink.

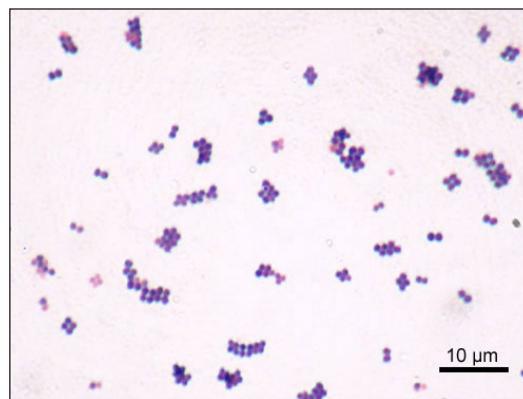


Figure 2. Gram stain showing gram-positive cocci under the microscope. Credit: Y Tambe, CC BY-SA 3.0, <<https://commons.wikimedia.org/w/index.php?curid=49534>>.

MANNITOL SALT AGAR (MSA) PLATES: MSA plates are growth plates that select for gram-positive bacteria and differentiate between mannitol fermenters and non-mannitol fermenters. If the bacteria ferment mannitol, the media to become acidic and the phenol red indicator turns yellow. (Figure 3)

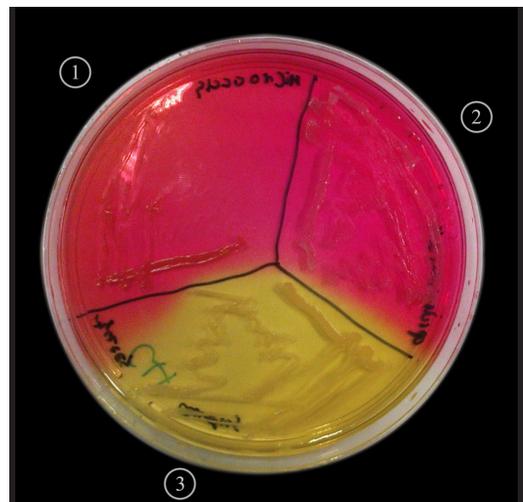


Figure 3. MSA plate with positive result (bottom section). Credit: Navaho, CC BY-SA 4.0, <<https://commons.wikimedia.org/wiki/File:Chapmanes.jpg>>.

DIAGNOSTIC LABORATORY 1 (cont.)

COAGULASE: Coagulase is an enzyme produced by certain bacteria that allows them to convert fibrinogen to fibrin (causing blood coagulation). To test for the presence of coagulase activity, a bacterial colony is added to a tube containing plasma, inoculated, and assessed for a fibrin clot. Presence of a clot indicates the bacterium is positive for coagulase. (Figure 4)



Figure 4. Positive coagulase test. Credit: Philippinjl, CC BY-SA 3.0, <<https://commons.wikimedia.org/w/index.php?curid=1968795>>.

CATALASE: Catalase is an enzyme used by bacteria to protect from oxidative damage caused by reactive oxygen species. If a bacterium produces catalase, when that bacterium is added to hydrogen peroxide on a microscope slide, bubbles form. (Figure 5)



Figure 5. Positive catalase test. Credit: CC BY-SA 3.0, <<https://commons.wikimedia.org/w/index.php?curid=1686190>>.

<i>Bacterium</i>	<i>Morphology</i>	<i>Mannitol</i>	<i>Coagulase</i>	<i>Catalase</i>
<i>Staphylococcus epidermidis</i>	Gram + cocci	Negative	Negative	Positive
<i>Staphylococcus aureus</i>	Gram + cocci	Positive	Positive	Positive
<i>Escherichia coli</i>	Gram – rod	Positive or Negative	Negative	Positive
<i>Bacillus cereus</i>	Gram + rod	Negative	Negative	Positive

DIAGNOSTIC LABORATORY 2

Patient: Alison

DOB: 01/02/1995

SEX: F

****FINAL REPORT****

LATEX AGGLUTINATION TEST: positive

DISC DIFFUSION TESTS:

<i>Antibiotic</i>	<i>Zone of inhibition diameter (mm)</i>	<i>Interpretation</i>
Methicillin	5	Resistant
Erythromycin	9	Resistant
Cephalexin	2	Resistant
Ciprofloxacin	10	Resistant
Gentamicin	4	Resistant
Vancomycin	22	Susceptible

DESCRIPTION OF TESTS

LATEX AGGLUTINATION TEST: The latex agglutination test is a rapid and specific test for methicillin resistant *Staphylococcus aureus* (MRSA). Rapid tests for MRSA rely on microscopic balls of latex coated with antibodies that specifically bind to the PB2A protein of MRSA. When mixed with a suspension containing MRSA, binding of the bacteria (via PB2A interaction with the antibody) cause the latex molecules to stick together and fall out of solution (similar to a blood typing test). This is called agglutination and can happen within our bodies as part of the immune response to infection.

DISC DIFFUSION TEST: The disc diffusion test is used to assess the antibiotic sensitivity of bacteria. Discs of paper infused with antibiotics are placed on an agar plate streaked with bacteria. Based on the size of the zone around the disc where bacterial growth is inhibited, one can determine whether a bacterium is susceptible or resistant to a particular antibiotic.

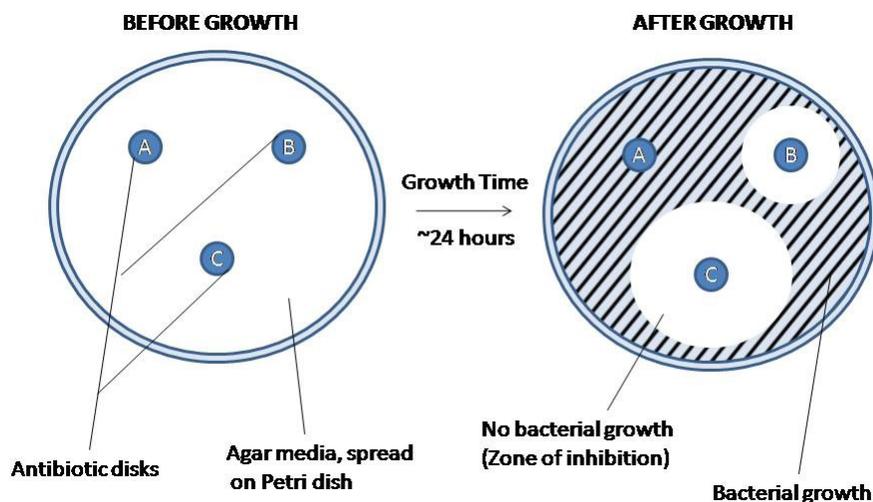


Figure 6. Disc diffusion method of antibiotic sensitivity testing. Credit: Sommer36. CC BY-SA 4.0, <https://commons.wikimedia.org/wiki/File:Agar_Diffusion_Method_1.jpg>.

Methicillin Resistant *Staphylococcus Aureus* (MRSA)

S. aureus is a ubiquitous bacterium and is frequently found in the nose, on the skin and in the respiratory tract. It does not usually cause disease, but can cause infections under certain circumstances. *S. aureus* is therefore considered an opportunistic infection.

In the rare case that *S. aureus* causes disease, the bacterium may travel to the heart, lungs or bone, causing respectively, endocarditis, pneumonia, or osteomyelitis. Staphylococcal infections can also lead to sepsis, a rapid progressing condition which leads to tissue damage, organ failure, and death in just a few hours. Rapid diagnosis is therefore not just convenient but perhaps lifesaving.

S. aureus has a troubling propensity to become resistant to many of the antibiotics commonly used to treat it. The acronym MRSA stands for methicillin-resistant *Staphylococcus aureus* and refers to a strain of the species that is resistant to methicillin, the most robust of all the antibiotic drugs used to treat such infections. In fact, MRSA is resistant to most antibiotics related to penicillin, called beta-lactam antibiotics. This family of antibiotics works by inactivating bacterial penicillin binding proteins (PBPs). MRSA encodes a PBP, PBP2a, which has decreased affinity for beta-lactam antibiotics, allowing MRSA to be resistant. Furthermore, MRSA tends to colonize polymononuclear leukocytes (PMNs), a class of white blood cells that includes neutrophils, eosinophils, and basophils. In burn patients, who tend to be immunocompromised, PMNs have decreased bactericidal function, which allows MRSA to survive longer than normal.* PMNs eventually undergo necrosis (a type of cell death). The infection also recruits phagocytic cells. As the infection continues, a fibrin capsule forms around the infected region, isolating the infected area. This fibrin capsule helps prevent spread of the bacteria, but also protects them from most treatments.

In addition to PML necrosis, MRSA can cause more systemic illness. Most strains of MRSA encode enterotoxins, which can cause gastroenteric and toxic shock syndromes. MRSA infections are common in hospitals, prisons, military barracks and child care facilities. Infections that are acquired from a hospital are called nosocomial infections.

Recommended Treatments

- *Chlorhexidine*: a cation that binds and disrupts bacterial cell walls.
- *Collagenase*: an enzyme to help break down necrotic tissue.
- *Mupirocin*: a chemical that inhibits bacterial protein synthesis.

* Steer, A.J., G.B. Hill, and A.P.R. Wilson. 1995. The effect of burn wound surgery and teicoplanin on the bactericidal activity of polymorphonuclear leucocytes against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 36(5): 851–5. <<https://doi.org/10.1093/jac/36.5.851>>.