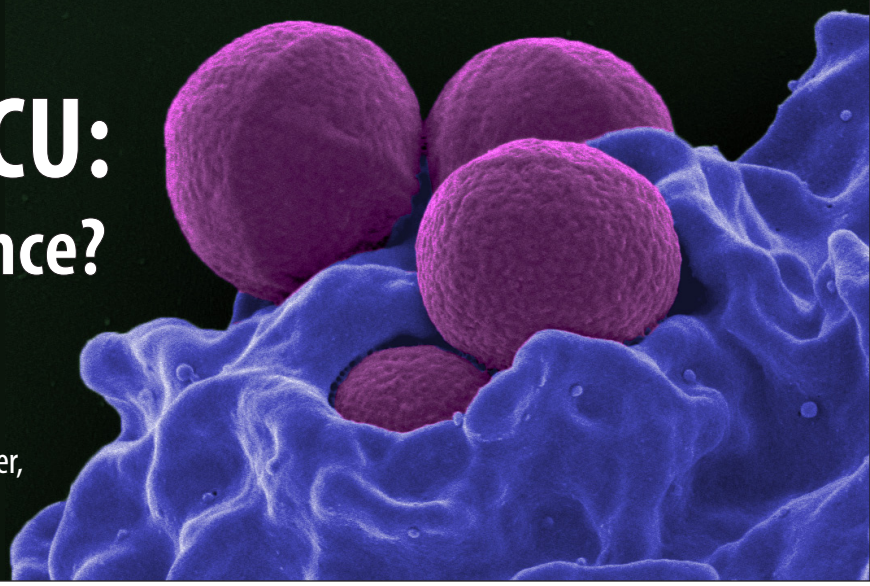
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

# MRSA in the NICU:
## Outbreak or Coincidence?



*by*
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## Part I – A Cluster of Disease Cases

Since the introduction of penicillin, the first antibiotic to be discovered, deaths resulting from bacterial infectious diseases have dramatically decreased, thanks in part to the use of antibiotics. However, the widespread use of antibiotics has had consequences: some bacteria are becoming resistant to these drugs. When these antibiotic-resistant bacteria cause infections, the infections can be challenging to treat, particularly if the bacteria are resistant to several different antibiotics. If the bacteria spread, the result can be an outbreak—an unusually elevated number of cases. In such instances, the source of the outbreak and the mechanism of its spread need to be identified in order to halt it in its tracks.

In this activity, you will explore the use of DNA sequencing technology in characterizing an outbreak that has been "ripped from the headlines." A hospital detected several cases of disease caused by antibiotic-resistant bacteria in a neonatal intensive care unit (NICU). In the course of a few months, over a dozen patients showed symptoms of infection (fever, redness and painful swollen areas on skin). Not all of them were in the NICU. To identify which patients were part of the same outbreak (that is, were infected by the same strain of bacteria), and to determine how the bacteria may be spreading in the hospital, researchers sequenced samples of bacterial DNA obtained from different patients and compared them to each other. You will be using data from the actual outbreak (Köser *et al.*, 2012) to track the outbreak yourself.

### *Background Information*

Please read the following information and answer the questions at the end before our in-class investigation.

*What exactly are antibiotics?*

Antibiotics are drugs that specifically target and kill bacteria, which they can do in a variety of ways. Because the structure of human cells is different than the structure of bacterial cells, antibiotics are able to interfere with bacterial cell structures and functions without harming human cells.

*Why are bacteria becoming increasingly resistant to antibiotics?*

The theory of evolution by natural selection states that individuals with the highest "fitness" will pass on more of their specific versions of genes (alleles) to the next generation. Such individuals are well-adapted to their environment, have more surviving offspring than other individuals, and therefore pass on more of their alleles to future generations. Because antibiotic resistance is associated with bacterial fitness in an environment with antibiotics, a

bacterial cell with a genetic mutation conferring resistance is more likely to survive, divide, and pass on the gene(s) that confers this trait. This means that antibiotic-resistant bacteria can reproduce more than other bacteria when challenged with the antibiotic, and thus become more and more common in a population growing in the presence of the antibiotic. Eventually, a bacterium can collect resistance to several antibiotics, making it "multi-drug resistant." A strain, for the purposes of this assignment, is a single isolation in pure culture—that is, it is made from several generations of pure culture (homogeneous as far as speciation) from a sample that's been isolated.

*What are the implications?*

Antibiotic resistant infections of hospitalized patients are now an emerging threat to public health, and such incidents are occurring more often than ever before. This results in lengthier hospital stays and higher mortality rates. For example, nosocomial infections are often found in places where antibiotic use is common, such as hospitals and nursing homes, making it increasingly likely that people in these environments will acquire an infection that can't easily be treated with antibiotics. For example, a patient may be admitted to a hospital for a simple surgery, but ultimately may have to extend his or her stay as the result of an antibiotic-resistant bacterial infection acquired during a hospital stay as a result of infection at the surgical site.

*What is this MRSA I've been hearing about?*

*Staphylococcus aureus* ("staph" for short) is a species of bacteria. Staph can cause diseases ranging from skin infections, such as boils, to serious respiratory infections and even potentially to fatal bloodstream infections (sepsis). It can also be found on the skin and in nasal passages of apparently healthy individuals. Such people are said to be carriers of staph.

MRSA (pronounced "mersa") is an acronym for methicillin-resistant *S. aureus*. MRSA strains are all resistant to the antibiotic methicillin but are also resistant to other antibiotics. In fact, MRSA can refer to any of several strains of *S. aureus* with multi-resistance to penicillins (including methicillin) and cephalosporins (Fitzgerald *et al.*, 2001). These two classes fall under the mechanism of β-lactam antibiotics, where β-lactam—a common ingredient in bacterial cell walls—is interrupted during bacterial synthesis. Because human cells do not have cell walls, methicillin doesn't damage the cells of a patient being treated with methicillin or a related antibiotic.

Bacteria can transmit the antibiotic resistance genes to their offspring during reproduction and to other, unrelated bacteria by transferring small DNA molecules called plasmids, many of which carry the antibiotic resistance genes. The latter is called horizontal gene transfer.

*What can current sequencing technology do to give us information about outbreaks as they happen? Does it have any shortcomings?*

DNA sequencing gives epidemiologists (scientists who study infectious disease and how it spreads) a powerful tool. It allows them to create what is essentially a family tree of the pathogen (or, as we will come to know it, a phylogenetic tree). Every time DNA is copied and passed down to another cell, little mistakes are made in the sequence. Over time these little mistakes add up. This means that the more time has passed, the more differences will be found in the DNA of two related organisms. Scientists can compare the number of differences between individuals and decide how closely related bacterial strains are (e.g., from two infected patients), and determine whether the patients were likely to have acquired their infections from the same or different sources. This strategy can be used to follow the spread of an infection during an outbreak, and to determine whether patients with the same disease are part of the same outbreak or just happen to be infected at the same time by unrelated strains.

*How do you "catch" MRSA?*

Most of the population has a relatively functioning immune system and need not worry about MRSA infection. MRSA is acquired when a person comes in contact with the bacteria. Touching the skin of an infected person or even just touching something they have used can be enough to infect you, particularly if you have minuscule breaks in your skin. Similarly, when health care professionals treat patients with MRSA, they can transfer the

bacteria to other patients if they are not vigilant about properly cleaning their hands (if they do not practice proper "hand hygiene") between patients. Approximately 1 in 50 people also "carry" MRSA in their nostrils or on their skin, usually with no evidence of illness, compared to 1 in 3 people who carry staph (<https://www.cdc.gov/mrsa/community/>). MRSA carriers, even if they don't have any symptoms, can transmit MRSA from their skin and hands to other people. Infections such as MRSA are very uncommon among people of typical health, but nevertheless proper germ etiquette (covering your mouth with your elbows, washing your hands frequently and correctly, and isolating yourself when ill) are the best means of prevention.

*Is everyone with MRSA ill?*

No. As noted above, some people "carry" MRSA without any illness. However, they can still spread the bacteria to other people. Some people have MRSA infections that are restricted to the skin. However, if the bacteria gain access to the bloodstream, the condition is known as *bacteremia*. Bacteremia is a life-threatening condition.

*How does a hospital respond to an increase in the number of multi-drug resistant cases?*

Hospitals are always working to prevent the spread of infections. If an increase in cases is observed, the infection control team needs to know the underlying reason in order to prevent additional cases. This can be complicated as there are a variety of possible reasons for an increase in cases. The following should be considered:

- Are the MRSA strains of the different cases related, or did a variety of unrelated cases just happen to occur at the same time? For example, a patient with an active MRSA infection could have brought in one strain, a temporary nurse who is a MRSA carrier could have brought in another, and an infected visitor could have brought in yet another.
- Did an infected patient bring a strain into the hospital, which then spread, e.g., on the hands of healthcare workers?

*What are phylogenetic trees?*

A phylogenetic tree represents evolutionary relationships among a set of genes or organisms. The tips of the tree represent the descendants, while the base (root) represents a common ancestor. The lines that connect ancestors and descendants represent evolutionary time (generations) between them. All trees contain branches, nodes, and leaves (tips). Most include a root node, or a common ancestor of the entire group of organisms that are represented by the leaf nodes. In Figure 1, the root node is represented by the red circle, and a leaf node by the blue circle. The green circle shows a common ancestor among a subset (or clade) of the organisms in the tree—in this instance, organisms B and G. Here B and G can be thought of as two closely related strains of *S. aureus* that have been found in patients.

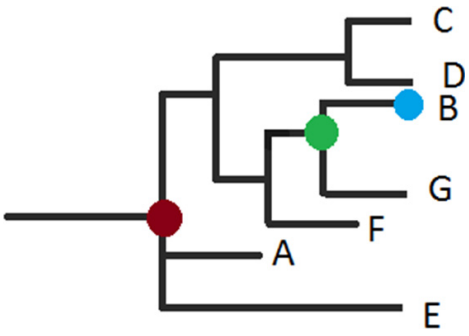



*Figure 1.* A simple phylogentic tree.

*What is Bioinformatics?*

Bioinformatics is a scientific field that uses the power of computers to analyze large biological datasets. Bioinformatics skillsets are becoming increasingly important as DNA sequencing technologies make DNA sequencing faster and less expensive, allowing scientific researchers to easily generate large amounts of DNA sequence data for answering scientific questions. Because DNA molecules, or chromosomes, are the "instructions" for an organism, looking directly at the DNA (genotype) can provide important biological information that is difficult to find any other way. In this case study, you will focus on using bioinformatics tools to find evolutionary relationships between bacterial strains to determine how an outbreak is spreading.

## Questions

1. A routine test reveals that Dr. Tashya Smith has just been identified as carrying a strain of *Staphylococcus aureus* in her nostrils. Dr. Smith is not sick, but the hospital is going to treat her with antibiotics to try and eliminate the *S. aureus* from her nostrils. Why are they treating Dr. Smith, even though she is not ill?

2. Nurse David Johnson is overworked, and has to take care of many patients on each of his shifts. Over the course of his shift, his hands get raw from washing and using antibacterial foam so he occasionally skips washing his hands between patients. One of his new patients is known to have a MRSA infection. After a few days, several of his patients are diagnosed with MRSA infections. What is the most likely source of the new infections, and what recommendations would you make in this situation?

3. You have isolated three strains of *Staphylococcus aureus* from Nurse Johnson's original patient's nose sample. You grow each strain on plates either without an antibiotic or with one of several antibiotics. One is not a MRSA strain (it is sensitive to methicillin, and most other antibiotics tested). Two are MRSA strains. One is resistant to just methicillin, one is resistant to methicillin and erythromycin. If you were to place these three strains into a tree, based on genetic relatedness, how would you organize them? Explain your reasoning.

## References Cited

Fitzgerald, J.R., D.E. Sturdevant, S.M. Mackie, S.R. Gill, and J.M. Musser. 2001. Evolutionary genomics of *Staphylococcus aureus:* insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proceedings of the National Academy of Sciences,* 98(15): 8821–6.

Köser, C.U., M.T. Holden, M.J. Ellington, E.J. Cartwright, N.M. Brown, A.L. Ogilvy-Stuart, L.Y. Hsu, C. Chewapreecha, N.J. Croucher, S.R. Harris, and M. Sanders. 2012. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *New England Journal of Medicine* 366(24): 2267–75.

## Part II – Assembling Phylogenetic Trees

There are many strategies to assemble a phylogenetic tree. Before DNA sequences were available, scientists used physical traits as an organizing principle. The underlying assumption is that the more physical traits two organisms share, the more closely related they are. Think feathers, beaks and wings versus hair, milk production and opposable thumbs. Even with today's relatively swift sequencing technology, it can be challenging to catch up with a fast-moving outbreak. So which way should we make these trees to sort out the MRSA cases in our hospital? Using DNA sequences, or figuring it out using some other kind of trait? Let's find out!

You are going to construct two trees using two types of data: genomic and phenotypic.

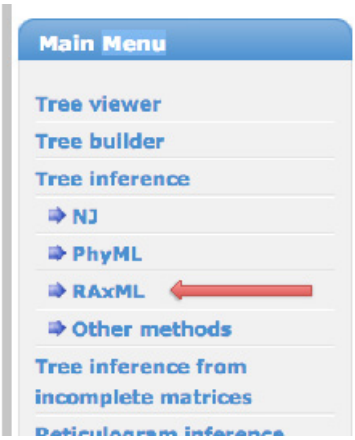
- *Genomic data.* This data is in the form of short DNA sequences from bacteria isolated from all the patients. Specifically, you will be working with SNPs (single nucleotide polymorphisms, pronounced "snips") that are present in the MRSA isolates from the hospitalized patients.
- *Phenotypic data of antibiotic resistance.* This data indicates the resistance *(R)* or susceptibility *(S)* to various antibiotics (the "resistome" for each strain). This data is binary (there are only two options for how each strain responds to a given antibiotic: *R* or *S* ). In the data you will be given, *R* is converted to 1 and *S* is converted to 0.

You are going to construct the trees using both datatypes with a program called RAxML.

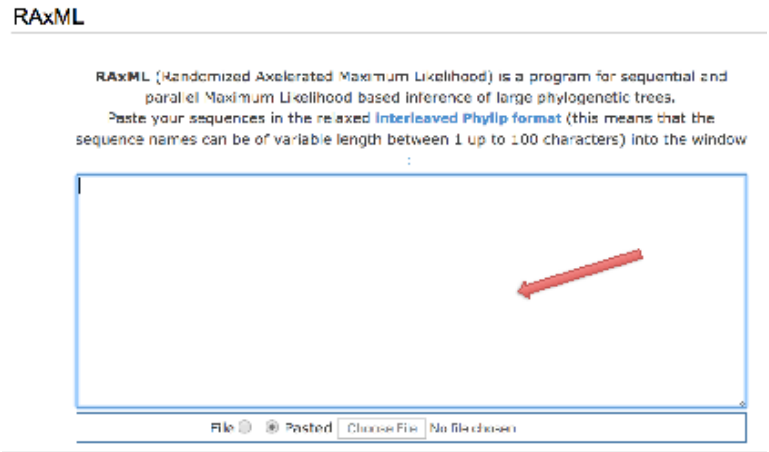
### *Tree 1 – Genomic Data*

You will make the first tree by using the instructions below, the DNA information provided to you in the file named *mrsa_sequencing_phylip.txt*, and the RAxML program.

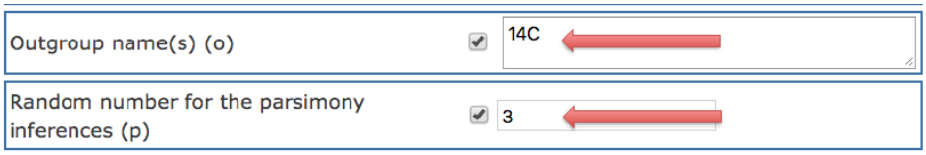
1. Go to: <http://www.trex.uqam.ca/>.
   Click on RAxML in the left hand menu.



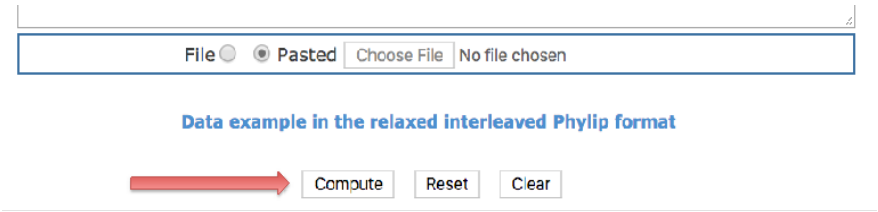

2. Paste the DNA data into the box.



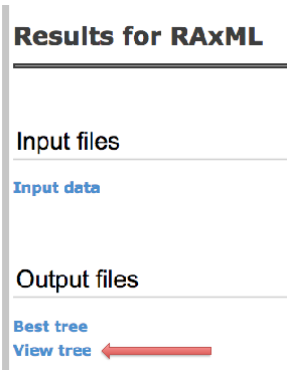
3. Scroll down. Near the end do the following:
   a. Click the checkbox by "Outgroup name(s) (o)" and enter "14C" in the box.
   b. Click the checkbox by "Random number for the parsimony inferences (p)" and enter "3" in the box.




4. Scroll back up and click "Compute."




5. Once it finishes computing, click on "View tree" in the left-hand menu.




## *Tree 2 – Phenotypic Data*

You will now make a tree using the phenotypic antibiotic resistance pattern (*mrsa_sequencing_antibiogram.txt*). Many of the steps are simlar to the process in costructing the first tree, so the instructions here are abbreviated.

1. Go to: <http://www.trex.uqam.ca/>. Click on RAxML in the left hand menu.
2. Paste the antibiogram data into the box.
3. Scroll down.
   a. Change "Data Type" to "Binary" (the "Substitution model" will automatically change to "BINCAT").


   b. Click the checkbox by "Outgroup name(s) (o)" and enter "14C" in the box.
   c. Click the checkbox by "Random number for the parsimony inferences (p)" and enter "3" in the box.
4. Scroll back up and click "Compute."
5. Once it finishes computing, click on "View tree" in the left-hand menu.

## *Questions*

1. Put your two trees side-by-side. Label each strain as NICU or non-NICU.
   a. Does each tree show the same general pattern of strain relationships? Describe how you came to your conclusion.

   b. In each tree, do all the NICU strains appear to be more closely related to one another than to the non-NICU strains?

2. Which tree appears to be most useful at determining which patient-derived samples are related and which are independent infections and why? That is, which tree seems to have more information about the relationships among these strains from the same hospital?

3. Based on the tree that is best able to differentiate the samples, which samples are part of the outbreak that originated with Patient 1 in the NICU? Are there other samples that appear to be independent of the NICU outbreak?

4. Based on your answers and analysis:
   a. Which tree allows scientists to better track this kind of outbreak, trees based on phenotypes or DNA sequence?

   b. Why produce an antibiogram tree?

   c. Historically, DNA sequence data has taken far longer to collect and analyze than phenotype data. Based on this activity, do you think phenotype-based trees were particularly informative when attempting to analyze outbreaks in the past?

   d. Advances in sequencing technology have reduced the time and cost needed to obtain DNA sequences. How do you think these advances will influence epidemiology (the investigation of infectious diseases)?