Elementary student outreach activity demonstrating the use of phage therapy heroes to combat bacterial infections

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INTRODUCTION

The public has recently gained an increased appreciation of “good” or “friendly” bacteria, including commensal bacteria that are part of the healthy human microbiome (1) and bacteria present in common probiotic food products such as yogurt (2). Despite this new perception of bacteria, the term “virus” still has a predominantly negative connotation that is almost exclusively associated with disease (3). Consequently, the public frequently asks questions such as “Are there any good viruses?” In fact, there are many viruses that have mutualistic relationships with their hosts (4), but one viral group of particular interest for benefiting human health is the bacteriophages (phages: viruses that infect bacteria), which can specifically kill disease-causing bacterial pathogens.

Battles between superheroes and villains easily capture the attention and enthusiasm of young learners, presenting an excellent opportunity to introduce interactions within the microbial world. This activity, which is appropriate for elementary-school children in a classroom or outreach setting, introduces learners to the concept of phage therapy, where certain phages (heroes) are used to destroy bacterial pathogens (villains) in a targeted and specific approach that does not negatively affect commensal bacteria (5). The specificity of phage therapy provides an advantage over antibiotic treatments, which disrupt the healthy microbiome. In addition, phage therapy presents an alternative strategy to treat bacteria that have developed resistance to antibiotics (6). In this activity, participants will learn about phage infection and host specificity by viewing photos of plaque assays performed in a laboratory with chromogenic bacteria, followed by a hands-on “fishing” activity using the phage heroes’ superpowers of specificity (hooks, Velcro, bacteria, followed by a hands-on “fishing” activity using the plaque assays performed in a laboratory with chromogenic phage infection and host specificity by viewing photos of antibiotics (6). In this activity, participants will learn about phage infection and host specificity by viewing photos of plaque assays performed in a laboratory with chromogenic bacteria, followed by a hands-on “fishing” activity using the phage heroes’ superpowers of specificity (hooks, Velcro, bacteria, followed by a hands-on “fishing” activity using the plaque assays performed in a laboratory with chromogenic phage infection and host specificity by viewing photos of

PROCEDURE

Preparation of supplies

<table>
<thead>
<tr>
<th>Supply Description</th>
<th>Amount Required</th>
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<tbody>
<tr>
<td>Plaque assay plates</td>
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</tr>
<tr>
<td>Phage stock solutions</td>
<td>3</td>
</tr>
<tr>
<td>Bacterial strains</td>
<td>3</td>
</tr>
<tr>
<td>String</td>
<td>5</td>
</tr>
<tr>
<td>Dowel</td>
<td>1</td>
</tr>
<tr>
<td>Magnets</td>
<td>3</td>
</tr>
<tr>
<td>Pipe cleaners</td>
<td>3</td>
</tr>
<tr>
<td>Coloring paper</td>
<td>5</td>
</tr>
</tbody>
</table>

Print (at scale), cut out, and laminate the following: (a) three bacterial villains (each representing a colony of many bacterial individuals): Staphylococcus aureus (Appendix 1), Vibrio coralliilyticus (Appendix 2), and Listeria monocytogenes (Appendix 3); (b) three phage heroes: Staphylococcus phage (Appendix 4), Vibrio phage (Appendix 5), and Listeria phage (Appendix 6); (c) twenty copies of the friendly bacteria (Appendix 7). A poster (Appendix 8) can also be printed that shows the environmental context of each of these bacterial infections (Staphylococcus aureus in the bloodstream, Vibrio coralliilyticus on coral reefs, and Listeria monocytogenes on lunchmeat). Optionally, custom temporary tattoos (e.g., www.stickeryou.com) can be made of the team of “phage heroes” displayed in Figure 1 as a reward for students who successfully complete the activity.

Affix a specific “receptor” (e.g., a pipe cleaner hook, a circle of Velcro) to each of the bacterial villains, and affix the corresponding phage with its respective attachment item to the end of a “fishing rod” constructed from a piece of string attached to a dowel (Fig. 2). A standard-size hula hoop covered with a sheet of black paper is used to provide boundaries for the activity and represents a petri dish in which a plaque assay is performed (7). White paper circles approximately the size of the bacterial villains are attached to the black background to represent the plaques, or death zones, that form in areas where bacteria are captured and killed by their specific phages. To demonstrate the laboratory analog of the game, print the plaque assays shown in Figure 3, which were performed according to standard protocols (7) with four colors of chromogenic bacteria (Escherichia coli) to increase plaque visibility. If safe laboratory facilities and supplies are available, activity leaders and/or participants can create their own plaque assays using chromogenic E. coli available from vendors such as Edvotek, or described in the literature (e.g., labeled with green fluorescent protein). The comic strips in Appendix 9 can be printed and colored by students during wait times or as a coloring activity.

activity rewards and for printing phage comic strips for a coloring activity.
Take-home activity. To enhance the superhero experience, instructors can wear capes and eye masks for the activity.

Teaching lesson and outreach activity

Define phages as viruses that infect bacteria and explain the concept behind phage therapy, where specific phages capable of infecting disease-causing bacteria (pathogens) are used to fight disease without disrupting the normal bacterial community (microbiome) associated with healthy animals. Using the poster (Appendix 8), introduce the learners to three bacterial villains for which the effectiveness of phage therapy has been demonstrated: *Staphylococcus aureus*, which frequently causes skin and blood infections in animals (8); *Vibrio coralliilyticus*, which causes bleaching and tissue loss in corals (9); and *Listeria monocytogenes*, a common source of foodborne human illness (10). While phage therapy has not been approved for human use in the United States by the Food and Drug Administration, other applications, including food safety, have been approved (11). Emphasize the concept of phage–host specificity and how phages will therefore only kill the targeted bacterial villains without negatively affecting any friendly bacteria. Stress that phages can be used as alternatives to antibiotics, which is becoming increasingly important given the rising rates of drug-resistant bacterial pathogens. Show photographs of plaque assays performed in a laboratory (Fig. 3), and explain that the clearings (plaques, or death zones) in the colored bacterial lawns are areas where large numbers of bacteria have been killed by phages (see https://www.youtube.com/watch?v=PHp6iYDi9ko for an age-appropriate description of lytic phage infection).
To emulate this process in a hands-on manner, learners will take turns using a phage hero fishing rod to capture the corresponding colony of bacterial villains from the hula hoop petri dish on the ground, creating their own plaque. Successful capture of the correct bacterial villains can be rewarded with a temporary tattoo, which prompts further discussion with friends and family members about the learning activity. Point out that the phages do not affect the friendly bacteria or even the other bacterial villains to stress specificity. Younger learners may also enjoy coloring comics about each of the phage heroes (Appendix 9), while older learners can engage in thought experiments regarding the logistics, ethics, and challenges of applying phage therapy to human health and coral reefs.

There are no safety issues associated with this activity.

CONCLUSION

This hands-on activity demonstrates the utility and advantages of phage therapy for treating bacterial infections in three different scenarios: skin infections, coral disease, and foodborne illness. Learners gain knowledge about how phages can benefit society, which is a critical first step in shifting the overwhelmingly negative perception of viruses and laying the groundwork for greater public acceptance of phage therapy as an alternative to antibiotics in the treatment of bacterial infections.

SUPPLEMENTAL MATERIALS

Appendix 1: Bacterial villain – Staphylococcus aureus. Artist: Kema Malki
Appendix 2: Bacterial villain – Vibrio coralliilyticus. Artist: Kema Malki
Appendix 3: Bacterial villain – Listeria monocytogenes. Artist: Kema Malki
Appendix 4: Phage hero – Staphylococcus phage. Artist: Kema Malki
Appendix 5: Phage hero – Vibrio phage: Artist: Kema Malki
Appendix 6: Phage hero – Listeria phage. Artist: Kema Malki
Appendix 7: Friendly bacteria. Artist: Kema Malki
Appendix 8: Poster showing the environmental context of each bacterial infection (Staphylococcus aureus in the bloodstream, Vibrio coralliilyticus on coral reefs, and Listeria monocytogenes on lunchmeat). Artist: Kema Malki
Appendix 9: Comic strips with three examples of phage therapy for a coloring activity. Artist: Natalie Sawaya

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REFERENCES