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Recent Developments To Use The Bacteriophage To Treat The Bacterial Infections (Case Study: Pseudomonas Aeruginosa Bacteria.

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ABSTRACT

The bacteriophages use the host in different ways for their duplication and survival. Some of them are lytic which cause bacterial licking after their duplication. These are used for treating the bacterial infections and phage typing. Which are more effective than antibiotics to treat the illnesses. This study aims to study treating effects and anti-microbial effects of the lytic phages derived from hospital resources for treatments against Pseudomonas aeruginosa bacteria. Population was selected among the clinical samples of outpatient and hospitalized patients of the different parts of the hospital of Imam Komeini in Tehran. After cultivation, antibiotic and resistance identification, 43 samples of isolated Pseudomonas aeruginosa resistant to the antibiotics were identified. The proper lytic bacteriophages were separated from the treated wastewater of the third pond of the septic sector of the same hospital. Their bacterial effect was investigated in two solid and liquid phases on resistant and isolated isolates. All samples were evaluated three times for different concentrations of phage under different conditions. The results were analyzed using Mann-Whitney test. In both tube and solid two layer cultivation methods, high concentration of the bacteriophages had complete bacterial effects on antibiotic-resistant bacteria. The results showed that the bactericidal effects of the bacteriophages are high and these effects appear in high concentration of the phagocytes.

Keywords: bacteriophage, antimicrobial agents, bacterial infections

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INTRODUCTION

Today, it is well-known that treatment with antibiotics with a wide spectrum of function can reduce and disrupt the function of the effective bacterial bacteria and create a suitable environment for replacing the other pathogens. Therefore, it is necessary to replace other therapeutic methods that can exclusively target only the pathogenic agents in the patient's body.

One of the most effective mechanisms that can be observed in bacterial groups, is the resistance to various types of the antibiotics.

Basically, the bacteria have genes within their genome that can easily withstand one or more antibiotics. As long as the bacteria are resistant to cope with a range of the antibiotics, so-called multidrug resistant bacteria are desired features.

Today, multidrug resistance is one of the most spectacular debates facing microbiologists and physicians to treat the bacterial infections.

Up to now, various drugs have been developed to address this problem throughout the world, but despite the advances made in this area (Salim, 2012), the scientists have not yet discovered a drug that destroy this drug resistance in bacterial groups. To overcome this issue, different methods are considered including the use of polymer compounds, nanotubes, synthesis of new drugs, and the use of proteomics and genomics methods (Qadir, 2012). Derived synthetic products of the plants is one of the proposed strategies by the microbiologists to reduce the issues derived from the bacterial infectious illnesses. The plant products and their derivatives allow extracting the pharmacological products, and they can be used as the anti-infectious drugs (Janbaz, 2011). Having anti-bacterial, anti-microbial, antioxidant, anti-inflammatory, pain killer features in herbal products have introduced them as limiting products for bacterial infectious illnesses and a good candidates for pharmaceutical industries. Although these methods were successful, but it can be seen that their side effects are more than their effectiveness, and it is possible that they bring some health problems for the patients who are using these products. So, it looks like that the effective and professional tools are needed to solve the bacterial resistance issue to reduce antioxidant resistance and reducing the infection too (Javed, 2013). Using the bacteriophages as the new targets to limit the growth of the cause of infectious diseases can bring us the new perspectives to develop the new drugs in order to reduce the bacterial infections. The bacteriophages are a bunch of viruses that infect the bacteria. Phages are intracellular parasites which can enter the bacterial cell system, and by incorporating bacterial replication and other components of the cell, will reduce the bacterial growth and ultimately restrict bacterial infections (Qadir, 2014). Considering this effective feature of the phages, they can be used to treat the different infectious illnesses which are occurred in human beings because of the pathogenic bacteria (Javed, 2013).

Bacteriophages or phages are a bunch of viruses that can infect and destroy the bacteria. More than a century ago, the first report was published on the recognition of the bacteriophages. As Hankin has discovered in 1898, a number of factors were identified in the Indian River which were able to pass through very tiny filters and exhibit antibacterial properties (Duckworth DH, 1976). Studying the bacteriophages was begun for the first time about their usefulness as a tool to confront the bacterial infections. But by discovering the penicillin and other broad-spectrum antibiotics, this application of phages was gradually forgotten (Brook Hard, 1991).

But with the identification of the method of proliferation of phages and their ability to kill bacteria at the end of the infectious cycle, the possibility of using phages as strengthened as a healing agent (Levin BR, 1996).

Currently, the use of phages for the treatment of the infection has been used in many treatment-resistant infections successfully. Even in some countries, including Georgia, physicians use phages in abundance in pediatric, surgical and burning cases (Livermore DM, 2004).

In a large number of studies, phage has been used as an antibiotic agent and has shown that it has the ability to leach out all bacteria. The phage is a proprietary agent that lays only bacteria and does not affect the eukaryotic cells. In other words, phage has a specific ligand that allows them to be attached to a specific antigen and this antibody receptor does not detect other bacteria. In other words, phage eliminates certain bacteria

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used while not affecting the natural flora bacteria. There are approximately 10 million phage particles per ml of sea water, it is estimated that there is close to 10^{30} phage particles in the world. Phages can be grouped according to the characteristics such as host range, physical properties such as: size of capsid, resistance to organic solvents, size and type of the genome, such as RNA or DNA (Figures 1 and 2).

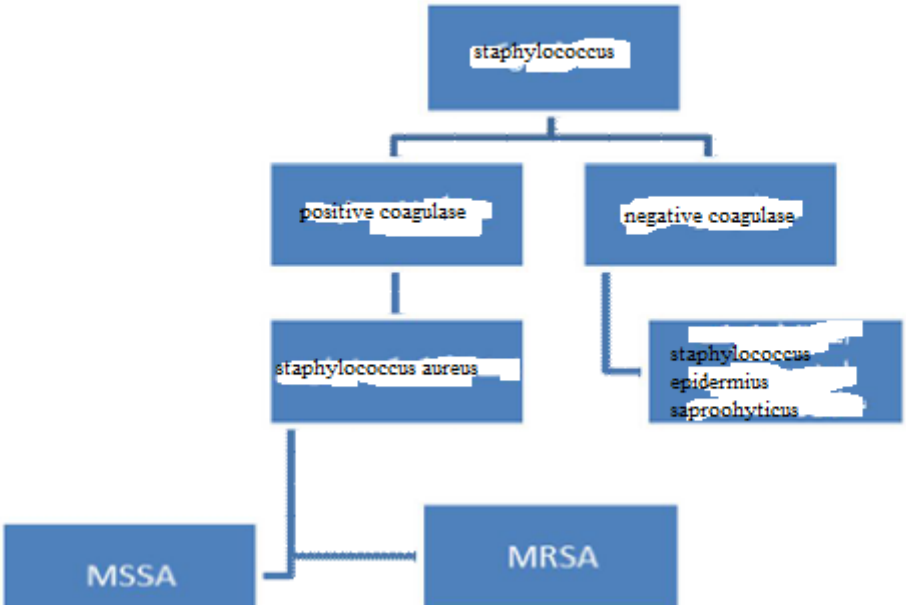

Fig. 1. Staphylococcal division based on coagulase and penicillin resistance gene

According to the classification by the International Taxonomy Committee of the virus (ICTV), the phages are all within a single order called Kodowara, which consists of three families. Most of the phages have tail and are DNA-shaped (Livermore DM, 2004).

There are at least 13 phage groups and each phage is dedicated to its host.

The phages can be divided into three types including: lysogenic, lytic and cchronic. (Sivera Marza JA, 2006). Lysogenic phage enters its genome inside the host's genome, while lytic phage begins to proliferate by injecting its own genome into the cell's cytoplasm, resulting in the destruction of host cell bacteria. Finally, the chronic phage can enter the extracellular space without causing the destruction of its host, which can therefore continue to split into the host.

Phages can remain outside the host cell, but their replication needs to be present within the bacterial cell (Ebrahimi, 2003). Another point about lysogenic phages is that they can be ligated under conditions and cause loss of the host (Imamipour, 2003). Due to the fact that most of the phages have their own host, they can be used to identify and determine the species and bacterial strain specifically referred to as phage typing.

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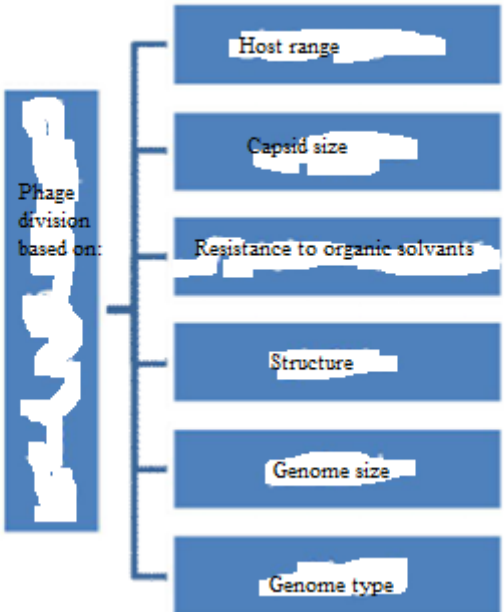

Fig. 2. Division of phages based genomic and structural content and the size of capsid

There are numerous problems in identifying phages, including the absence of their cultivation outside the host, since it is not present, it will not be able to reproduce (McManus AT, 1982). Phage structure can be tail-shaped, strain, polymorph or multi-dimensional (Livermore DM, 2004). The phage has a structural design including capsid, disk plates, spike, base plate or fiber. In addition to phage therapies, the phage has different applications, phage can be used for industrial uses including purification of possible pathogens in agricultural and livestock feed as prophylaxis and hospitals for controlling biofilms (Inal JM, 2003). Nowadays, many companies operate on the phage and its products, either commercially or in medical form (Table 1).

Pseudomonas aeruginosa causes 10 to 20% of hospital infections and is often isolated from people with cancer or people who have large burns. This bacterium can cause infection in all tissues and in different parts of the body. The mortality rate for people with immunodeficiency is about 80%. Also, pneumonia caused by this bacterium causes 70% of mortality in patients. This mortality rate is very high compared to other pneumonia-causing microorganisms, where mortality is about 35%. Pseudomonas aeruginosa can cause epidemic diarrhea in children, ocular infections, osteomyelitis, skin infections, ear infections, bacteremia, endocarditis, and meningitis (Imamipour, 2003).

Table 1. Phage therapy and its results

In this study, the staphylococcus aureus was treated by phagitis in close proximity to the site of the infection.	Essai de thérapeutique au moyen du bacteriophage. 1921	BRUYNOGHE R. and MAISIN J.
Successful treatment of oculogyric infections in mice without side-effects by phage, which indicates the specificity of phage function on a dedicated host.	Successful treatment of experimental Escherichia coli infections in mice using phages: its general superiority over antibiotics. 1982	Smith, H. W., and M. B. Huggins
The use of a single dose of phage in oocyte treatment reduced the dose of bacteria and stopped diarrhea caused by oocytes.	Effectiveness of phages in treating experimental E. coli diarrhoea in calves, piglets and lambs. 1983	Smith, H. W., and M. B. Huggins.

Phage therapy was used to treat 518 paatients with inflammation of resistant bacteria, with success rate of 75 to 100%.	Results of bacteriophage treatment of suppurative bacterial infections in the years 1981–1986.	S ´LOPEK S. et al
Phage is a safe and effective agent in the treatment of existing living infections.	Immunobiological properties and therapeutic effectiveness of preparations from Klebsiella bacteriophages. 1992	Bogovazova, G. G., et al.
The use of phage as a prevention pf pseudomonas aeruginosa infection is effective and usable and cannot prevent the infection of this bacterium in the skin.	Bacteriophage prevents destruction of skin grafts by Pseudomonas aeruginosa. 1994	Soothill, J. S. et al.
In this study, bacteriophage d29 mycobacterium was used to treat the heel of the mouse, and it was observed that the use of this phage effectively cured the infection caused by mycobacterium olerance.	Phage Therapy Is Effective against Infection by Mycobacterium ulcerans in a Murine Footpad Model. 2013	Gabriela Trigo et al.
In this study, 13 different phages were used to eliminate MRSA and it was found that LizAnk phage had the most effect against MRSA and had no effect on fibroblastic cells	Identification of a novel lytic bacteriophage obtained from clinical MRSA isolates and evaluation of its antibacterial activity. 2013	Sahin F et al

METHODOLOGY & MATERIALS

This study was performed on different specimens of phlegm, urine, vaginal discharge, ear secretion and wound secretion of patients referred to the special clinic of Imam komeini Hospital in Tehran as well as patients in different parts of this hospital. Samples were cultured in agar-agar culture media and McCongie Agar culture media. Then incubated at 37 ˚C for 24 hours.

Pseudomonas aeruginosa was diagnosed with suspicious colony culture on different differential environments and biochemical tests. Evaluation of antibacterial sensitivity to antibiotics was performed using disc diffusion, tobramycin 30 µg, gentamicin 10 µg, amikacin 10 µg, nalidixic acid 30 µg, rifampin 30 µg, kanamycin 30 µg, cephalexin 30 µg, amoxicillin 25 µg, chloramphenicol 30 µg, tetracycline 30 µg, sulfamethoxazole 25 µg, nitrofurantoin 300 µg, doxycycline 30 µg, ampicillin 10 µg (antibiotic medicine, Tehran, Iran).

After 24 hours incubation, the non-growth diameter was measured and the results were recorded in susceptible and stable manner using the Clinical and Laboratory Standards.

The refined sewage of the septic basin of Imam Komeini Hospital was used for the separation of lytic bacteriophages. First, a broth culture medium was prepared with a concentration of 10x. 5 ml was mixed with 45 ml of refined wastewater. Then, 5 ml of fresh suspension of 4 hours of pseudomonas aeruginosa and a few drops were added to the tube containing the treated sewage treatment medium. After mixing the contents of the tube, it was incubated at 37˚C for 24 hours.

FINDINGS

A total of 43 isolates of pseudomonas aeruginosa were collected from different samples of the patients. The percentage of isolates by patient sample is shown in Fig. 1.

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The overall results of the isolate drug reference were as follows: resistance to amikacin in 20 isolates was 47.51%, gentamicin resistance in 14 isolates 35.56%, resistance to tobramycin in 14 isolates 34.56%, resistance to potassium in 29 isolates 64.42%, resistance to rifampin in 24 isolates 50.84%, nalidixic acid resistance in 24 isolates 54.81%, chloramphenil response in 38 isolates 88.34%, resistance to amoxicillin in 43 isolates 100%, resistance to cephalexin in 43 isolates 100%, 100% resistance to nitrovironthmonium in 43 isolates, 70.42% resistance to sulfamethoxazole in 38 isolates, tetracycline resistance in 43 isolates was 95.51%, resistance to ampicillin in 43 isolates 100%, doxycycline resistance in 36 isolates was 90.23% (Figure 1).

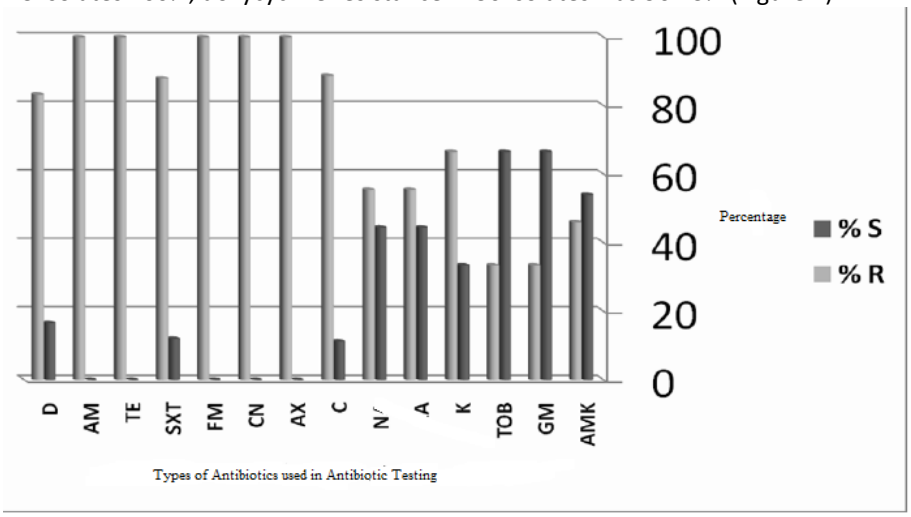

Fig. 1. Percentage of sensitivity and resistance of pseudomonas aeruginosa isolates isolated from samples of patients with different antibiotics (s: sensitivity, R: resistance)

The effects of phages on pseudomonas aeruginosa isolates were thus divided into: negative (-), which means that the concentration of the phage used had no antibacterial effect. Positive response (+), meaning low antibacterial effect and decrease in number of bacteria less than 50% in comparison with the control group. The two-positive response (++) meant an average antibacterial effect and a 50 to 75% reduction in number of bacteria in comparison to the control group. Three positive responses (+++), which meant high antibacterial activity and a reduction of 75-100% of the bacteria l count comparing with the control group (Fig. 2 and 3). As shown in fig. 2 for the tube method, in the high concentration of phagia, all of 43 isolates showed a triple positive response (+++). At medium and low concentrations, the responses are more likely to be seen as double positive (++) and one positive (+). Fig. 2 also shows the results of the two-layered solid-state culture method. In this method, in high concentrations, the response in all samples was positive (+). Double positive and triple positive (+++) responses were observed in medium and low concentrations, respectively.

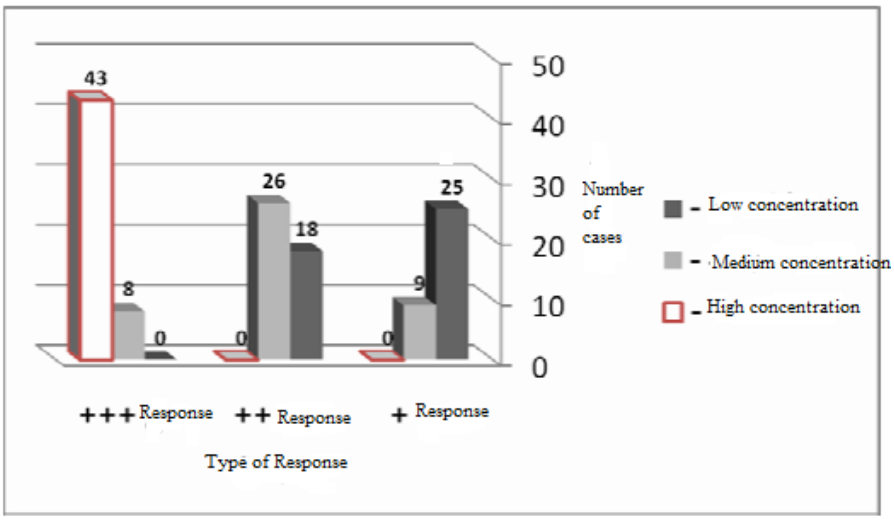

Fig. 2. Antibacterial effects of different concentrations of phage cells in tube method

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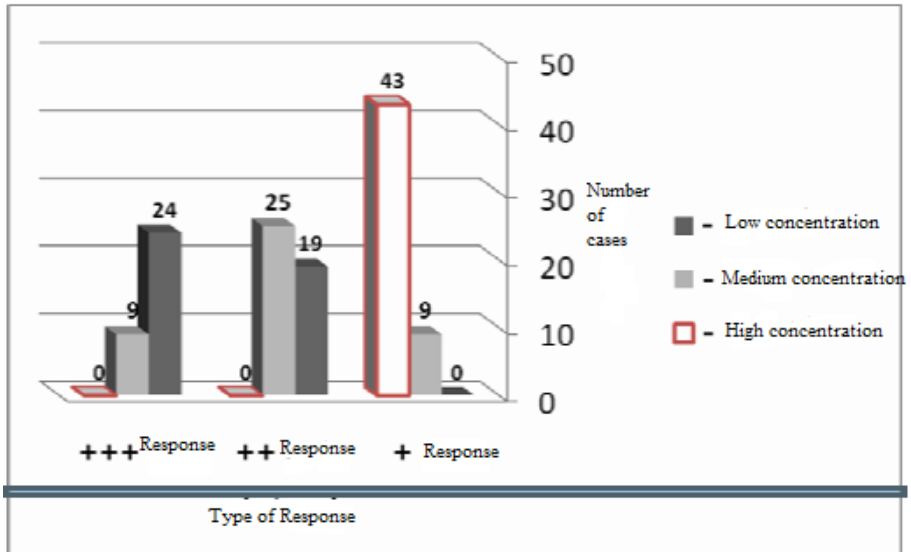

Fig. 3. Antibacterial effects of different concentrations of phage samples in two-layered solid culture method

DISCUSSION

One of the problems with the treatment of pseudomonas aeruginosa is its resistance to various antibiotics. The resistance of the isolated bacteria to the antibiotics was mentioned in this study and various studies and in some cases, they were resistant to all antibiotics used. This study showed that bacterial effects on pseudomonas aeruginosa isolates also increase bacterial effects. Considering the importance and the role of bacteriophages in the prevention and treatment of bacterial infections, it has been mentioned in various studies, especially antibiotic-resistant bacterial infections. The results of this study like other studies, show the bactericidal effect of bacteriophages. Also in this study, bactericidal effects of different phage concentrations were investigated. The results showed that the bacterial effects also increased by increasing the phage concentration. These results are similar to previous studies (Church D, 2006). This increase does not depend on the method used, but the severity (+ or +++) is different is different methods. It is noteworthy that the bacteriophage used to treat antibiotic resistance of isolated bacteria has a relatively similar bacterial effect. Therefore, it can be a good alternative to bacterial treatments. Another use of bacteriophages is to use them instead of strong disinfectants in various departments of the hospital. Due to the specificity of phages and the sensitivity of different bacteria to them, the use of bacteriophages for human pathogens in these places prevents the high use of disinfectants in hospitals. This method does not destroy the natural bacteria and we do not witness the replacement of human pathogens in these places. In addition, various studies have shown that the removal of a problematic bacteria from the environment has been observed by chemical disinfectants has been fruitless despite repeated attempts and bacterial colonization. In such cases, the use of bacteriophages is a good solution for controlling the agent in the environment.

CONCLUSION

In a large number of studies, phage has been used as an antibiotic agent and has shown that it has the ability to leach out all bacteria. Phage is a proprietary agent that only leaches out the bacteria and has no effect on eukaryotic cells. In other words, the phage has a specific ligand that allows them to be attached to a specific antigen and this antibody receptor does not detect other bacteria. In other word, phage is used to kill certain bacteria while not affecting natural flora bacteria. Separated bacteriophage samples have acceptable bactericidal effects on antibiotic resistant pseudomonas aeruginosa isolates. Antibacterial effects of bacteriophages in high concentrations do not depend on the type of method used. At least the application of this technique involves the use of bacteriophage isolated from the sewage system to remove bacteria to environmental disinfection. Of course, one of the disadvantages of using bacteriophages is that they need special conditions and a long time to apply their antibacterial effects, which is longer than antibiotics.

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