Assessment of Therapeutic Potential of Bacteriophages to Control *Escherichia coli* Infection in Swiss Mice Model

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**ABSTRACT**

*Escherichia coli* is one of the causes of infections in human and farm animals. Bacteriophages (or phages) have been proposed as natural antimicrobial agents to kill bacterial cells within human or animal bodies. The study aimed to isolate phages against *E. coli* from sewage sources and used it as a candidate for therapeutic purpose against lethal dose of bacterial infection in Swiss mice model. Lytic phage was isolated from sewage water collected in Jimma town following standard enrichment method against *E. coli*. The bacterium was obtained from Jimma University Specialized Hospital bacteriology laboratory. Phage characterization as well as *E. coli* identification was performed. Lytic phage was used to assess survival of mice health from lethal dose (LD100) of *E. coli* infection. Three bacteriophages were isolated from among 5 different sewage sources processed. One of the three selected bacteriophages has a mean of 5 mm plaque size and resistant to chloroform and ether. This phage was selected and used in mice model and inoculation of mice with high-dose of φJS3 phage alone produced no adverse effects. Then swiss mice injected intraperitoneally with the lethal dose of *E. coli* (10⁹CFU/ml) was grouped into three categories. Group one with six mice was given standardized number of lytic phages (10⁹PFU/ml), the second with antibiotic treatment and the third as control group was given sterile physiologic saline. 10⁹CFU/ml *E. coli* cells caused death in the control mice within 5 days. In contrast, intraperitoneal administration of purified bacteriophage or antibiotic suppressed *E. coli* lethality in mice (100% survived). This study supports the view that bacteriophages are common in Jimma area and could be useful in the treatment of animal infections caused by pathogenic bacteria.

**Key words:** Bacteriophage; *Escherichia coli*; Lethal dose; Phage therapy; Survival of mice
INTRODUCTION

Bacteriophages (phages) are non-hazardous self-replicating viruses of bacteria that increase their numbers as they destroy target hosts (Ackermann et al., 2011). Phages infect bacteria and use the bacterial cell’s genetic apparatus to produce more viral particles. In the process, the virulent lytic phages kill their host and as a result bacterial infections can be cleared (Abedon et al., 2011). These viruses are highly abundant and chiefly concentrated in the niches of almost all natural environments on this planet (Bruttin and Brussow, 2005) and up to 2.5 x 10^8 plaques forming units per millilitre (PFU/ml) number of phages was determined in natural unpolluted water (Bergh et al., 1989). Currently, more than 6000 different bacteriophages have been discovered, morphologically described including 6196 bacterial and 88 archael viruses (Jensen 2006). The first clinical studies of phage therapy began in 1919 when d’Herelle used a phage preparation to treat a twelve-year old boy suffering from dysentery and completely recovered within a few days (Sulakvelidze et al., 2001). Bacteriophages were considered to be safe during the long therapeutic history in Eastern Europe, and former Soviet Union. More recently, British scientists reported on the successful veterinary application of E. coli phages in the 1980s, and excellent studies on phage therapy were carried out by Smith and colleagues, using E. coli infection in mice and farm animals (Merril et al., 2003).

Since, bacteriophage can target against pathogenic specific bacterial strains (Wagner and Waldor, 2002), they can be the solution against antibiotic resistant bacteria. They are self-limiting with decrease in the number of the specific bacteria concomitantly leading to their decrease and elimination from the body without any harm. The phages are capable of faster increase than bacterial growth rate. As a result, they can accumulate and replicate at the site of infection. Moreover, unlike bacterial resistance to antibiotics, phages can mutate in stepwise with evolving bacteria. If the bacteria become resistant to one phage strain, there is a natural abundance of phage species which can infect the bacteria having a similar target range (Sulakvelidze et al., 2001).

Escherichia coli exist as a normal flora in the lower gastrointestinal tracts of warm-blooded organisms in addition as a pathogen. It causes a third of cases of childhood diarrhoea in developing countries as well as the most prominent cause of diarrhoea in travellers to developing countries (Reid et al., 2001). E. coli is also associated with diarrhoea in pet and farm animals. The World Health Organization estimates that 5 million children die each year as a consequence of acute diarrhoea (Donnenberg, 2002; Boschi-Pinto et al., 2008). The bacterium is also the leading causes of urinary tract infection, septicaemia and neonatal meningitis.

In the current time, due to the excessive use of antimicrobial drugs in bacterial infections, the increasing prevalence of antibiotic-resistant bacteria has become a worldwide health security issue. Hence, the search for alternatives to antibiotics is now one of the top priorities in the field of microbiology. Antibiotics kill bacteria rather non-specifically and can therefore lead to numerous side effects. But phages have already been proven to be a good natural antimicrobial treatment. The use of phages as bacterial therapeutics may have brought back to existence as a promising alternative (Fiorentin et al., 2005). Even though phages are commonly quoted as one of the major assets of phage therapy (Schuch et al., 2002), the classical protocols of isolating phages and use them in experiments are limited in developing countries.

The implication of this study is to establish the basis for additional information of the therapeutic effect of bacteriophage in treating diseases caused by E. coli in mice model. In Jimma area
this approach was not attempted at all. Moreover, this work would show the way for further investigation of bacteriophages to target bacterial aetiologies on various animal, human beings and plant organisms. The main objective of the study was to isolate bacteriophages from environmental sewage sources, and evaluate their therapeutic efficacy to treat lethal dose of E. coli infection in mice model.

MATERIALS AND METHODS

Study area and study period
The study was conducted at Jimma University during the period from March to July, 2014. The university is one of the leading universities in Ethiopia. Geographically, it is located in Jimma town which is 354 Km away from Addis Ababa. The town is characterized by tropical climate i.e. heavy rainfall and warm climate having a mean annual temperature and rainfall of 24.9 °C and 800-2500 mm³, respectively. The town has a total population estimated to be 194,139. Out of these male is 98,907 and female 95,233 (Anon, 2008; Personal communication with Zone health department during the study period, June 2014). Moreover, the university has specialized hospital that provides referral health services for an estimated of 15 million populations.

Host Bacterial strains
The bacterial strain used in this study was obtained from Jimma University Specialized Hospital bacteriology laboratory. Escherichia coli was biochemically identified using standard protocol (Cheesbrough, 2006) including growth characteristics on MacConkey agar medium (Lactose feremneter), Gram reaction (Gram negative rod), Indole production (+), Citrate Utilization (+), Kligler Iron agar (Acid slant, Acid butt with gas production), Lysin decarboxylase (-), Motility (+), Urease (-) and oxidase (-) tests. Pure culture was grown from single colony on Nutrient agar (Oxoid, Hampshire,UK) and then transferred into Nutrient broth (Oxoid, Hampshire,UK) to be grown at 37°C to an absorbance of 1.0 (approximately 10¹⁰ CFU/ mL) at 600 nm. Culture was centrifuged (10,000 x g for 10 min) and the pellet was suspended in phosphate buffer solution (pH 7.2) to a concentration of 10¹⁰CFU/mL. Appropriate dilutions from this preparation were made in sterile normal saline solution and used for in vivo and in vitro experiments. The host bacterium was grown aerobically in Nutrient broth at 37°C and used in logarithmic phase in all the experiments.

Bacteriophage isolation
Isolation of Bacteriophages specific against E. coli was carried out from sewage sample according to the standard enrichment protocol described by VanTwest and Kropinski (2009). Briefly, sewage samples were collected in sterile 200ml glass containers from five different randomly selected sewage locations in Jimma town [i.e. Jimma University Hospital (1 point), College of Natural Sciences (2 points) and Jimma Kochi area (2 points)]. The samples were processed for phage isolation at Jimma University Medical Microbiology Laboratory. Fifty millilitre of each sample was centrifuged at 10,000xg for 10 minutes to remove particulate materials. The supernatant was filter sterilized by passing through a 0.45 μm membrane filter (Merk, Eurolab [PTFE], U.S.A) to remove microorganisms except viruses. The filtrate (50 ml) was mixed with equal volume of sterile double strength nutrient broth in a 250 ml Erlenmeyer flask. The flask was incubated with 5 ml of the indicator strain, prepared as described in the above section, overnight at 37°C in static incubator shaking in between every 2 to 4 hourly. The following day, the mixture was centrifuged at 10,000xg, 4°C for 15 minutes. Then the supernatant containing phage was passed through a 0.45 μm pore membrane filter under aseptic
condition whereas the pellet was discarded.

**Amplification of Bacteriophage**

Amplification step was run on the filtrate from the above last step by mixing 50 ml of the filtrate with equal volume of double strength nutrient broth containing 2mmol MgSO$_4$ and incubated with 5ml of the indicator strain (E. coli). The mixture was incubated at 37°C in the incubator overnight shaking every two to four hours. The next day, the mixture was centrifuged at 10,000 x g, 4°C for 15 minutes. The supernatant considered to contain phages was filter sterilized through a 0.45 μm pore membrane filter under aseptic condition. The amplified filtrate obtained was used for phage activity testing (VanTwest and Kropinski, 2009).

**Detection of Bacteriophage Activity**

Bacteriophage activity against the host bacterium was checked using spot assay as described by Cerveny et al (2002) and Kumari et al (2010). The indicator cells (0.1ml) were added to sterile molten soft agar (0.75%) prepared and maintained at about 45°C in a water bath and quickly mixed. The contents were poured onto previously prepared nutrient agar plate. Around 12-15 μl of amplified filtrate was spotted on each plate at 2 different places. The plates were allowed to dry at room temperature and then incubated overnight at 37°C. The following day, the plates were examined for clearance at the spotted area.

**Phage Purification**

All the isolated phages were purified by successive single plaque isolation until homogenous plaque was obtained per the standard procedure described by Sambrook and Russel (2001). For purifying a single strain from a heterogeneous stock, a single plaque was picked aseptically and transferred into tube containing 5ml broth containing fresh log phase grown E. coli strain. Another tube containing indicator strain left as a control. Both tubes were incubated at 37°C under shaking condition until complete lysis occurred in the test preparation. Phage host mixture and control preparation was centrifuged at 10,000xg for 15 minutes at 4°C. The supernatant was filter sterilized by passing through 0.45μm pore membrane to remove any bacterial contaminants. The filtrate was serially diluted and assayed for plaques in order to quantify the number of plaques. The procedure was repeated for three times to ascertain the purity of isolated phage. The plaque recorded and scored as: confluent lysis with some halo formation. Purified phage was stored for the subsequent purpose at 0 - 4°C.

**Titration of Bacteriophage**

Titre of the phage preparation [plaque forming units per millilitre (pfu/ml)] was estimated by the soft agar overlay method as described by Adams (1959). High titer was prepared by adding phages to early log phase host culture at an MOI (multiplicity of infection) of 0.1 and incubating at 37°C, until complete clearance was obtained. MOI is the ratio of phage to infection targets of the bacterium cells.

\[
\text{Multiplication of infection (MOI)} = \frac{\text{Plaque forming units (pfu) of phage used for infection}}{\text{number of cells}}
\]

Large plaque forming phage type was selected for the present subsequent study. Serial dilution of the bacteriophage sample was made in sterile phosphate buffer solution. A 0.1ml of bacteriophage suspension from each dilution was mixed with 0.1ml of the indicator host cells of E. coli and added to about 8ml of molten soft agar held at 45°C. The mixture was thoroughly mixed without producing air bubbles and quickly poured over previously moisture dried nutrient agar plate. The pellets were swirled gently to ensure even distribution of the mixture. The overlays
allowed to solidify upright for 30 minutes at room temperature and then incubated at 37°C for overnight in inverted position. The following day plates with 30 to 300 plaques were selected and counted. Original phage count (titre) was determined by using the following formula to calculate phage number.

\[ \text{Number of plaques (PFU/ml)} = 10 \times \text{plaques counted} \times \frac{1}{\text{reciprocal of the dilution}} \]

It is assumed that each phage particle infects a cell, multiplies and releases mature phages that proceed to a second round of infecting neighbouring cells leaving a clear area on the lawn called plaque.

**Phage characterization**

The adsorption rate, burst size, sensitivity to chloroform and temperature and its host range was determined essentially according to the method used by Adams (1959).

**Mice infection model development and phage toxicity testing**

In vivo experiment was carried out on female Swiss mice of 8 to 10 weeks old with an average weight of 30g at Jimma University Veterinary pharmacology and toxicology laboratory. All mice were fed antibiotics free diet and given water ad libitum. For the animal handling in this experiment, ethical handling of the mice experiment was done following standard animal welfare guidelines.

*E. coli* cells were grown in 5ml nutrient broth medium at 37°C and were centrifuged at 10,000xg for 10 min at the logarithm phase. The cell pellet was washed with normal saline, centrifuged again under the same conditions, and finally resuspended in 1mL normal saline. Dilution adjustment was made from the suspension after the number of Colony Forming Units per millilitre (CFU/ml) of the bacterial cells was determined. Bacterial cell numbers each with \(10^6, 10^7, 10^8, 10^9\) and \(10^{10}\) CFU/ml were prepared in sterile tubes. The bacterial cells in 0.2 mL volume were used to inject into one group of 6 Swiss mice to evaluate for lethal dose over 5 days observing every day. Another group of 6 mice was injected with 0.2 ml of sterile normal saline intraperitoneally alone and retained as control. LD100 is defined as the lethal dose of *E. coli* cell counts that kill 100% of the test mice population. The following points were given to determine the signs of sickness on mice in 3 to 5 days: Point (pt) 5=normal, unremarkable condition, Pt 4=slight illness, lethargy and ruffled fur, Pt 3=Moderate illness, severe lethargy, ruffled fur and humbled back, Pt 2=severe illness above signs plus exudative accumulation around closed eye, Pt 1=Moribund state, and Pt 0=Death.

For assessment of the toxicity of the phage, mice were injected with 0.2 ml phage suspension (\(10^{11}\) PFU/ml) through the intraperitoneal (i.p.) route in one group of 6 mice and followed for five days for any signs of illness. Sterile normal saline injected mice (n=3) were retained as controls.

**Measuring the efficacy of phage to rescue mice from *E. coli* infection in vivo:**

Three categories with 6 mice in each group were prepared and all of the mice were exposed to lethal doses of *E. coli* (\(10^9\) CFU/mouse) via intraperitoneal (i.p.) injection. Group I: followed by an immediate sterile normal saline injection and used as a control; Group II: immediately given with Bacteriophage (\(10^9\) PFU/mouse) i.p. at MOI of 1; and Group III: immediately followed by antibiotic drug (Ciprofloxacin) injection. The mice were observed for signs of illness every hourly during the first 5 hours after injection and then daily for the next 5 days. The mortality rate between mice treated with phage, ciprofloxacin and those not protected was compared. Moreover, reduction of mice weight was followed daily for five days. Points were given as follows: Point (pt) 5=normal, unremarkable condition, Pt
4 = slight illness, lethargy and ruffled fur, Pt 3 = moderate illness, severe lethargy, ruffled fur and humped back, Pt 2 = severe illness above signs plus exudative accumulation around closed eye, Pt 1 = Moribund state, and Pt 0 = Death.

Statistical methods

Statistical analysis of the data was done using Microsoft Excel 2007. Most data are expressed as descriptive with mean of more than two experiment value for every variable. Student’s ‘t’ test was used to compare different variables and p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Isolation of *E. coli* – specific phages

Three lytic bacteriophages were isolated against pathogenic *E. coli* from among the five sewage samples collected. This is possible since bacteriophages are extremely common in every environment particularly in bacterial contaminated environments (Bergh et al. 1989). One of the three phages demonstrating large clear plaque size was selected for subsequent study, indicating that this phage can adsorb efficiently to the indicator host (Fig.1). This bacteriophage was named as bacteriophage фJS3 because the virus was recovered from the university compound sample (phage Jimma natural science Sample 3).

![Figure 1 - Purified *E. coli* bacteriophage фJS3 plaques after 24 hours of incubation at 37 degree Celsius isolated from sewage samples in Jimma town, 2014.](image)

The indicator host used in this research was *Escherichia coli*. This species is responsible for intra- and extra- intestinal infections in humans and animals. Furthermore, it is among known antimicrobial resistant threats in the world (Gaschignard et al., 2011; Clermont et al., 2011).

Phage characterization

The *in vitro* characteristic of lysis rate at MOI of 1 has a burst plaque size of averagely 5mm after overnight incubation at 37°C (Fig. 1). In samples where virulent bacteriophages were present, they replicate and lyse the bacteria, causing a zone of clearance (a plaque) on the plate. Theoretically, each plaque is formed by one virus and the plaque size is proportional to the efficiency of the virus (Ackermann, 2011).

The size of the plaque is proportional to the efficiency of adsorption, and the burst size of the phage. Moreover, a clear plaque was formed because the phage is completely virulent or the host bacterium is susceptible to the phage (Gallet et al., 2011). The number of plaques multiplied by the dilution factor is equal to the total number of viruses in a test suspension. This is analogous to bacterial cell enumeration and the same guidelines for colony forming unit (CFU’s) apply to plaque forming units (PFU’s) in defined volume of solution (Adams, 1959).

The phage host range was checked against clinical isolates of *Staphylococcus aureus*, *Psudomonas auriginosa* and *Klebsiella pneumoniae* forming no plaques, except on the specific host *E. coli*. This is in agreement with the biology of bacteriophages where they have a remarkable specificity to their hosts, unlike antibiotics (Wagner and Waldor, 2002).

In this study, Phage фJS3 is resistant to chloroform and ether application maintaining its number and infectivity as equal as the control phage maintained in normal saline after one hour of exposure. However, фJS3 phage was easily inactivated in absolute ethanol after one
hour of exposure. On the basis of its tolerance to chloroform and ether, it can be deduced that the phage was devoid of lipid component (non-enveloped) in its structure as these solvents are known to show effect on enveloped phages (Ackermann, 2007). This observation also suggests that chloroform can be used to prevent bacterial contamination of phages during storage or it may be employed for phage isolation from environmental samples (Kutter and Sulakvelidze, 2005; VanTwest and Kropinski, 2009). The phage inactivation in absolute ethanol may be because of denatured protein coat of the virus.

**Determination of lethal dose of *E. coli* in infected mice**

Mice challenged with *E. coli* doses ranging from $10^6$ to $10^8$ CFU/ml was not lethal within five to seven days. Injections of $10^9$ and $10^{10}$ bacterial cells lowered the survival rate of the mice in a dose-dependent manner. The number of bacteria when adjusted to $10^9$ CFU/ml, all the mice died in 3 to 5 days. It was lethal in more than 66% of the mice within 4 days and 100% died within 5 days of injection (Table 1). This dose was recorded as minimum lethal dose (LD100) of the bacterial cell in five days time. All infected mice receiving normal saline treatment only (control) did not show any signs of slight illness or bacteraemia over a period of 5 days. This was scored to develop a phage therapy system that relaxes time to assess continued activity of phage or antibiotic against the bacteria in the mice system.

**Table 1. Effect of defined dose of *Escherichia coli* cells after intraperitoneal injection into Swiss mice and followed for five consecutive days.**

<table>
<thead>
<tr>
<th>CFU/ml Dose</th>
<th>Number of Mice used in the experiment</th>
<th>Death of mice</th>
<th>Modal day of death</th>
<th>Average point of mice health</th>
<th>Mice Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>$10^{10}$</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>$10^9$</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>$10^8$</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4.3</td>
<td>0</td>
</tr>
<tr>
<td>$10^7$</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td>$10^6$</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>$10^5$</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5.0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Only Sterile physiological saline was injected intraperitoneally

$^5$ Point (pt) 5: Normal, unremarkable condition; Pt 4: Slight illness, lethargy and ruffled fur; Pt 3: Moderate illness, severe lethargy, ruffled fur and humbled back; Pt 2: Severe illness above signs plus exudative accumulation around closed eye; Pt 1: Moribund state; and Pt 0: Death.

**Toxicity testing of phage in mice**

From the result obtained, there was no observable changes found due to administration of high titre bacteriophage ($10^{11}$ PFU/ml) or normal saline into the mice group. All the tested were given 5 point (i.e. normal with no remarkable condition). Thus the phage was decided to be safe to study for treatment experiments. Similar study has been reported indicating that phages themselves, at least when injected into the peritoneal cavity, did not give rise to any observable adverse effects (Soothill, 1992).

Another study reported by Bogovazova and his colleagues (1991) showed that purified phage preparations were nontoxic in mice and guinea pigs after intravenous, intranasal, and intraperitoneal administration, even after a dose approximately 3,500-fold higher
(estimated by body weight) than the human dose was given to mice during acute toxicity studies. Therefore, the result obtained in our study gave similar finding although only intraperitoneal systemic administration was used.

Mice treatment experiment

The control, phage and antibiotic categories of mice in each group observed on day 2 of post infection showed that all the mice were still alive. However, there was slight sign of illness in the bacteria plus normal saline injected group (Control). On the third day of post bacterial infection, there were distinct differences in survival and morbidity.

Since one of the six mice from Group I died whereas the remaining 5 mice showed 3 point (remarkable illness). On the fourth day of post infection, four of the six (67%) mice died and on the next day (day 5) all of the remaining (unprotected) mice died. The phage and Ciprofloxacin administered mice groups (Group II and III) were 100% rescued from the lethal infection due to E. coli after five days (with 5 point observation) compared to the unprotected group. (Fig 2). Those mice which have taken LD100 E. coli injection showed a mean weight of reduction compared with the phage protected ones, p<0.05. After fifth day of post infection, all mice were killed. On the other hand, the weight of mice injected with LD100 E. coli and immediately protected by bacteriophages or Ciprofloxacin were showed comparatively the same weight at day zero and all the mice were remained alive after five days. As such, determination of the appropriate dose of phage is a prerequisite for successful phage therapy.

All mice injected with lethal dose E. coli were all survived with little or unremarkable illness condition due to φJS3 phage. Possible mechanisms behind the lifesaving effects of phage may be that the in vivo kinetics of the phage was amplified locally as the specific bacteria resided there in the intra-peritoneal compartment. Under these circumstances, the phage may continue to search the target bacterial cells locally and eradicated. Otherwise the bacteria would have been disseminated and result death in the mice model as observed in the control group (Nakai et al., 1999).

Similar study was performed by Biswas et al., (2002) who used mice model infected with vancomycin-resistant Enterococcus faecium and showed that a phage administered intraperitoneally 45...
minutes post-infection was able to liberate mice from the bacterium. The rescue was associated with a significant decrease in bacterial numbers in the bloodstream. They also demonstrated that phage administration up to 5 hours post-infection still fully rescued the mice.

This study investigated whether the direct bactericidal activity of phage was actually responsible for its protective effects in in vitro and in vivo environments. In the in vitro environment, being a virulent lytic bacteriophage have shown a clear plaque formation or lyses of the indicator host in nutrient broth. Whereas the in vivo parameters were the mice model used. The therapeutic efficacy of phage was even predictable in mice treated two days after injection with bacteria, when all the control (unprotected) mice exhibited signs of physical deterioration, such as reduced activity and ruffled hair but mice (Merril et al., 2003).

One researcher has investigated the possibility of curing E. coli infections in poultry. Barrow et al. (1998) prevented morbidity and mortality in chickens using bacteriophages lytic for E. coli. When chickens were challenged intramuscularly with E. coli and simultaneously treated with 10^6 to 10^8 PFU of bacteriophages, mortality was reduced by 100%. This study demonstrated that bacteriophages can cross the blood brain barrier, and furthermore can amplify in both the brain and the blood. The team also stated that, phage therapy was even effective when given at the onset of clinical symptoms.

In this study we observed that phages can be selected in days or weeks time against even to a multidrug resistant bacteria species. In contrast, the process of discovering and testing a new antibiotic can take decades. Like with antibiotics, bacteria can also develop resistance to phages. But unlike antibiotics, phages are dynamic and can evolve alongside bacteria. As antibiotic resistance mechanisms do not affect phages, phage therapy provides an ideal way to treat highly antibiotic resistant microorganisms (Donlan, 2009). In conclusion, the results of this study support the view that phages are easily recoverable from our environment. Its use as therapy has a potential to control the growth of pathogenic E. coli within animal body if initiated at appropriate time. The ability of lytic and virulent phages to rapidly kill bacteria, the specificity of phages for pathogenic bacteria and its safety in toxicity in the animal body make phages an excellent therapeutic candidate for fighting bacterial disease. The authors believe that this study can open a broad research interest in Ethiopia that will enable future researchers to use phages as an alternative therapeutic agent in various disciplines. Appropriately selected, well-characterized and purified phages will be desirable for therapeutic use.

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