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Short Communication

Breadth of Depth of Bacteriophage Antibacterial Activity

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Abstract

Bacteriophages (phages) are viruses that specifically infect bacteria. Many phages are obligate killers of bacteria, making them valuable antibacterial agents that have been used clinically for over a century. However, bacterial resistance to phages remains a significant limitation of phage therapy. This resistance can be categorized into two distinct types: pre-existing resistance that must be addressed at treatment initiation, and resistance that evolves during ongoing therapy. Phage cocktails, combinations of multiple treatment phages, can address both forms of bacterial resistance. The "breadth of activity" of phage cocktails refers to their capacity to overcome phage resistance present at treatment outset, which is particularly important for empirical treatment of unidentified bacterial infections. Conversely, "depth of activity" describes a cocktail's ability to combat bacterial resistance as it evolves throughout treatment. The concept of "breadth of depth" combines these two parameters by providing a comprehensive measure of a phage cocktail's capacity to suppress resistance evolution across diverse bacterial strains, including those targeted empirically.

Introduction

A phage cocktail by definition consists of more than one type of bacteriophage [1-18]. In a phage therapy context [19-26], phage cocktails thus represent a kind of phage-only combination treatment. As considered here, these phage combinations can possess a breadth of activity against different bacterial types or a depth of activity against specific bacterial types [6], but also a 'breadth of depth' of activity against different bacterial types. These concepts correlate with the additional concepts of 'Community' resistance to phages, which is bacterial resistance to phages that is observed at the beginning of treatments and which must be addressed if treatments are going to be effective, versus 'Treatment' resistance, which is bacterial resistance to phages that evolves in the course of therapies [27,28]. Here we consider how cocktail breadth of activity addresses issues of bacterial community resistance while a cocktail's depth of activity serves instead to address treatment resistance. Cocktail breadth of depth of activity, in turn, quantifies an ability to address treatment resistance to phages across a diversity of potential bacterial targets. Thus, while breadth of activity describes the ability of a phage cocktail to be used empirically to kill a diversity of bacterial strains, breadth of depth of activity describes instead the ability of a phage cocktail to both kill a diversity of phage-sensitive bacteria and interfere with their evolution of phage resistance.

Breadth of Activity

The breadth of activity of a phage cocktail is a function of the host ranges of the constituent phages [6,8-10,29-31]. This breadth can be quantified in terms of the diversity of bacterial host strains against which the cocktail is effective, especially bactericidally. However, this effectiveness is typically assessed without considering the resistance to phages that can arise during treatments. When this 'treatment resistance' is taken into account [27,28], more than just cocktail breadth of activity becomes important. Specifically, it becomes necessary for a cocktail to contain at least two phages that are active against specific bacterial targets [6,27,28], rather than just one active phage per targeted bacterium, the latter being the minimum requirement for determining cocktail breadth of activity alone. Thus, cocktail breadth of activity is explicitly defined as the fraction of bacterial strains that are lethally impacted by at least one cocktail phage.

Depth of Activity

The concept of cocktail depth of activity takes into account the idea that having more than one phage that is able to infect an individual bacterium is necessary for a cocktail to combat the evolution of treatment resistance [6], see equivalently [32-34]. Furthermore, the targeted bacterium should not be able to mutate to cross-resistance to both (or more) of those phages pleiotropically, i.e., as a function of only a single mutation [35]. If within a phage cocktail only a single phage is present that can target a given bacterium, then that cocktail can be described as having a depth of only 1 against that bacterium. This is also true even if more than one phage is able to target that bacterium, should both or more of those phages be found within the same cross-resistance group. A depth of one thus implies that while the cocktail could be effective as a primary treatment, including in terms of addressing community resistance via its breadth of activity, it will not also be effective in preventing treatment resistance, unless other anti-resistance mechanisms are present [28].

A cocktail with a depth of 2, by contrast, will by definition have some potential to address treatment resistance, versus none for a cocktail of depth 1 (for the latter, at least in most cases [28]). Specifically, this means that mutation to resistance against two treatment phage types will be of low probability, particularly relative to the likelihood of mutation to resistance against one of those phages alone. Consequently, upon mutation to phage resistance, a bacterium will tend to remain vulnerable to at least one phage type found within the treating cocktail. To the extent that it is possible to design a cocktail containing three phages, all found within different cross-resistance groups and all of which are able to infect the specific targeted bacterium, then that cocktail can be described as having a depth of 3 against that bacterial strain, and so on. The key point is that with every increase in cocktail depth of activity against a specific bacterium, the likelihood that the bacterium will mutate to complete resistance against all treatment phages will by definition substantially decline: Greater cocktail depth of activity thus implies greater cocktail potential to combat treatment resistance against a specific, targeted bacterium. By contrast, greater cocktail breadth of activity instead implies greater cocktail potential to combat community resistance.

Breadth of Depth of Activity

These ideas become more complicated when considering that a cocktail which possesses a depth of 2 or 3 against a specific bacterial strain will not necessarily possess the same depth of activity against a different bacterial strain. This leads to the concept of breadth of depth of activity. That is, how many bacterial strains, or what fraction of bacterial strains, are susceptible to some number of phages that are found within a cocktail, with each of those phages being found within a different cross-resistance group? If 25% of bacteria tested are susceptible to at least two of those different phages, then the breadth of activity for a depth of 2 would be 25%, whereas the breadth of activity for a depth of 1 could be much higher, e.g., perhaps 75%. Put simply, it generally is harder for a given phage cocktail to target many bacterial strains with multiple phages, let alone multiple phages for which bacterial mutation to cross-resistance is unlikely-han it is for the same cocktail to target many bacterial strains with only a single phage.

"Ideally", we would determine phage cross-resistance groups by testing phages against every potentially targeted bacterial strain. However, this would be extremely burdensome, particularly in comparison to simply determining the individual host range of each phage making up a cocktail. Thus, as an approximation for addressing treatment resistance proactively, cocktail preferably should have a depth of activity of greater than 1 against a breadth of as many potentially targeted bacteria as possible. Starting with two phages for which at least one bacterial strain fails to display cross-resistance, 'breadth of depth' would then be approximated by how many bacterial strains both of those phages are effective against [6].

Conclusion

For the sake of addressing treatment resistance, a prêt-à-porter [36] phage cocktail, one that has been developed in advance, ideally will possess a substantial breadth of depth of activity against all potentially targeted bacteria. This would require at least two phages from different cross-resistance groups that are effective against a wide variety of these potentially targeted bacterial strains. This, though, will tend to be much more difficult to achieve than for a personalized, sur-mesure [36] cocktail that is obliged to treat only a single bacterial strain. For a prêt-à-porter cocktail, however, that depth of 2 or more can be spread over multiple pairs of cocktail phages, making generation of substantial breadth of depth at least slightly easier. The key to substantial breadth of depth, in other words, is for many bacteria to be affected by more than one phage type, without constraining which specific phages are responsible for that depth against each targeted bacterial strain. Crucially, though, for a cocktail to have a depth of activity of greater than 1, bacteria must be impacted by more than just a single phage type. Depths of 1 thus contribute only to 'breadth of activity'. In contrast, 'breadth of depth of activity' at a minimum is a function of per-bacterium depths of activity of 2 or more, with each of those phages affecting a given bacterium derived from a different cross-resistance group.

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