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CLINICAL SIGNIFICANCE OF DETERMINING BACTERIAL SUSCEPTIBILITY TO PHAGES

Sodiqov Sardorbek Maxmudjon ugli
Kokand University Andijan Branch
Kokand University Andijan Branch, student in the General Medicine program.
e-mail: *sodikov9632305@gmail.com*
Andijan, Uzbekistan
Mirzayeva Saidaxon Abdusalomovna
Professor Department of Microbiology, Virology and Immunology.
e-mail: *mirzayevasaidaxon999@gmail.com*
Andijan, Uzbekistan
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Abstract. *The increasing number of bacteria resistant to antibiotics requires the introduction of new diagnostic and therapeutic approaches in clinical microbiology.*

Bacteriophages are viruses that selectively infect bacteria; they do not target human cells and, in many cases, affect the normal microbiota less than antibiotics. Therefore, determining bacterial susceptibility to phages, especially in infections caused by multidrug-resistant microorganisms, is considered an important laboratory step for selecting individualized treatment.

Keywords: *bacteriophage, phage susceptibility, phagogram, spot test, double-layer agar, antibiotic resistance, clinical microbiology.*

Bacteriophages are viruses that infect bacteria. They bind to specific bacterial receptors, inject their genetic material into the cell, and may cause bacterial lysis at the end of the lytic cycle [1,6]. The specific ability of bacteriophages to target bacteria makes them biologically important agents for diagnostics, epidemiological surveillance, and therapy in medical microbiology [5].

Determining bacterial susceptibility to phages refers to a set of tests used to evaluate whether a clinical or laboratory isolate undergoes lysis, growth inhibition, or productive infection under the effect of a particular phage or a set of phages [2]. This process may support clinical decision-making in a manner similar to antibiotic susceptibility testing; however, because phages are highly specific, their matching at the “one bacterium - one phage” level requires separate testing [3].

Antibiotic resistance is currently a global threat to health-care systems and limits treatment options for many bacterial infections. According to the World Health Organization, phages are being considered as alternative or combined treatment options for drug-resistant bacterial infections; however, strong evidence regarding their safety, efficacy, and practical implementation is required for their broad clinical use [1,7].

Traditionally, antibiotics have been viewed as more universal agents because many antibiotics can affect several bacterial species. Phages usually have a narrow host range. This, on the one hand, may help preserve the normal microbiota, but on the other hand, it creates the need to find a suitable phage for each patient isolate [3].

Therefore, the success of phage therapy depends not only on the availability of a phage preparation, but also on correctly performed phage susceptibility tests.

There are also opposing views regarding this topic. Some researchers emphasize that in vitro lysis does not always lead to clinical effectiveness because bacterial biofilms, immune response, the site of infection, and pharmacokinetic factors can influence the final outcome. At the same time, clinical experience and compassionate-use cases suggest that phage therapy may be beneficial in certain severe and treatment-refractory infections [1,5].

The aim of this article is to systematically analyze methods for determining bacterial susceptibility to phages from the perspective of medical microbiology, to describe their advantages and limitations, and to clarify their role in clinical decision-making. The scientific novelty of the article is related to evaluating methods such as the spot test, double-layer agar method, efficiency of plating, and growth kinetics as a single diagnostic algorithm, with special attention to the clinical interpretation of each test.

This work was designed not as a laboratory experiment, but as an analytical article based on the literature. Therefore, the “results” section does not present new clinical sample data, but summarizes the diagnostic value, areas of application, and interpretation problems of the tests described in the literature [2,3,4].

The analysis was carried out in three stages. In the first stage, sources related to the clinical significance of bacteriophages and antibiotic resistance were selected. In the second stage, phage susceptibility testing methods, including the spot test, double-layer agar method, efficiency of plating, and growth kinetics, were compared. In the third stage, their clinical interpretation, limitations, and need for standardization were evaluated [2,3,4,5].

The spot test is regarded as a convenient method for the initial screening of many phages within a short time because several phage samples can be assessed on a single plate. However, a clear zone observed in the spot test may sometimes be related not to true phage replication, but to a high concentration of phage components, diffusion, or the phenomenon of lysis from without [2,4].

The double-layer agar method helps evaluate productive phage replication in bacteria by observing plaque formation. This method allows the phage titer to be expressed as plaque-forming units, or PFU/mL, and also enables evaluation of plaque morphology [4,8].

Efficiency of plating (EOP) is used to compare how effectively a phage propagates on different bacterial strains. A high EOP indicates that the phage forms plaques on the tested isolate at a level close to that observed on the reference strain, whereas a low EOP may indicate limited productivity or only partial compatibility of the phage [4].

Growth kinetics in liquid medium assesses how a bacterial population changes over time under the influence of a phage. This approach is clinically important because, in some cases, even when the plaque test is positive, bacterial growth may not be completely suppressed or a resistant subpopulation may emerge after a certain period [3,4].

A practical algorithm for determining phage susceptibility usually begins with the correct identification of the clinical isolate.

The next step is the initial screening of the isolate using an available phage collection.

The purpose of this stage is to quickly identify potentially active phages against the clinical isolate.

Comparative characteristics of the main methods used to determine phage susceptibility

Method	Main purpose	Advantage	Limitation	Clinical significance
Spot test	Initial screening	Rapid method; allows comparison of several phages at the same time	Does not always prove productive phage infection	Useful for the preliminary selection of phages potentially active against a clinical isolate
Double-layer agar method	Assessment of plaque formation and phage titer	Allows determination of PFU/mL and evaluation of plaque morphology	Requires time and laboratory experience	Helps confirm productive replication of the phage in the bacterial host
EOP	Comparison of phage propagation efficiency on different bacterial strains	Allows assessment of phage host range quality	Results depend on the reference strain	Evaluates how efficiently the phage works against the clinical isolate
Growth kinetics	Evaluation of bacterial growth over time	Provides dynamic and clinically relevant interpretation	Difficult to standardize	Shows whether the phage can suppress bacterial growth over time

Table 1.

The initial positive result should be confirmed by additional tests. When plaque formation, EOP values, and growth kinetics are assessed together, the reliability of the laboratory result increases. If a phage is positive in one test but shows weak results in another, the clinical decision should be made with caution.

At the final stage, the result is correlated with the clinical situation. If the site of infection, bacterial antibiotic susceptibility, possible biofilm formation, the patient's immune status, and the route of administration are not considered, a laboratory conclusion of "susceptibility" alone may be insufficient.

The literature analysis shows that the most appropriate approach to determining bacterial susceptibility to phages is the sequential and complementary use of several methods. Accepting the result of a single spot test as sufficient for clinical selection may lead to errors because a lysis zone does not always indicate phage replication inside the bacterial cell.

The first important finding is that the high specificity of phages has a dual clinical significance.

On the one hand, they may selectively infect the target bacterium and cause less disturbance to the normal microbiota. On the other hand, this same specificity requires the separate identification of a suitable phage for each clinical isolate.

The second finding is that the lack of standardization of phage susceptibility testing methods is one of the major gaps in clinical practice. While many standards exist for antibiotic susceptibility testing, a single universal “gold standard” for phage susceptibility testing has not yet been fully established. For this reason, different laboratories may reach different conclusions for the same bacterium-phage pair.

The third finding is that plaque formation and actual suppression of bacterial growth are not identical concepts. Some phages may form plaques, but may fail to control the bacterial population for a long period in liquid medium. Therefore, the growth kinetics test is important as an additional source of evidence that strengthens clinical selection.

The fourth finding is that the clinical effectiveness of phage therapy does not depend only on phage-bacterium compatibility. The site of infection, the presence of biofilm, the patient’s immune status, the route of administration, dose, phage stability, and combination with antibiotics also influence treatment outcomes.

The fifth finding is that the phage-antibiotic combination may be a promising strategy in certain cases. WHO materials indicate that phages may be used together with antibiotics to improve treatment effectiveness. However, clinical decisions in this direction should be based on individual microbiological testing and medical supervision.

The scientific significance of determining bacterial susceptibility to phages is that it is not merely a “pre-treatment test.” It also helps to understand infection etiology more deeply, assess heterogeneity within the bacterial population, and develop principles of individualized therapy.

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