Part I – Infections

On the first day back to class at the local university, Brent noticed a small abscess on his right elbow but didn’t think much about it. Over the next couple days, however, the abscess became more swollen and painful.

“Gross, this sore is looking nasty, and it hurts too,” thought Brent.

Brent decided to visit the school’s health center to get it checked out, and hopefully get rid of it.

“It looks like a nasty spider bite, but I can’t recall getting any bites and haven’t seen any spiders at home,” he told the doctor.

“I do see lots of cases of spider bites,” replied the doctor. “I’m just going to take a swab of this lesion to check it out. Here’s a prescription for an antibiotic cream. It looks infected. The cream should take care of that.”

Another student, Kristen, had to have “routine” surgery on her right knee because of previous sports injuries. Everything about the surgery seemed to go smoothly, but just one week after the surgery Kristen wasn’t feeling well.

“I have a fever and my knee hurts,” Kristen told her mom on the phone. “And it’s all red around the incision.”

“Why don’t we get you back to your doctor to ask him about it,” Kristen’s mom replied.

After visiting the doctor, Kristen was frustrated. “Why can’t they be more careful? I could die with this infection because they didn’t clean their equipment,” she told her mother.

“Well the antibiotics should take care of it, and they should identify what the infection is soon,” Kristen’s mother responded.

Both Brent and Kristen were prescribed the drug oxacillin. Within a few days Brent’s lesion was gone, but Kristen’s symptoms didn’t improve. She continued to have a high fever with increased lethargy; the antibiotics apparently weren’t working for Kristen.

Questions

1. Record similarities and differences between the two patients.

2. What ideas do you have as to why the antibiotic did not work for Kristen?

3. What, if any, diagnoses could you make for Brent and Kristen?
Part II – Identifying the Pathogen

A sample obtained from both Brent and Kristen was cultured using trypticase soy agar with 5% sheep red blood in the local hospital lab. The lab microbiologist observed yellow colonies characteristic of the common pathogen *Staphylococcus aureus*. It was also concluded that both cultures were coagulase positive, another characteristic of *S. aureus*.

“**So, I just had a nasty Staph infection?”** Brent asked his doctor.

“Yes, and I’m glad the antibiotic took care of it,” said the doctor.

“Me too,” said Brent.

“**They think I just have a Staph infection,”** Kristen told her mom.

“Yes, but why didn’t your antibiotics get rid of it?” asked Kristen’s mom.

“I don’t know. I just hope it doesn’t get any worse,” said Kristen.

Questions

1. What do you already know about *S. aureus*? (See note #1 on the Case Notes Handout.)
2. What additional info about *S. aureus* can you find? (Use the Internet or your textbooks).
3. List hypotheses that would explain why *S. aureus* was susceptible to antibiotics in Brent’s case but not in Kristen’s case.
Part III – DNA Analysis

To learn more about this pathogen and why it behaved differently in the two patients, David, the staff microbiologist, decided that analyzing the DNA from each bacterial culture would provide some answers. David had seen a lot of Staph infections that were actually caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (see note #2 in the handout) and now he wanted to know if Kristen and Brent had become infected with MRSA. MRSA is unique in that it contains one gene that provides the bacterium with resistance to several antibiotics. David decided to search for this gene in each bacterial strain using genetic tools.

- First, DNA was isolated from each bacterial strain—the sample from Brent and the sample from Kristen.
- Second, since only a small amount of DNA was obtained, PCR (see note #3 in the handout) was utilized to create about a million copies of a unique segment of the DNA. This segment of DNA that was copied is unique to MRSA. *S. aureus* cells that do not contain this segment or gene are not antibiotic resistant. In MRSA, this gene codes for a protein that provides antibiotic resistance to all bacterial cells that contain this gene in their DNA.
- Third, the copied DNA segment was sequenced. Having a DNA sequence allows for a comparison between the copied DNA sequence to other DNA sequences contained in a database that is available at the National Center for Biotechnology Information (NCBI) website. Matching DNA sequences by using a BLAST search (see note #4 in the handout) helps to identify the presence of the MRSA antibiotic resistance gene and allows further insight as to why *S. aureus* behaved differently in Brent and Kristen.

David obtained the results recorded in Table 1. You now need to analyze them. Your job is to confirm the presence or absence of the antibiotic resistance gene, and to further explore why the two pathogens behaved differently.

Table 1. Results of DNA Analysis

<table>
<thead>
<tr>
<th>Sequence for Brent’s pathogen:</th>
<th>No sequence obtained.</th>
</tr>
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| Sequence for Kristen’s pathogen: | tttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt...
bases that are most similar to the sequence you entered in the BLAST search. A description of each sequence is given along with an accession number, which is a way to keep track of all the sequences in the database. The percentage and scores listed refer to the similarity of your sequence compared to the sequence in the database.

8. Specifically, the gene that identifies \textit{S. aureus} as MRSA is the \textit{mecA} gene. Scroll till you find \textit{mecA} and, if it has 100\% similarity, then it is definitely the gene we’re looking for. The others are not exactly called the \textit{mecA}, but are referring to it as SCCmec or type I to type IV SCCmec. \textit{mecA} is the specific name of the gene.

9. Click on the accession number of the first line you see containing \textit{mecA}. You will see a page that gives a full description of the DNA sequence, including the names of the researchers who submitted this sequence to the database, the name of the protein encoded by this sequence and other details about the sequence. On the right of the page, there are links to related information about the protein that this gene codes for.

\textbf{Questions}

1. Because Brent’s pathogen was not MRSA, it did not contain the \textit{mecA} gene. Explain why PCR and DNA sequencing provided no results for Brent’s pathogen.

2. Does the presence of the \textit{mecA} gene confirm that Kristen is infected with MRSA? Why?

3. What protein does the \textit{mecA} gene encode for? How does this allow MRSA to be antibiotic resistant?

4. What are some benefits of having a national database for nucleotide sequences and what could you use this site for in the future?
Part IV – Exploring Bacterial Resistance

Due to the presence of the mecA gene, the microbiologist, David, confirmed that Kristen was infected with MRSA. Brent was infected with S. aureus that did not contain the mecA gene, making it susceptible to a topical antibiotic. Luckily, several other antibiotics do exist for MRSA that were used to allow Kristen to fully recover.

Kristen was thankful that she recovered, but being a bit curious she wants to know why there are these “super bugs” that defy treatment, and why she had to get infected with one.

What is antibiotic resistance? Explore antibiotic resistance by visiting the following sites:

- http://www.cdc.gov/drugresistance/

Questions

1. What artificial or selective pressures could influence prevalence of antibiotic resistant microbes?
2. How could MRSA or other “super bugs” become antibiotic resistant?
3. Read note #5 in your handout about horizontal gene transfer. Discuss how Staphylococcus aureus could have acquired the mecA gene.
4. Is MRSA the result of a fundamentally different process than evolution? Explain.
5. What can be done to prevent MRSA from becoming more dangerous?

To help Kristen understand antibiotic resistance and specifically MRSA, write a one-page, double-spaced letter to Kristen explaining what MRSA is, why it is common today, and what can be done about it.

References


Case Notes Handout

**Note #1: Staphylococcus aureus**

*Staphylococcus aureus* is a bacterium that is commonly found on the skin of humans. On the surface of the skin it is harmless to the host and may even provide protection against other foreign pathogens. The danger with this organism is that it is a pathogen that can cause disease when it enters the body. It typically enters a host through a break in the skin and may further result in symptoms of pus along with redness and swelling of the surrounding skin. If the bacterium enters the bloodstream, it may cause pneumonia, fever, chills, and malaise. In serious causes, it can also lead to death (Lowy 1998).

**Note #2: MRSA**

Today, a more serious form of *S. aureus* called methicillin resistant *Staphylococcus aureus* (MRSA) abounds that is resistant to many antibiotics, specifically oxacillin in this case, along with several others. MRSA is different from *S. aureus* in that it contains what is known as a *meca* gene in its genome. This gene provides MRSA the ability to be resistant to many antibiotics that are used today. MRSA may be created when *S. aureus* acquires the *meca* gene by horizontal gene transfer from other bacterial cells. Today, there are several types of MRSA, including hospital associated (HA) MRSA strains and community associated (CA) MRSA strains (Noto & Archer 2006).

**Note #3: PCR**

Polymerase chain reaction (PCR) is a technique used to increase the number of copies of a segment of DNA or gene coding region of DNA. Through the use of enzymes, a specific DNA segment is copied once to produce two copies, which are both in turn copied to produce four copies and so forth, resulting in the original DNA section being copied exponentially to produce up to a million copies. PCR will only amplify a section of DNA that the researcher wants copied. If that unique sequence is not found in the original DNA, then nothing will be copied. PCR has many applications, but for this situation it provides multiple copies of a fragment of the bacterial DNA of interest to be used for DNA sequencing. Having more copies of a DNA fragment to use in DNA sequencing can provide more accurate sequencing results (Micklos & Freyer 2003).

**Note #4: BLAST**

BLAST (Basic Local Alignment Search Tool) is a set of tools for comparing sequence information in proteins or DNA by using all available public sequence databases. It is designed to find matches in sequence information by looking for close matches of a small portion of DNA or protein within a larger whole sequence (McGinnis & Madden 2004).

**Note #5: HGT (Horizontal Gene Transfer)**

Natural selection can drive the phenotypes and genotypes present in a species population over generations. Environmental forces may allow only individuals with a certain characteristic to survive. Bacteria have a way of passing DNA to one another in a process known as horizontal gene transfer (HGT). HGT can occur through a process called conjugation in which one bacterial cell directly passes DNA to a recipient cell. In the case of MRSA, one MRSA cell may contain a gene that protects it from antibiotics. This cell may then conjugate with a recipient cell that does not have the antibiotic resistant gene and pass a copy of that gene to the recipient cell, resulting in another antibiotic resistant cell (Amabile-Cuevas & Chicurel 1993). HGT allows existing *S. aureus* cells to gain DNA that allows them to become MRSA or antibiotic resistant cells.

References


“Just a Spider Bite?” by Wayne O. Hatch