Review Article, Phage Therapy: An Alternative to Antibiotics

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Abstract: The idea of phage therapy to treat bacterial diseases was conceived with the revelation of the bacteriophage right around a century back. After a checkered history, its present renaissance is energized by the unsafe appearance of antibiotic resistant bacteria on a worldwide scale. As a characteristic of this restored intrigue, the unanswered issues of phage therapy are presently being tended to, particularly for human use. Phage treatment in the farming, nourishment preparing and fishery businesses are as of now being in use effectively. While staying alert of the potential downsides of this novel development, this review is undertaken for promoting painstakingly controlled empirical data on its viability and safety in treating human diseases, particularly in perspective of its various advantages over antibiotics. Also, it highlights some studies that support the effectiveness of phage therapy on some bacterial genera of *Enterobacteriaceae*. Lastly, the review aims to promote scientists to start human trials for phage therapy and compare it with antibiotic treatment results.
[Sana Alattas, Malak Abu Samha, Wid Al-Rashidi. **Review Article, Phage Therapy: An Alternative to Antibiotics.** *Life Sci J* 2019;16(4):97-107]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 12. doi:10.7537/marslsj160419.12.

Key words: Phage therapy, bacteriophage, *Enterobacteriaceae*, antibiotic resistant.

Introduction

Infection Control and the Spread of Antibiotic-Resistant Bacteria

One of the greatest achievements in modern medicine is infection control. At the beginning of the first half of the 20th century, antibiotics were discovered and have contributed to the treatment of many serious diseases that were considered fatal in the past. However, abusive uses of antibiotics have caused the emergence of antibiotic- resistant bacterial strains. This occurs when the bacteria change their response to the drug, and with this phenomenon increasing in countries where there are no standard treatment guidelines health workers over-prescribed antibiotics for use by the patients. The world today faces a growing problem with antibiotic-resistant bacteria, and we are now heading towards the post-antibiotic era. We urgently need to find an alternative treatment. Before the antibiotic revolution, there were some studies [reference is required here. At least two] on the use of bacteriophage for treatment, but most of them stopped when antibiotics appeared, although scientists are now generating their attention towards bringing back the use of bacteriophages as a safer alternative for antibiotics. (Rea, 2013).

Bacteriophages: Life cycle and properties

Bacteriophages or bacterial viruses or phages (from the Greek word *phagein*, "to eat") are the most abundant type of viruses known to scientists. Although bacteriophages were discovered in 1915 by Frederick Twort and Felix d'Herelle in 1917, researches on them weren't conducted frequently until the 1980's (Puniya, 2015). Broadly, phages can be classified as virulent or temperate. Virulent phages destroy the cellular organelles of its host to multiply, typically ending its cycle with culminating cell lysis (for obligate lytic phages) and then release progeny virions. Temperate phages have alternative replication cycles: a reductive infection, in which the phage remains latent in the host – lysogeny or a productive lytic infection. The overall importance of phage-host interactions in relation to the ecosystem remains elusive, but these viruses are likely to impact on the bacterial population balance and the flow of genetic material between microorganisms within the ecosystem. (Puniya, 2015).

Phage Therapy

As scientists interest shifts away from antibiotics, phage research is becoming more focused on phage therapy based treatments for bacterial diseases. Phage therapy based on virulent phages has the advantages over conventional antibiotics and antimicrobials in that phage (a) are naturally occurring biological agents which do not develop antibiotic resistance in the environment; (b) it can be applied without negative health side effects associated with some antibiotics; (c) it can be delivered directly to living tissue without harm; and (d) it can attach, self-propagate and penetrate microbial biofilms which is not possible by other chemicals antimicrobial agents. In general, phages tend to have a narrow spectrum of activity directed against target organism, giving them an advantage over antibiotics and lower the risk factor while using them in therapy (Carlton, 1999).

Limitations of phage therapy and possible solutions

Problems and solutions of phage therapy

Table 1 - Key problems with phage therapy, and how the problems can be overcome (Carlton, 1999)

Problems and limitations	Solutions
Bacterial debris present in the phage preparations	Modern technology allows density centrifugation, banding, and other methods of purification.
Attempts to remove host bacteria from therapeutic preparations, often using chemicals that may cause denaturation of phage protein coat	Sterile filtration. If chemical agents must be used, re-titrate the preparation over time to ensure that the phage remain viable
Rapid clearance of phages	Minor variations in coat proteins might enable some variants to be less easily recognized by the reticulo-endothelial system (RES) organs and to remain in the circulation for longer periods of time than the wild-type phage
Lysogeny: phage could not provide the rapid lysis and exponential growth in numbers that are needed for full efficacy.	Only use lytic phages and sequence the phages that are possible for clinical trials to indicate any lysogeny known gene
In chronic treatment, the neutralizing antibodies might prevent some proportion of the administered dose of phages from being able to adhere to the bacterial target (anti-phage antibodies)	Administer a higher dose of phage, to compensate for those that are rendered non-viable by interaction with neutralizingantibodies

Phage Therapy vs. Antibiotics

Table 2 - Attributes of phages that tend to favor a therapeutic response (Carlton, 1999)

Problem	Disadvantage of antibiotics	Advantage of phage therapy
The fate of the "treatment" molecule	Metabolic destruction of the molecule	Exponential growth in numbers, so that the phage makes more of itself at the site of infection and where it is needed
The concentration of the "treatment" required to kill a given bacterium within the spectrum	A relatively high dose of antibiotic is needed to kill a given bacterium. During initiation of therapy, the sub-lethal dose that bacteria experience affords them the opportunity to express resistance genes	Phage works by the principal that one phage particle is sufficient to kill a given bacterium
The ability to overcome bacterial resistance	Antibiotics are fixed, immutable chemicals that cannot adapt to a bacterial mutation and therefore become obsolete. Bacteria that have resisted them can pass along the resistance trait within and between species	Phages are organisms that undergo mutations, some of which can overcome bacterial mutations.
The spread of bacterial resistance	Antibiotics tend to have a wide spectrum, thereby provoking resistance in several species and genera of bacteria (in addition to the one targeted)	Although there are some exceptions, phages don't cross species boundaries. So even when the targeted bacterial species may become resistant to the phage, it is unlikely that other species will

Examples of Phage Therapy Trials for Some *Enterobacteriaceae*

Shigella sp.

Shigella is a group of Gram-negative, facultative intracellular pathogens. Perceived as the etiologic operators of bacillary dysentery of the bowels or shigellosis in the 1890s, Shigella was embraced as a class in the 1950s and sub grouped into four species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* (likewise assigned as serogroups A to D). The bacteria are fundamentally transmitted through the fecal-oral course and subsequently keep on threatening general wellbeing principally in developing nations where sanitation is poor. The evaluated yearly number of cases of shigellosis is 160 million, with 1.1 million deaths, generally kids less than 5 years of age in developing nations.

Phage therapy for *Shigella sp.*

A Phage inactivation of foodborne *Shigella* on ready-to-eat (RTE) spiced chicken

Table 3 - Phage inactivation of foodborne Shigella on ready-to-eat (RTE) spiced chicken (Zhang, Wang, & Bao, 2013)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of isolation	Antibiotic that is currently used
Shigella flexneri* 2a, *S. dysenteriae*, and *S. sonnei	Meat processing effluent and inpatient feces samples in different regions in China	SF-A2 SD-11 SS-92	Spiced chicken	Quinolones, ampicillin, trimethoprim-sulfamethoxazole, or, in patients over 17 years old, a 4-fluorquinolone such as ciprofloxacin.

Methodology

1. The bacterial strains were isolated from samples and confirmed as Shigella by a series of biochemical tests.

2. The Shigella isolates were resuspended in Luria-Bertani broth containing 15% glycerol and sorted at -80°C for phage isolation.

3. Isolated phages were prepared for Transmission Electron Microscope, the tested for thermal and pH tolerance.

4. Phages were applied to RTE spiced chickens in the presence of their respective hosts at 4°C to represent refrigeration, and a high host concentration (104 host cells/gram) and a ratio 104 phages to host cells. Incubation lasted to 72 hours to allow possible regrowth of the host.

Infection

Shigellosis (bacillary dysentery)

Results summary

1. No phages or host cells were isolated from the control sample.

2. The host concentration on samples treated by phages decreased by approximately 1 log10 after 2 hours from phage addition.

3. After 72 hours incubation, *S.dysenteriae* and *S.sonnei* concentration were reduced below the detection level.

4. *S.flexneri* and mixture group were declined below the detection limit.

5. Host concentration from control sample remained close to initial concentration level after 72 hours incubation. (Zhang, Wang, & Bao, 2013)

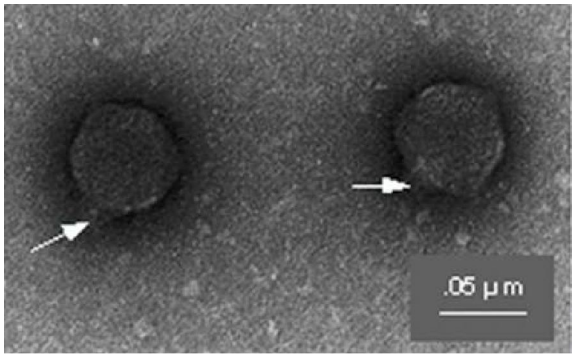

Figure 1 - Electron micrograph showing the morphology of *S. dysenteriae* type 1-specific phage SF-9 (Zhang, Wang, & Bao, 2013)

A* Isolation and genomic characterization of SfI, a serotype-converting bacteriophage of *Shigella flexneri

Table 4 - Isolation and genomic characterization of Sf1, a stereotype- converting bacteriophage of *Shigella flexneri* (Sun, 2013)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used
***Shigella flexneri* Stereotypes**	Diarrheal patients in China or purchased from National Collection of Type Cultures (NCTC), UK	SFl (17 serotype 1a, 5 serotype 1b, 10 serotype 2a, 10 serotype 2b, 10 serotype 3a, 2 serotype 3b, 5 serotype 4a, 5 serotype 4b, 4 serotype 5a, 10 serotype Y, 24 serotype X and 30 serotype Xv)	Raw meet	Quinolones, ampicillin, trimethoprim-sulfamethoxazole, or, in patients over 17 years old, a 4- fluorquinolone such as ciprofloxacin.

Methodology

1. Bacterial strains were serologically identified using Shigella antisera Kits and monoclonal antibody reagents.

2. S. flexneri strains were routinely cultured on LB agar or in LB broth with shaking at 37°C.

3. After the induction of phage SFl, a freshly grown colony of strain 019 was incubated in 10 ml LB broth overnight with vigorous shaking. After being induced for 30 min at 56°C with aeration, the cultures were centrifuged, and the supernatants were filtered through a 0.22 mm membrane filter (Promega) to remove bacterial cells. The filtrates were either used directly for phage infection assay or stored at 4°C with addition of 10% (v/v) chloroform.

4. S. flexneri strain 036 cells were prepared using the methods for phage lambda infection and lysogenization. The serotypes of isolated colonies were identified by slide agglutination assay.

5. The purified phages were absorbed on carbon-coated copper grids (300 mesh) and negatively stained with 2% (w/v) sodium phosphotungstate (pH 7.0). Samples were visualized with a Hitachi 600 electron microscope at 80 kV.

6. To determine the host range of phage SfI, one hundred and thirty two S. flexneri strains of 12 serotypes were infected with SfI. The preparation of component cells; phage infection and lysogeny isolation were performed as methods for strain 036 above. The SfI host range was determined by observing the presence of plaques and serologically identification of the lysogeny.

Infection

Shigellosis (bacillary dysentery)

Results summary

1. The SfI phage was induced and purified from a *S. flexneri* serotype 1a clinical strain 019.

2. Electron microscopy showed that the SfI phage has a hexagonal head and a long contractile tail, characteristic of the members of *Myoviridae* family.

3. SfI can convert serotype Y to serotype 1a and serotype X to serotype 1d, but cannot convert 10 other *S. flexneri* serotypes (1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, Xv) tested, suggesting that SfI has a narrow host range. (Sun, 2013).

C. Characterization of new Myoviridae bacteriophage WZ1 against multi- drug resistant (MDR) *Shigella dysenteriae*

Table 5 - Characterization of new Myoviridae bacteriophage WZ1 against multi-drug resistant (MDR) *Shigella dysenteriae* (Jamal, 2015)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used
S. dysentriae, S. dysentriae 208, S. dysentriae 209, Achromobacter xylosoxidans and Klebsiella pneumonia	Clinically isolated from Microbiology lab, Railway General Hospital, Pakistan.	WZ1	Water sample from Pakistan.	***Shigella sp.*:** Quinolones, ampicillin, trimethoprim-sulfamethoxazole, or, in patients over 17 years old, a 4-fluorquinolone such as ciprofloxacin. ***Achromobacter sp.***: Piperacillin, piperacillin/tazobactam and the carbapenems ***Klebsilla pneumonia***: Cefotaxime, gentamicin, amikacin and ceftriaxone.

Methodology

1. The Bacteriophage WZ1 was isolated from a wastewater sample, S. dysenteriaea culture was grown in Luria-Bertani broth (LB) for 24 h at 37 °C. About 100 ml wastewater was centrifuged to remove the suspended particles and algae, and was aseptically poured into a sterile 500 ml flask.

2. After filtration, filtrate was serially diluted (10 1–10 9) in phage buffer (1 M Tris pH 7.5, 5.8 gram of NaCl, 2.0 gram of MgSO4.7H2O, 50 ml/L of 5.0 ml/L of gelatin in distilled water), which was used for storage and dilution of bacteriophage stocks.

3. The plates were incubated overnight at 37 °C and were examined for the presence of plaques.

4. Phage suspension was pellet down by ultracentrifugation at 32,000 rpm for 4 h. Phage WZ1 morphology was examined by transmission electron microscopy of purified phage particles.

5. Bacterial reduction assay was performed: two flasks containing 100 ml LB broth media were inoculated with S. dysenteriae and incubated at 37 °C in incubator with constant shaking. One flask (OD.0.6) at 600 nm was inoculated with phage filtrate with multiplicity of infection (MOI) of one (2.1$\blacklozenge$1010 PFU/ml) and the other flask was left as control. Both flasks were then incubated at 37 °C with shaking and the OD at 600 nm readings were taken after every 2 h and recorded for up to 24 h using SP300 spectrophotometer by Optima.

Infection

Shigellosis (bacillary dysentery), Cystic Fibrosis, Pneumonia.

Results summary

1. The phage produced clear plaques on the lawn of the host, indicating that it was a virulent phage. The phage has a plaque size ranging from 0.4 to 2 mm in diameter and well-defined boundaries. The isolated phage was designated WZ1.

2. All 34 strains of bacteria used to determine the host range of phage WZ1 by using the spot test method, only *S. dysenteriaea, S. dysenteriaea 209, S. dysenteriaea 208, Achromobacter xylosoxidans,* and *K. pneumonia 3206* were susceptible. (Jamal, 2015)

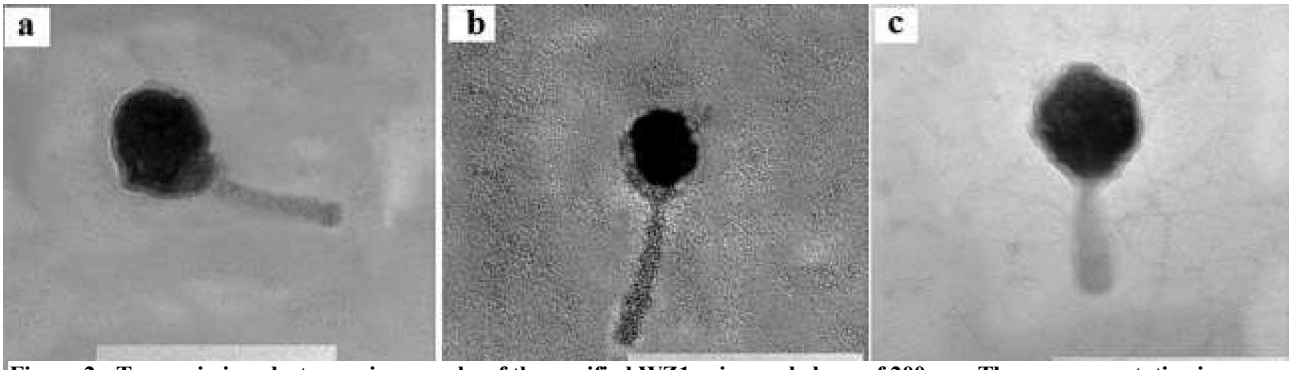

Figure 2 - Transmission electron micrographs of the purified WZ1 using scale bars of 200 nm. Three representative images a, b and c are shown. (Jamal, 2015)

Clostridium sp.

Clostridium sp. are Gram-positive spore-forming anaerobes discovered universally in the earth (soil and water) and the gastrointestinal tract of people and animals. There are a few noteworthy human and animal infections causing Clostridium species including: *Clostridium difficile* (pseudomembranous colitis), *Clostridium botulinum* (newborn child botulism), *Clostridium tetani* (lockjaw), and *Clostridium perfringens* (intense watery looseness of the bowels/necrotising enterocolitis [NEC]), with related pathology credited to the poisons they deliver. (Kiu, 2017)

Phage therapy for *Clostridium sp.*

A Evaluation of bacteriophage therapy to control *Clostridium difficile* and toxin production in an *in vitro* human colon model system

Table 6 - Evaluation of bacteriophage therapy to control Clostridium difficile and toxin production in an in vitro human colon model system (Meader, 2013

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used
C. difficile	Filter sterilized stool supernatants of patients suffering from CDI	ΦMMP01, ΦMMP02, ΦMMP03 and ΦMMP04	Prophages that naturally coexist with host but can kill it	Metronidazole and Vancomycin

Methodology

Available in referenced article. (Meader, 2013)

Infection

Hospital-acquired diarrhoea.

Results summary

1. Phage treatment reduced vegetative *C. difficile* cells in 2 of 3 experiments.
2. Phage treatment consistently reduced *C. difficile* toxin production.
3. Lysogenisation of the phage was demonstrated.
4. Commensal flora was not negatively affected by phage treatment. (Meader, 2013)

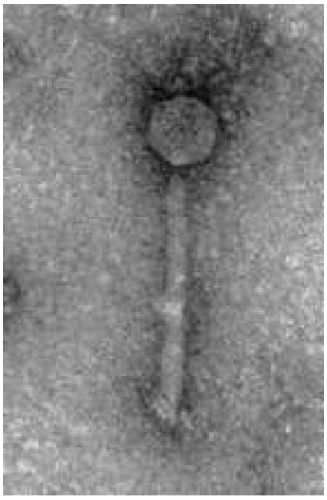

Figure 3 - *Clostridium difficile* bacteriophage ΦCD27 (Rea, 2013)

B Gut solutions to a gut problem: bacteriocins, probiotics and bacteriophage for control of *Clostridium difficile* infection

Table 7 - Gut solutions to a gut problem: bacteriocins, probiotics and bacteriophage for control of Clostridium difficile infection (Rea, 2013)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used
***Clostridium difficile* strains NCTC 11204 and 12726**	National Collection of Type Cultures (PHL, London)	ΦCD27	Induced from *C. difficile* NCTC 12727	Metronidazole and Vancomycin

Methodology

Available in referenced article. (Rea, 2013)

Infection

Hospital-acquired diarrhoea.

Results summary

1. The current knowledge regarding the use of phage therapy for the control of CDI phage is limited. This is mainly due to the lysogenic nature of *C. difficile* bacteriophages studied and isolated to date.
2. Thus far, no lytic phages specific for *C. difficile* has been isolated, with all reports to date showing that phage were recovered only after induction of the host with mitomycin C. (Rea, 2013).

Salmonella sp.

Salmonella sp. diseases are a noteworthy general medical issue. In the United States, they cause sickness in more than one million individuals consistently, bringing about evaluated restorative expenses of $ 365 million. In the European Union, they are the second most regular reason for gastrointestinal disease, with an affirmed case rate of cases for each 100,000 people in 2011. In China, it is assessed that 22.2% of foodborne maladies are because of *Salmonella*. Around the world, *Salmonella* contaminations are the second driving reason for foodborne bacterial illnesses.

They are frequently the etiological operators of gastroenteritis related with the utilization of crustaceans and contaminated meat items. *Salmonella* gastroenteritis is a noteworthy reason for food poisoning in developed nations, and neonatal mortality in developing nations. (Dougnon, 2017)

Phage Therapy for *Salmonella sp.*

A Bacteriophage P22 to challenge *Salmonella* in foods

Table 8 - Bacteriophage P22 to challenge Salmonella in foods (Zinno, 2014)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used	Bacterial Sample
Salmonella enterica serovar Typhimurium	18 isolates of *S.enterica* from chicken samples	Phage P22	Retail meat sample	Amoxicillin, ampicillin, ceftriaxone, cefotaxime.	Five liquids (energy drink, apple juice, pasteurized whole and skimmed milk, whole egg) and two solids (sliced chicken breast and chicken mince)

Methodology

1. Tryptone Soya Agar for the bacterial growth.

2. Bacteriophage P22 was amplified adding PFU. Suspension to TSB containing dilution of an overnight culture of its host LT2.

3. The titer of the phage stock was determined by the double-layer plaque titration method.

4. All food samples were preliminarily analyzed.

5. Samples of liquid foods previously were contaminated with diluted over-night cultures of LT2.

6. Food samples were incubated at 4 °C for 1h.

7. An aliquot of a P22 phage suspension was added.

8. Solid foods, previously conditioned at 4 °C, were treated, chicken breast were superficially spiked with a suspension of LT2.

Infection

Typhoid fever, food poisoning, gastroenteritis, enteric fever.

Results summary

1. Phage application was not enough to inactivate the entire population of bacteria; combination of phage with other antimicrobials (e.g. bacteriocin or essential oils) should be encouraged.

2. Bacteriophages are a promising tool to control food pathogens both in liquid and solid foods. (Zinno, 2014)

B Use of a lytic bacteriophage to control *Salmonella Enteritidis* in retail foods

Table 9 - Use of a lytic bacteriophage to control Salmonella Enteritidis in retail foods (Thung, 2017)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used	Bacterial Sample
Salmonella Enteritidis	Salmonella enterica serovar Enteritidis strain isolated from retail chicken meat	Phage SE07	Retail chicken meat	Amoxicilli, ampicillin, ceftriaxone, cefotaxime.	Fruit juice and fresh eggs (liquid form), beef and chicken meat (solid form)

Methodology

1. Bacterial survival was determined by performing viable count on selective CHROM agar.

2. All samples were further incubated at 4 °C for up to 48 h.

3. A lytic bacteriophage, SE07, was successfully isolated from retail chicken meat.

4. Prepare bacteriophage suspension.

5. Liquid Food samples were contaminated with diluted overnight cultures of *S. Enteritidis*.

6. Phage suspension was added to the samples.

7. Six slices of the meat samples were spiked with a suspension of *S. Enteritidis*.

8. Phage suspension was inoculated by using a small spray dispenser.

9. All samples were incubated for 12, 24 and 48 h at 4 °C.

10. All measurements were carried out in

triplicates and mean value of control was compared to that of treated samples by Student's t-test.

Infection

Typhoid fever, food poisoning, gastroenteritis, enteric fever.

Results summary

1. Some characteristics as structure and composition can affect bacteriophage activity.
2. Bacteriophage preparations use for bio-control must be free of undesirable genes, to prevent or limit bacteriophage-mediated emergence of new pathogenic bacterial strains.
3. Bacteriophage SE07 might serve as a promising tool for the bio-control of *S. Enteritidis* in either solid or liquid food. (Thung, 2017)

C. Use of a bacteriophage cocktail to control *Salmonella* in food and the food industry

Table 10 - Use of a bacteriophage cocktail to control Salmonella in food and the food industry (Galarce, 2014)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used
Salmonella enterica serovar Typhimurium, Salmonella Enteritidis	Human	UAB_Phi20 UAB_Phi78 UAB_Phi87	Amoxicillin, ampicillin, ceftriaxone, cefotaxime.	Pig skin, Chicken breast, Fresh eggs, Packaged, lettuce.

Methodology

1. Overnight cultures of S. Typhimurium and S. Enteritidis strains were diluted in fresh Luria Bertani broth.

2. The cultures were appropriately diluted in NaCl 0.9% to achieve the bacterial concentration used to contaminate the different food matrices.

3. Viable counts of salmonella strains in food matrices were routinely determined.

4. The lytic bacteriophages were based on their broad host range and the high killing kinetics observed in in vitro Salmonella cultures.

5. Preparation of food sample (pig skin, poultry, fresh eggs, fresh lettuce) then they were contaminated with salmonella.

6. Bacteriophage cocktail used to treat.

Infection

Typhoid fever, food poisoning, gastroenteritis, enteric fever.

Results summary

A bacteriophage cocktail composed of a 1:1:1 mixture of the bacteriophages UAB_Phi20, UAB_Phi78, and UAB_Phi87 was evaluated for *Salmonella* bio control in pig skin, two representatives of poultry production (chicken breasts and fresh eggs), and ready to eat product, i.e., packaged lettuce. (Galarce, 2014).

Escherichia coli

Escherichia coli is an ordinary occupant of the digestive organs of most creatures, including people. Some *E. coli* strains can cause a wide assortment of intestinal and extra-intestinal sicknesses, for example, diarrhea, urinary tract infections (UTI), septicemia, and neonatal meningitis. (Clermont, 2000). *E. coli* has one of the most extensive spectra of infection of any bacterial species. The *E. coli O157:H7* that as of late developed as a noteworthy food pathogen is a live case. Moreover, there is an absence of compelling treatment for *E. coli* diseases. Oral rehydration goes about as the pillar of treatment. Innumerable lives have been spared by this basic and modest measure. In any case, it doesn't influence the common course of the sickness or the inherent action of hostile to bacterial. (Xu, 2015).

Phage Escherichia

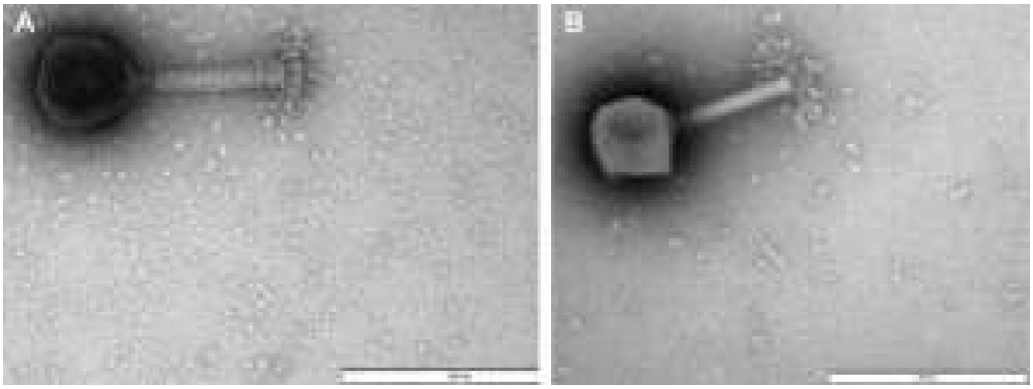

Figure 4 - Electron micrographs showing structure of phage FAHEc1. Scale bar shows 200 nm. (Sukumaran, 2015)

Therapy for coli

A Use of a bacteriophage to inactivate *Escherichia coli* O157:H7 on beef

Table 11 - Use of a bacteriophage to inactivate *Escherichia coli* O157:H7 on beef (Hudson, 2013)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used	Bacterial Sample
E.coli O185:H7	Sewage sample	FAHEc 1	Raw screened sewage	Ciprofloxacin (Cipro), Trimethoprim-sulfamethoxazole (Bactrim).	Beef

Methodology

1. Isolation of FAHEc1
2. Phage host range determination
3. Phage DNA isolation and analysis
4. Inactivation of E. coli O157:H7 in vitro
5. Demonstration of the control of E. coli O157:H7 on raw meat by phage FAHEc1
6. Control of E. coli O157:H7 with varying phage concentration and time of exposure
7. Demonstration of phage replication on beef
8. Control of E. coli O157:H7 on beef subject to simulated chilling
9. Scenarios
10. Statistical analysis

Infection

Diarrhea, Hemorrhagic colitis and Hemolytic uremic syndrome.

Results summary

1. The phage lysed 28 of 30 of *E.coli* O185:H7.
2. Phages are useful for the control of foodborne pathogens, and this is being reflected by the introduction of commercial phage products for use in food. (Hudson, 2013).

B Characteristics of coliphage ECP4 and potential use as a sanitizing agent for biocontrol of *Escherichia coli* O157:H7

C

Table 12 - Characteristics of coliphage ECP4 and potential use as a sanitizing agent for biocontrol of Escherichia coli O157:H7 (Lee, 2013)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used	Bacterial Sample
E.coli O185:H7 NCTC1207 9	Sewage sample	ECP4	Sewage	Ciprofloxacin (Cipro), Trimethoprim-sulfamethoxazole (Bactrim).	Vegetable juice and cabbage

Methodology

1. *E. coli* was grown in Luria Bertani broth (LBC) or agar at 37 _C overnight in a shaking incubator.
2. To isolate coliphage for E. coli, bovine fecal samples were analyzed by plaque assay.
3. Three temperatures (55, 65 and 70º C) were selected to study the thermal tolerance of coliphage ECP4 in LBC.
4. To confirm the shiga toxin-carrying bacteriophage, PCR was performed for detection of shiga toxin encoding gene in coliphage ECP4.
5. Out of 6 tested coliphages, coliphage ECP4 exhibited a broad host range spectrum, which appeared the characteristic to impede E. coli O157:H7 growth, infecting 27 out of 28 E. coli O157:H7.

Infection

Diarrhea, Hemorrhagic colitis and Hemolytic uremic syndrome.

Results summary

The morphology, stability, and molecular characterization of coliphage ECP4 were experimentally confirmed.

Coliphage ECP4 might serve as an effective form of biocontrol to reduce *E. coli* O157:H7 in foods and remove biofilm- formed *E. coli* O157:H7 at the same time. (Lee, 2013).

Computational determination effects of virulent *Escherichia Salmonella* bacteriophages on gut

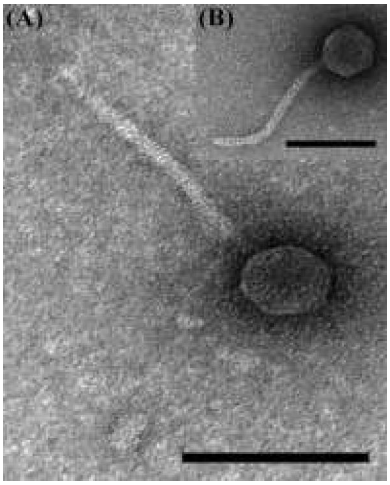

Figure 5 - Electron micrograph of (A) coliphage ECP4 for *E. coli* O157:H7 (Lee, 2013) of the ***coli* and human**

Table 13 - Computational determination of the effects of virulent Escherichia coli and Salmonella bacteriophages on human gut (Mostafa, 2016)

Bacterial species	Sample of bacterial isolation	Phage name	Sample of phage isolation	Antibiotic that is currently used	Bacterial Sample
Ecoli O17:H7 Ecoli O104:H4 Salmonella typhimurium	Undercooked meat Drinking impure water	Phage 933W Phage VT2SA Phage P22	Sewage	Ciprofloxacin (Cipro), Trimethoprim-sulfamethoxazole (Bactrim).	Computational

Methodology

Based on domain-domain interactions (DDIs) Model

Infection

Diarrhea, Hemorrhagic colitis and Hemolytic uremic syndrome.

Results summary

Several effects were detected such as: antibacterial activity against a number of bacterial species in human gut, regulation of cellular differentiation and organogenesis during gut, lung, and heart development, ammonia assimilation in bacteria, yeasts, and plants, energizing defense system and its function in the detoxification of lipopolysaccharide, and in the prevention of bacterial translocation in human gut. (Mostafa, 2016)

Conclusion

After listing the previous examples of phage therapy for several *Enterobacteriaceae*, we would like to conclude our review with the following recommendations.

First, we strongly suggest that biologists who are interested in phage therapy should conduct a comparison experiments. As shown in the previous tables in this article, there was no information about the efficiency of antibiotic treatments in comparison with the efficiency of phage therapy for a given bacterial species. Such experiment could be conducted in both in vitro methods (directly exposing bacteria to both treatments), and in vivo methods (injecting lab mice with bacteria then give them the treatments).

Secondly, we also suggest that after the comparison is made, a human trial should be given for selected group of volunteers and patients.

Acknowledgments

The authors would like to express their gratitude to the King Abdulaziz University Research Endowment Fund and the Science Research and Innovation Unit at the Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, for supporting this work financially. We are also thankful for Dr. Sanaa Al-Attas for her effort in helping us complete this article.

References

1. Puniya, A.K., Singh, R. and Kamra, D.N. eds. 2015. *Rumen microbiology: from evolution to revolution*. Springer India.
2. Rea, M.C., Alemayehu, D., Ross, R.P. and Hill, C., 2013. Gut solutions to a gut problem: bacteriocins, probiotics and bacteriophage for control of Clostridium difficile infection. *Journal of medical microbiology*, *62*(9), pp.1369-1378.
3. Carlton, R. M. (1999). Phage therapy: past history and future prospects. *ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS-ENGLISH EDITION-*, *47*, 267-274. (Carlton, 1999)
4. Yang, F., Yang, J., Zhang, X., Chen, L., Jiang, Y., Yan, Y.,... & Xue, Y. (2005). Genome dynamics and diversity of Shigella species, the etiologic agents of bacillary dysentery. *Nucleic acids research*, *33*(19), 6445-6458.
5. Zhang, H., Wang, R. and Bao, H., 2013. Phage inactivation of foodborne Shigella on ready-to-eat spiced chicken. *Poultry science*, *92*(1), pp.211-217.
6. Sun, Q., Lan, R., Wang, Y., Wang, J., Wang, Y., Li, P., Du, P. and Xu, J., 2013. Isolation and genomic characterization of SfI, a serotype-converting bacteriophage of Shigella flexneri. *BMC microbiology*, *13*(1), p.39.
7. Jamal, M., Chaudhry, W.N., Hussain, T., Das, C.R. and Andleeb, S., 2015. Characterization of new Myoviridae bacteriophage WZ1 against multi - drug resistant (MDR) Shigella dysenteriae. *Journal of basic microbiology*, *55*(4), pp.420-431.
8. Kiu, R., Caim, S., Alcon-Giner, C., Belteki, G., Clarke, P., Pickard, D.,... & Hall, L. J. (2017). Preterm Infant-Associated Clostridium tertium, Clostridium cadaveris, and Clostridium paraputrificum Strains: Genomic and Evolutionary Insights. *Genome Biology and*

Evolution, *9*(10), 2707-2714.

9. Meader, E., Mayer, M.J., Steverding, D., Carding, S.R. and Narbad, A., 2013. Evaluation of bacteriophage therapy to control Clostridium difficile and toxin production in an in vitro human colon model system. *Anaerobe*, *22*, pp.25-30.
10. DOUGNON, T. V., LEGBA, B., DEGUENON, E., HOUNMANOU, G., AGBANKPE, J., AMADOU, A.,... & de SOUZA, M. (2017). Pathogenicity, Epidemiology and Virulence Factors of Salmonella species: A Review. *Notulae Scientia Biologicae*, *9*(4), 460-466.
11. Zinno, P., Devirgiliis, C., Ercolini, D., Ongeng, D. and Mauriello, G., 2014. Bacteriophage P22 to challenge Salmonella in foods. *International journal of food microbiology*, *191*, pp.69-74.
12. Thung, T.Y., Premarathne, J.M.K.J.K., San Chang, W., Loo, Y.Y., Chin, Y.Z., Kuan, C.H., Tan, C.W., Basri, D.F., Radzi, C.W.J.W.M. and Radu, S., 2017. Use of a lytic bacteriophage to control Salmonella Enteritidis in retail food. *LWT-Food Science and Technology*, *78*, pp.222-225.
13. Galarce, N.E., Bravo, J.L., Robeson, J.P. and Borie, C.F., 2014. Bacteriophage cocktail reduces Salmonella enterica serovar Enteritidis counts in raw and smoked salmon tissues. *Revista Argentina de microbiologia*, *46*(4), pp.333-337.
14. Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and simple determination of the Escherichia coli phylogenetic group. *Applied and environmental microbiology*, *66*(10), 4555-4558.
15. Xu, Y., Liu, Y., Liu, Y., Pei, J., Yao, S., & Cheng, C. (2015). Bacteriophage therapy against Enterobacteriaceae. *Virologica Sinica*, *30*(1), 11-18.
16. Hudson, J. A., Billington, C., Cornelius, A. J., Wilson, T., On, S. L. W., Premaratne, A., & King, N. J. (2013). Use of a bacteriophage to inactivate Escherichia coli O157: H7 on beef. *Food microbiology*, *36*(1), 14-21.
17. Sukumaran, A.T., Nannapaneni, R., Kiess, A. and Sharma, C.S., 2015. Reduction of Salmonella on chicken meat and chicken skin by combined or sequential application of lytic bacteriophage with chemical antimicrobials. *International journal of food microbiology*, *207*, pp.8-15.
18. Lee, Y. D., Kim, J. Y., & Park, J. H. (2013). Characteristics of coliphage ECP4 and potential use as a sanitizing agent for biocontrol of Escherichia coli O157: H7. *Food control*, *34*(2), 255-260.
19. Mostafa, M.M., Nassef, M. and Badr, A., 2016. Computational determination of the effects of virulent Escherichia coli and salmonella bacteriophages on human gut. *Computer methods and programs in biomedicine*, *135*, pp.27-35.

4/25/2019