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The Hunt for MRSA and ESBLs: An Examination of Pathogenic Bacteria on a University Campus

Tara Halterman, Kaylee Brown, Sarah E. Ruffell

University of Pittsburgh at Bradford, Biology, 300 Campus Dr, Bradford PA 16701 USA
(**tarahalterman@gmail.com; kab359@pitt.edu; ruffell@pitt.edu**)

The purpose of this laboratory exercise is to collect bacterial samples and identify Methicillin-resistance *Staphylococcus aureus* (MRSA) and bacteria containing Extended Spectrum Beta-Lactamases (ESBLs) on a university campus. In this workshop, participants will experience the laboratory exercise through a combination of hands-on activities, visual aids and discussions. Students formulate a hypothesis in regards to the presence of MRSA and ESBLs within campus buildings. Over the semester, students make growth media, collect and culture bacterial samples from door handles, and evaluate the bacteria present using morphology. The bacterial samples are collected from door handles, a fomite capable of harboring and allowing the spread of virulent bacteria. Students utilize skills such as aseptic techniques, microbiological culture media preparation, and quantifying bacteria using the standard plate count method. After the completion of the experiment, they analyze their data and create a resolution to the hypothesis they formulated.

Keywords: hands-on learning, aseptic technique, fomite, microbiology, differential media, MRSA, ESBLs, laboratory exercise

Introduction

Overview of Laboratory Exercise

The purpose of this laboratory exercise is multi-faceted: 1) students will be able to perform common skills associated with preparing a microbiology experiment, such as growth media preparation and plate pouring, 2) students will experience a variety of bacterial sample collection, plating and identification techniques, and 3) students will perform basic statistical tests and interpret the data in the context of their hypothesis. Students will achieve these three objectives by first creating a hypothesis related to the presence of MRSA and ESBLs within a building on campus. Within the lab, students will first create nonselective, selective, and differential media, which will allow for the bacteria to be identified as gram-negative, gram-positive, MRSA and/or ESBLs. For example, nonselective media was used in the form of TSA, supplementing the growth of a diverse range of bacteria. Selective media was used in the form of PEA and EMB identifying gram-positive and gram-negative bacteria, respectively. Additionally, two differential media were used in the form of CHROMagar MRSA and CHROMagar ESBL medium, which use color indicators to distinguish MRSA and ESBL-containing colonies. Once all the media types have been created and poured on plates, students will begin collecting bacterial samples. Students will collect bacterial samples by swabbing door handles within a predetermined building and streak the nonselective, selective, and differential media. After a short 24hr incubation period, students can examine the morphology of the colonies formed. Students will identify colonies using morphology and then count total CFUs, MRSA-specific CFUs and ESBL-containing CFUs. Once students have collected all of their results, they will then compare their bacterial identification and CFUs to the door locations. Students can then perform basic statistical tests, to determine if there is a significant relationship present within the data. Additionally, students can also use this time to examine the data in the context of their hypothesis. For example, if a student is trying to compare the MRSA levels on doors handles of office vs bathrooms they could perform a t-test to determine whether there is a significant difference. Notably, students would need to perform repeat trials if they are planning to perform any statistical analysis on their data.

Recommended Student Background

This experiment is designed to be a component of the lab portion of an undergraduate microbiology course. Students should have prerequisite knowledge of the role of bacteria in society, antibiotics, and drug-resistant bacteria.

The required laboratory skills are not difficult, however; due to the many components of the project, time management is an important skill that may make the activity more difficulty for certain age groups and class sizes. Upon completion of this activity, students should be competent in the following microbiology curriculum: bacterial morphology, colony counting, plate pouring, streak plating, aseptic techniques, media preparation, and culture media types and usage.

Flexibility in Required Time

This lab activity could be completed by students over two 3hr lab periods, if all the culture media plates were purchased premade and only 1 or 2 door handles were swabbed. In this scenario, the instructor would need to prepare sterile BBL medium in test tubes and autoclaved Q-tips®. This would take approximately 3 hrs of preparation and one autoclave cycle (~2 hrs). Additionally, the number of media types can be reduced, depending on the focus of the lab. For example, if the focus of the class is to only examine the presence of MRSA, only one media type would be required (Methicillin-resistance *Staphylococcus aureus* (MRSA) identifying agar (CHROMagar MRSA)). Reducing the number of media types used would reduce time and cost. Preparation of a single media type from scratch (ie. students would weight out each compound separately, and then combine and mix) will take ~3 hrs (not including the 1-2hr autoclave cycle required). However, each group in the class could prepare a different media, which would be shared with all of the students in the class. Thus, only one lab period would be needed for this. In this scenario, the only preparation required by the instructor is verifying that all supplies are present for students. Once the media is autoclaved, students can pour plates of their medium. The number of plates required will vary, based on the number of doors swabbed and repeats collected. Thus, students will require ~1hr for every 30 plates poured. In this scenario, the instructor would need to place the autoclaved media in a water bath, multiple hours before the lab period, to melt the solid media in to a liquid state for pouring. As the number of door handles swabbed and media types used increases, so does the time required to complete the lab (ie. number of lab periods). For the field testing, students created, autoclaved and poured plates for all 5 media types, prepared Q-tips and collection tubes, and swabbed 20 doors, resulting in the activity occurring over five 2-3hr lab periods.

Student Outline

Objectives

- To compare and analyze different bacterial samples collected from door handles.

Introduction

Bacteria are primarily round, spiral, or rod-shaped single-celled prokaryotic microorganisms. The majority of bacteria are not harmful however, approximately one of eight bacteria are capable of causing health issues in individuals with a healthy immune system (Immune 2006). These harmful bacteria are known as pathogens. Pathogenic bacteria are capable of causing disease and illness when they enter the body (Piso et al. 2017). Historically, pathogenic bacteria have been responsible for outbreaks such as the bubonic plague which resulted in the death of approximately 20,000,000 people in Europe (McEvedy 1988). More recently, pathogenic bacteria have led to tuberculosis becoming one of the top 10 causes of death worldwide (Tuberculosis 2018).

Two types of pathogenic bacteria that this lab exercise will focus on are Methicillin-Resistant *Staphylococcus aureus* (MRSA) and pathogenic bacteria that produce extended spectrum beta-lactamases (ESBL). The MRSA strain is a type of *Staphylococcus* bacteria that is highly resistant to many antibiotics. This highly resistant strain most commonly causes skin infections, and when not treated promptly it may lead to sepsis (General 2006). Sepsis is known to be one of the leading causes of death in hospitals, occurring when toxins released from a pathogenic infection build up in the blood and begin to travel to other areas in the body. (Lever and Mackenzie 2007). Bacterial strains containing ESBL enzymes can more quickly gain resistance to antibiotics. Examples of ESBL-containing bacteria include *Escherichia coli* and *Klebsiella pneumoniae*. Individuals with ESBL infections may experience fever, diarrhea and nausea (Hadziyannis et al. 2000). The spread of these resistant bacteria within a population can be very detrimental to a community and have serious negative effects in populations with weakened immune systems, such as immune-compromised patients in hospitals.

The spread of bacteria within a population can occur through direct and indirect methods, such as person-to-person contact or contact with fomites. Fomites are non-living, porous and nonporous objects that can carry pathogenic bacteria, resulting in transmission of the bacteria when the fomite comes in to contact with a person. For example, doorknobs are a very common and effective fomite used to spread bacteria (Boone n.d.). A recent study targeting MRSA on a Midwestern university campus demonstrated that door handles were the most common fomite containing MRSA, and overall 27% of campus surfaces sampled contained MRSA (Thapaliya et al. 2017).

Methods and Data Collection

Part A: Door Sampling

1. Read over MRSA and ESBL material provided by you instructor, then create a hypothesis for this lab.
2. This lab is designed to be explorative. Identify how many door handles and the number of times you wish to examine each handle. Once you determined the number of samples you would like to collect, grab the corresponding number of autoclaved Q-tips® and sterilized test tubes containing BBL Transport Medium. You should also bring distilled water parafilm ® squares, a sterile sponge, and ethanol.
3. For the first round of testing, you will need to sterilize the doorknob with your sponge and ethanol. Let dry for 30 seconds. Make note of the time you sterilize and swab the handle. For repeated testing, you do not need to sterilize the doorknob again.
4. Once the handle is dry, dip the autoclaved Q-tip® into the distilled water then swab the door. Try to streak both vertically and horizontally to cover the surface for 10 seconds.
5. Place the sampled Q-tip® in a sterilized test tube containing BBL Transport Medium. Seal the tube with parafilm®.
6. Label the test tube with the time and location of the door handle. If it would be easier, designate a number for each location. Example: 1: gym, 2: room 103, 3: room 204.
7. Repeat steps 3-6 for each door sample obtained.

Part B: Creating Agar Media

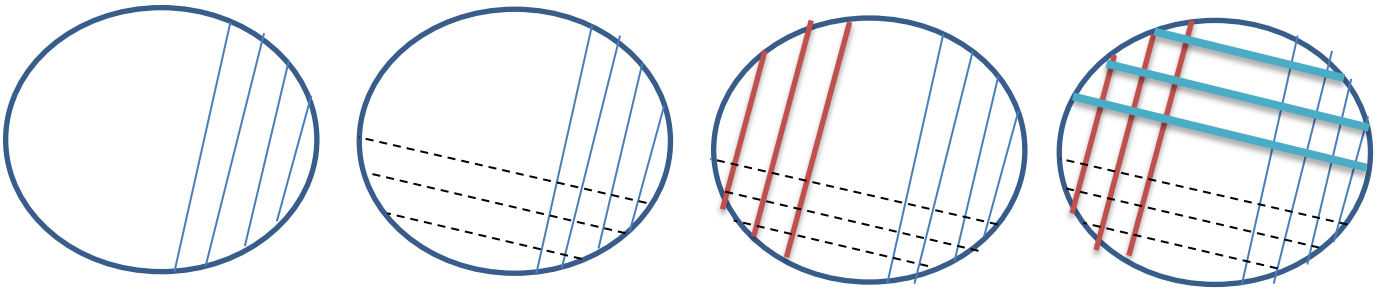
A variety of agar plates will need to be made. The number of plates will depend on how many samples you choose to collect. Each sample will need to be tested on each type of agar made. For example, Samples 1: 8:00am will need to be swabbed on TSA, EMB, MRSA identifying agar, and CHROMagar ESBL. Sample 1: 10:00am will need to be swabbed on TSA, EMB,

MRSA identifying agar, and CHROMagar ESBL ect. They type of agar and the instructions to make each will be provided by your instructor.

Part C: Inoculating

Note: As mentioned above, each sample will need to inoculate each agar plate made. Please refer to the following streak plate technique for each inoculation.

1. Obtain a sterile petri plate and one of the sampled test tubes you collected.
2. Remove the parafilm® on the top of the test tube. To keep the plate sterile, only lift the lid high enough to insert the Q-tip®. Referring to the diagram below, remove the Q-tip® and streak in a straight line to the right of the plate. This area will now be called area 1.
3. To create area 2, streak the Q-tip® perpendicular to the streaks in area 1. Allow a small section to overlap between the two bacteria.
4. Continue to work your way around the petri plate until the whole surface is streaked.
5. Seal the agar plate with parafilm® to prevent contamination.
6. Label the plate with the type of agar, door handle location or number, and time collected.
7. Repeat steps 1-6 for each sample and each plate (Harley 2017a).
8. Once all plates are inoculated, incubate for 48 hours at 37˚C.



Area 1 Area 2 Area 3 Area 4

Data Analysis

1. After incubation, select all petri plates containing 25 to 250 colonies.
2. Record your results below and any other characteristics of each plate (Harley 2017b).

Table 1. Data

Plate	# Colonies	Color	Size	Shape

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Materials

Supplies and Sources

- Trypticase soy agar medium (TSA) - Ward's, Rochester NY
- Eosin methylene blue agar medium (EMB) - Carolina Biological Supply Co., Burlington NC
- Phenylethyl alcohol agar medium (PEA)
- Methicillin-resistance *Staphylococcus aureus* (MRSA) identifying agar medium - CHROMagar MRSA
- Extended Spectrum Beta-Lactamase (ESBL) identifying agar medium - CHROMagar ESBL
- Autoclaved Q-tips®
- Test tubes filled* with BBL- There should be enough BBL present so the cotton tip of the Q-tip® rests on the surface of the medium when the test tube cap is on
- Transport Medium - BD, Sparks, MD
- Parafilm ®
- Sharpies® for labelling
- Petri plates
- Autoclaved Q-tips®

Equipment

Autoclave
Dissecting microscopes
Incubator at 37ºC

Notes for the Instructor

Developing a Hypothesis

Students should be exposed to scientific literature regarding the presence of pathogenic bacteria, such as MRSA and ESBLs, outside of healthcare settings. Students should also review the process of formulating a hypothesis which they will subsequently use to develop their own hypothesis relating to pathogenic bacteria within a campus building.

Creating Agar Media

Students will first need to create different forms of agar media. A multitude of agar media were used during the field testing of this study including trypticase soy agar (TSA), eosin methylene blue agar (EMB), phenylethyl alcohol agar (PEA), Methicillin-resistance *Staphylococcus aureus* (MRSA) identifying agar (CHROMagar MRSA), and Extended Spectrum Beta-Lactamase (ESBL) identifying agar (CHROMagar ESBL). Once the agar media are created they can be poured onto plates and properly labeled. The number of plates to be created is determined by the number of door handles being swabbed, number of sampling times, and number of repeats. Additionally, this number will also determine how many sterile swabs and collection tubes containing BBL Transport medium will be to perform the initial bacterial collection via swabbing of door handles.

Door Handle Sampling

Students must first identify and number the door handles from which the bacteria samples will be taken as well as the time points at which the samples will be taken. Immediately prior to the first sampling time, door handles should be sterilized using a sterile sponge and ethanol for a period of 30 seconds. Then, at each sampling time a sterilized cotton swab should be dipped into distilled water and used to swab the door handle using both horizontal and vertical strokes, each for a period of 10 seconds. Next, the cotton swab should be placed into a sterilized test tube containing BBL Transport Medium (BD, Sparks, MD) and sealed with parafilm®strips. Each test tube should be properly labeled with the door handle number and time. This process should be completed for all door handles and at each sampling time point. For example, during field testing of this experiment students decided to sample 21 door handles at the Sport and Fitness Building at eight time points over the course of the day.

Inoculation of Bacterial Samples

The cotton swabs containing the bacteria samples from the door handles should be used to inoculate each form of agar media. The plates should be inoculated with the cotton swab using the streak-plate technique. This technique allows the bacteria sample to be at different densities on the agar media to create isolated colonies. This will be beneficial when the morphology of the bacteria was examined. The inoculated agar should be labeled with the type of agar, the door handle number, and the time at which the sample was taken. Then, the sample should be sealed with parafilm strips to prevent contamination and be placed in an incubator for a period of 48 hours at 37°C.

Morphology and Data Collection

Students should first be instructed on morphology and quantifying the number of bacteria colonies using the standard plate count method. The morphology should consist of color, size, and other notable features of the bacteria colony. Students should follow the standard plate count method by counting the number of colonies on each plate.

After incubation, the plates should be examined and the morphology of bacteria recorded. Data collected from the samples should include: door handle number, time point, type of agar media, number of colonies, and number of strains based upon color.

Data Interpretation

Students should be instructed on how to use Excel to manage the data that was collected. Students should be

aware of how to enter the data into Excel using appropriate headers and labels. Students should also be aware of how to manipulate the data using the sort tool, such as to group each numbered door handle. This data should be compiled and students can then analyze the results of the experiment and any trends applicable to their hypothesis.

Safety Issues

Students should wear a lab coat and gloves throughout the activity. Additionally, student should thorough when using parafilm® to seal their petri plates, to present future exposure to unknown bacterial strains. Additionally, once a petri plate has been inoculated with bacteria, it should never be opened to prevent exposure to unknown bacteria.

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About the Authors

Tara Halterman is currently pursuing an advanced certificate program.

Kaylee Brown is currently a senior at the Univerisyt of Pittsburgh at Bradford. She studies Biology, Pre-Medicine.

Sarah Ruffell is currently teaching and has research interested in science education and community outreach. As of 2020, she has begun a new position at the University of Waterloo and would be happy to answer any questions you have at her new email sruffell@uwaterloo.ca.'

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