Characterization of Lytic Phages Isolated from Effluent Water and Soil against E. coli O157:H7

Anayochukwu C. Ngene (ngene.anayochukwu@mouau.edu.ng)
Michael Okpara University of Agriculture Umudike
John O. Egbere
University of Jos
Isaac A. Onyimba
University of Jos
Chinedu G. Ohaegbu
Michael Okpara University of Agriculture Umudike
Michael M. Dashen
University of Jos
Uzal Umar
University of Jos
Nnaemeka E. Nnadi
Plateau State University
John C. Aguiyi
University of Jos

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Abstract

Escherichia coli O157:H7 commensally lives in the rumen of food animals, making it an important foodborne pathogen. It can be transmitted through direct and indirect contact with animals or manure of animals carrying *E. coli* O157:H7, mediating its transfer to water and food products, which could cause infections to human upon consumption. *E. coli* O157:H7 is currently an emerging food-borne pathogen of severe public health concern. To this effect, bacteriophages can provide alternative measures for its control. This study was aimed at isolation and characterization of lytic phages isolated from effluent water and soil against *E. coli* O157:H7 as potential biocontrol agents. Fifty environmental bacteria and four phages were isolated from six different locations; Student Village Hostel_1and 2, Old JUTH_1and 2 and Angwa Rukuba_1 and 2 and were examined for their physiological and morphological characteristics. The fifty environmental bacteria isolates were characterized and screened to determine the permissive host for the four phages as the antibiotics susceptibility profile of the environmental isolates were determined. Three isolates EIA_18 (*Escherichia coli*), EIA_19 (*Bacillus* spp) and EIA_26 (*Pseudomonas aeruginosa*) were observed to be permissive host for the phages. The phages were screened against *E. coli* O157:H7 and other eleven selected pathogenic bacteria. The four phages displayed broad lytic activity against shiga toxin-producing *E. coli* O157:H7 and some of the selected pathogenic bacteria. These four lytic phages were designated as ECPA_1, ECPA_2, ECPA_3 and ECPA_4. Despite isolation from different locations, further characterization showed that they displayed a high degree of similarities. While all the phages were relatively stable over a wide range of salinity, temperatures, NaCl, Chloroform and pH values, their range of infectivity or lytic profile was rather wide against *E. coli* O157:H7 and some of the selected pathogenic bacteria, while few of the selected pathogenic bacteria showed no activity. This study showed that the isolated bacteriophages are the dominant *E. coli* O57:H7-infecting phages harboured in the selected areas within Jos metropolis and due to their favourable characteristics can be exploited in the formulation of phage cocktails for the bio-control of *E. coli* O157:H7.

Introduction

Between the urine and faeces, we all are born as Latin proverb states. Faecal flora of our mothers is thus, acquired from birth. Over a century ago, Escherich explained the bacteria he isolated from a human neonates faeces as Bacterium coli commune. He revealed that the organisms now called as *Escherichia coli* were present in the intestinal contents of humans and faeces and were considered as commensal organisms (Ngene *et al*., 2020; Bettelheim, 1986; Escherich, 1988). In California, in 1975, *E. coli* O157:H7 was first identified as a possible human pathogen in a patient with bloody diarrhea and in 1982, was first associated with a foodborne (ground beef) outbreak of disease (Riley *et al*., 1983). Some non-O157 serotypes and the serotype (defined by its O and H surface antigens) of *E. coli* produce Shiga-like toxins called verocytotoxins because of their similarity to toxins produced by *Shigella dysenteriae*. These *E. coli* are called VTEC (verocytotoxin-producing *E. coli*), STEC (Shiga-toxin producing *E. coli*), and also EHEC (enterohemorrhagic *E. coli*) because of the symptoms they produce (Wells *et al*., 1983; Brown *et al*., 1997).

Escherichia coli is a bacterium that normally lives in the gastrointestinal tract of animals and humans. Although most types of these bacteria produce toxins that cause illness while others are harmless. Some strains of *E. coli*, including *E. coli* O157:H7 are shiga toxin-producing *E. coli* (STEC), producing toxins known as Shiga toxins. These may cause severe diarrhoea and kidney damage (Sulakvelidze, 2005). In Argentina in 1977, the first reported *E. coli* O157:H7 was isolated from cattle of less than 3 week old calf with colibacillosis was. In 1982, when it was associated with two food borne outbreaks of Hemorrhagic colitis, the bacterium was first identified as a human pathogen. Since then, in many outbreaks and in sporadic cases of bloody diarrhoea in North America and Great Britain, O157 VTEC have been identified and a close association has been established between and haemorrhagic uremic syndrome (HUS) and VTEC (Ngene *et al*., 2020; Fernandez, 2008).

As the causative agent of hemorrhagic colitis in humans, Enterohemorrhagic *Escherichia coli* (EHEC) has predominantly emerged in recent years. With symptoms of abdominal cramps and bloody diarrhea, can advance into hemolytic uremic syndrome (HUS), a more severe, life-threatening complication. The pathogenicity of EHEC appears to be associated with a number of virulence factors, including the production of several cytotoxins (Brown *et al*., 1997; Griffin *et al*., 1991). *Escherichia coli* strains that cause diarrhoea includes; enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), and enteroinvasive (EIEC) strains. Recently, a diarrhoegenic strain, enteroaggregative *E. coli* (EAggEC) has been established.

One of the most studied foodborne pathogens is *E. coli* O157:H7 among EHEC strains, because of their tolerance to some chemical and physical treatments, severity of illness, low dose infectiveness and widespread diffusion (Karmali, 1989). Consumption of undercooked meat is the most common source of outbreaks. Consumption of ground beef is the most common pathway among *E. coli* O157:H7 outbreaks. *E. coli* O157:H7 has also been found in pigs, chickens, turkeys, wild animals, seafood, and leafy vegetables (Beneduce *et al*., 2003).

In current scenario, serious medical and social problem results from the increasing antibiotic resistance of bacterial strains (WHO, 2014). Due to unprofitability of the venture and the risks of development of resistance in bacteria, pharmaceutical industries are withdrawing from research and development on new antibiotics (Clarke, 2003), which over conventional and current system of microbial control has generated interest in alternatives therapy. Lytic phages are the possible replacement for antibiotics to treat bacterial infections not responding to conventional antibiotic therapy (O'flynn *et al*., 2004). The application of phages to control a certain bacterial pathogen is complicated by the high degrees of phenotypic diversity within populations of both phages and bacteria (Holmfeldt *et al*., 2007). To different co-occurring phages, individual strains of a pathogen may be resistant or more or less susceptible. Before therapeutic application, it is necessary to understand in detail the phage and host interaction, which is affected by both biological and physical factors (Beke *et al*., 2016). Temperature and pH are the main physical factors affecting the phage adsorption and bacterial growth whereas the biological aspect is related to bacterial resistance.

As bacteriophages are viruses capable of lysing bacteria, specific lytic phages can kill pathogenic bacteria in their own habitat. Phages are ubiquitous in nature and can often be found in a variety of environments related to their host such as soil, sewage, water, manure, animal and farms produce, as well as different food processing plant effluents (O'Flynn *et al*., 2006; Wang *et al*., 2017).

Bacteriophages been a bacterial viruses is considered to offer a great advantage over antibiotics. Firstly, bacteriophages target only the pathogens of interest, so the normal gut microflora are not affected. Secondly, bacteriophages are self-replicating in the bacterial host and kill the bacteria. (Connerton and Connerton, 2005). Against zoonotic pathogens in live animals, bacteriophages have been used (Smith and Huggins, 1983; Atterbury *et al*., 2003; Huff *et al*., 2005; Tanji *et al*., 2005). Phage therapy can be as efficient as antibiotics in treating bacterial infections as demonstrated with these instances.

As recently investigated, the application of bacteriophages as a food safety intervention with a few commercial preparations have been approved and marketed. Bacteriophages are often used in high concentrations to inactivate foodborne pathogens, such as *Escherichia coli* O157:H7, *Salmonella, Listeria, and Campylobacter* etc. in different foods. Also, in production facilities, phages also have been used to control specific bacteria at pre-harvest and post-harvest stages of food production and storage (Carlton *et al*., 2005; Greer, 2005). Bacteriophages application is highly dependent on the biological properties of the phages which affect their performance in the biological system.

Materials And Methods

Sample collection

Under aseptic conditions, a total of 12 samples, including wastewater and soil (2 samples for each location) were obtained from 6 different locations (Student Village Hostel 1 & 2, Old Jos University Teaching Hospital, JUTH 1 & 2, and Angwa Rukuba 1 & 2), within Jos North Metropolis, Plateau State, Nigeria. Latitude and Longitudes of their various locations were noted as follows: latitude; 9.96565, 9.96571, 9.918695, 9.918323, 9.93922, 9.934003 and longitude; 8.87116, 8.87128, 8.890219, 8.890219, 8.909185, 8.908757 respectively. A 50 ml sterile vials with cover tops were used for this purpose. The containers were immediately disinfected with 70% ethanol at the point of collection, labeled, and kept in a super cool flask for transportation to Microbiology Laboratory Research Unit, Africa Center of Excellence in Phytomedicine Research and Development (ACEPRD) University of Jos, for analysis.

Laboratory Isolation of Environmental Isolates

According to the method of (Ibrahim and Hameed, 2015) modified, a total of 10 ml of each sample (after mixing the wastewater and sand and allowed to decant in a conical flask) was diluted in 90ml of sterile 0.9% NaCl Normal Saline and homogenized. Then, 100 µl of the fourth and fifth diluent of the samples were inoculated on Eosin Methylene Blue Agar (EMB) agar plates for the isolation of enteric bacteria, MacConkey agar plates are used for both lactose and non-lactose fermenters and Sorbitol-MacConkey for *E. coli* O157:H7, using the spread plate method. All the bacteria plates were incubated at 37˚C for 24 hours.

Total viable count for Environmental Isolates

The total viable count was determined using the spread plate technique on nutrient agar and counting the colonies developed after incubation at 37˚C for 24 hours (Harley and Prescott, 1996).

Identification of Isolates

Gram stain

Gram staining was done by making a thin smear of each isolate on a clean grease-free glass slide, air dried and heat-fixed by passing briefly over flame three times. Each smear was covered with crystal violet stain for 1 minute and rinsed with water, then Lugol's iodine for a minute too and then washed with water. The dyed smear was then decolorized with acetone for 20 seconds and rinsed with water. Finally, the smear was covered with safranin for 1 minute, washed with water, bloted and air dried. Each slide was then viewed under microscope using oil immersion at X100 objective lens (Forbes *et al*., 2016).

Motility

A straight needle was touched to a colony of a young (18- to 24-hour) culture growing on agar medium. A depth of only $^{1/3}$ to ½ inch was stabbed once in the middle of the tube. It was ensured that the needle was kept in the same line it entered as it is removed from the medium. They were incubate at 37°C and examined daily for 7 days. A diffused zone of growth flaring out was observed from the line of inoculation (Forbes *et al*., 2016). .

Haemolysis Test

After incubating an inoculated blood agar plate, the media around the bacteria growing on it was observed for changes in the opaque, red colour. If the area around the bacteria turns transparent, that strain displays complete haemolysis (Forbes *et al*., 2016).

Biochemical Test

Methyl-red test

A loopful of bacterial culture was inoculated in glucose phosphate peptone water then incubated at 37^{O}C for 24 hours. About eight drops of methyl red were added. A distinct red ring formed at the top of the cryo-vial meant organism was positive.

Voges-Proskauer (VP) test

The isolates were inoculated into 2 ml of sterile glucose phosphate peptone water medium and incubated anaerobically at 37^{O}C for 48 hours. To the culture, a very little amount of creatinin was added and properly mixed. Also added and mixed properly was a 3 ml of the sodium hydroxide (NaOH)

reagent. The preparation was left to stand for 1 hr at room temperature as the bottle cap was removed, a positive VP test was a slow development of a pink-red colour.

Catalase test

A loopful of bacterial culture from nutrient slant smeared onto a drop of hydrogen peroxide on a clean grease-free slide. An immediate effervescence of gas bubbles from the culture indicated a positive reaction.

Coagulase test

One drop each of normal saline and serum were put on a clean, grease-free slide, and a loopful of a bacterial culture was added and smeared together. The slide was then rocked and observed for agglutination/clumping. Clumping of the culture cells indicates a positive reaction.

Oxidase test

A filter paper was soaked with 1% oxidase reagent and a loopful of bacterial culture was then smeared on the treated filter paper and observed for color change. A color change to dark purple or dark blue within 10 to 30 seconds indicated a positive result.

Indole test

A loopful of bacterial culture was inoculated in freshly peptone water and incubated at 37^{o}c for 24 hours. About 5 drops of kovas reagent were added to the culture and observed for colour formation. Formation of a pink to red ring at the top of the cryo vial indicated a positive result.

Citrate-utilization test

Simmons citrate agar was prepared, poured into flavor bottles and then slanted. Each bacterial culture from a nutrient slant was inoculated on the surface of one simmons citrate slant. The slants were then incubated at 37^{o}c for 24 hours. A change of colour from green to Prussian blue on the slant surface and in the medium indicated a positive result.

Urease test

Christensens's urea agar was prepared, poured into tubes and slanted. Culture from each nutrient slant were streaked on the slant surface and incubated at 37^{o}c for 24 hours. A colour change of the slant or/ and butt to pink indicates a positive result (Church, 2016; Cheesbrough, 2006).

Fermentation Test

The medium was allowed to warm to room temperature prior to inoculation. The Purple Broth (with carbohydrate of choice) was inoculated with isolated colonies from an 18-24 hour pure culture of the organism. A control tube of Purple Broth Base was inoculated in parallel with the carbohydrate based media. The inoculated media was incubated aerobically at 37ºC for 3-5 days. Observation was done daily for development of a yellow color in the medium (Forbes *et al*., 2016).

Triple Sugar Iron (TSI) A Test

The center of a well-isolated colonies obtained from solid culture media was picked with an inoculating needle. With a straight inoculation needle, the top of a well-isolated colony was touched. The TSI was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. The cap on loosely and incubate the tube was left at 35°-37°C in ambient air for 18 to 24 hours. The reaction of medium the medium was examined. (Forbes *et al*., 2016; Cheesbrough, 2006).

For further identification, the isolates were sent to Westerdijk Fungi Biodiversity Institute (Medical Mycology), Netherlands for Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) analysis.

Preservation of Isolates

The isolates were subcultured on nutrient agar, incubated at 37^{0}C for 24 hours. A single colony was inoculated into a sterile nutrient broth, incubated in a shaker incubator (ZHP-100) at 180 rpm for 24 hours at 37^{0}C. The isolates were also incubated on a nutrient agar slant at 37^{0}C for 24 hours. They were all stored at 4^{0}C in a refrigerator.

Antibiotics Susceptibility Profile

The antibiotic susceptibility profile of the test organism and the environmental isolates were determined using the standard Kirby-Bauer disk diffusion method (Bauer, 1966). The antibiotics used has the following disk concentrations: Ceftazidime (10 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (10 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg), Ofloxacin (10 µg), and Augmentin (30 µg). Bacterial culture suspension equivalents of 0.5 tube McFarland turbidity standards were spread on Muller-Hinton agar plates using sterile swabs and incubated aerobically at 37˚C for 24 hours; then, the zone of inhibition diameters around the antibiotic disks was measured. Obtained results were compared with the standard performance chart for antimicrobials disc susceptibility testing provided by CLSI (2012), and the frequencies of sensitivity and resistance were recorded. According to the criteria recommended by (CLSI, 2012), the results were expressed as susceptible or resistant.

Phage Isolation

(Ezemokwe *et al.*, 2021)

Direct phage isolation from samples

Phage isolation from water/sewage samples

50 ml of water/sewage was centrifuged at 4,200 rpm for 10 min to remove debris and other undissolved particles.The supernatants were divided into portions A and B (saved portion B for enrichment). Portion A was further divided into 2 portions, A1 and A2. To portion A1 0.5 ml chloroform was added to lyse any bacteria sustained. Stored at 4^{o}C for 30 minutes. After 1 hour, it was centrifuged at 21,000g for 10mins and filtered through 0.45 µm filters. The other portion A2 was centrifuged at 21,000g for 10mins and filtered through 0.45 µm filters. **P**ortion B was also centrifuged at 21 000 g for 10mins and filtered with a 0.22 µm filter.

Phage isolation through enrichment

Phage isolation from enrichment of water/sewage samples

The following was added to a conical flask:

a. 10 ml of the filtered sample B
b. 10 ml double strength LB broth
c. 40 µl 1 M $CaCl_2$
d. 100 µl of overnight broth culture of bacteria

It was incubated at 37^{o}C up to 48 h with shaking at 50 rpm. After 24 h, 5 ml of the sample (B1) was collected and centrifuged at 4,200 rpm for 10 min. The supernatant was centrifuged at 21,000 g for 10mins and filtered through 0.45 µm filters. After 48 h, the other remaining portion was centrifuged and filtered (Sample B2).

Phage isolation from soils

The following was added to a conical flask:

a. 1 g of soil/mud
b. 5 ml LB broth
c. Mixed continuously by gentle inversion at 20 rpm for 30 min
d. Incubated at 4^{o}C for 30 min

For 10 min., the sample was centrifuged at 4,200 rpm. The supernatants were divided into 2 portions, C and D. Portion C was centrifuge at 21,000g for 10mins and filtered through 0.45 µm filters (direct soil phage isolation).

To a flask, the following was added:

a. 5 ml double strength nutrient broth supplemented with $CaCl_2$ (see **Phage isolation from enrichment of water/sewage samples**).
b. 5 ml soil/mud centrifuged supernatant D
c. 100 ul overnight bacterial culture

It was incubated at 37^{o}C up to 48 h with shaking at 50 rpm. After 24 h, 5 ml of the sample (D1) was collected and centrifuged at 15, 000 x g for 10 min. and was filtered through 0.45 µm filters. After 48 h, the other remaining portion was centrifuged and filtered (Sample D2).

Phage detection from extracted supernatants

Lawns of bacterial isolates were prepared. When the overlays were set, added 10 ul of the filtered samples A1, A2, B1, B2, C, D1 and D2 to a section of the agar plate. They were allowed to dry and incubated at 37^{o}C overnight. The next day, zones of lysis/clearance on the bacterial lawns was observed. Using sterile loop, the lysed zones were scrapped out and transfer to 1 ml SM buffer in Eppendorfs and mixed by inversion. Vortex was avoided and was incubated at 4^{o}C for 30 min. They were centrifuged at 15,000 x g for 10 min and filtered.

Phage purification

1. Filtrates from lysed zones from (B1 and D1 filtrates).

2. Two filtrates were selected to work with while the remaining filtrates were saved as a back-up).

3. One overnight bacterial culture

4. Standardized *E. coli* O157:H7 (fron NVRI Vom)

4. Warm semi-solid nutrient broth 0.7 % agar

5. SM buffer and nutrient broth

6. Nutrient agar plates

Plaque assay

Day 1

A 10-fold serial dilution of 10^{-1} to 10^{-8} of the lysed zones filtrates from two samples of B1 and D1 in cold SM buffer was conducted using 96 wells micro-titer sterile plate. Labelled one nutrient agar plate for each tube marked as 10^{-1} to 10^{-8}. 100 µl of overnight standardized culture of (*E. coli* O157:H7 host bacteria) was added to sterile Eppendorf tubes marked 10^{-1} to 10^{-8}. From the 10^{-1} serial dilution of the filtrates, 100 µl was removed and add it to the Eppendorf tube containing 100 µl of overnight culture labeled 10^{-1} and was mixed by pipetting up and down several times. All 200 µl was removed from this tube and added it to 3 ml overlay agar (for 90 mm Petri dishes). Mixed by inversion. The mixture was poured onto the appropriately labeled nutrient agar plate and allowed to set. This was repeated for each serial dilution and incubated aerobically at 37^{o}C overnight.

Day 2, 3, 4

Each plate was visually examined and the one which contains individual distinct plaques were selected. For each distinct plaque morphology present on the plate, an Eppendorf tube with 500 µl of diluent (SM buffer) was prepared. From the selected plate, a pipetteman with pipette tip was selected and gently penetrated the overlay agar in the middle of the plaque. Immediately added the cored plaque to the tube prepared. This was repeated for a total of three times, using the most recently cored plaque in 500 µl of diluent (SM buffer) instead of the enriched phage lysate each time. It was ensured to pick plaques of the same plaque morphology of the parent plaque (i.e. the previous plaque picked).

Titer calculation

Formula: $\frac{N}{D} \times \frac{1}{v}$

Where; N = Number of plaques

D = Dilution factor

V = Volume of virus pipetted

Unit = PFU/ml

Where; PFU = Plaque Forming Unit

Phage propagation (Bulking/Multiplication)

The following was added into a sterile conical flask; 2ml *E. coli* O157:H7 broth, 20ul of the phage lysate, 10ul of nutrient broth and 40ul of 1mM $CaCl_2$. Incubated at 37^{o}C for 24h in a shaker incubator at 180rpm.

STABILITY TEST

(Manohar *et al*., 2018 modified; Ahmadi *et al*., 2017; Ateba, and Akindolire, 2019 modified**)**

Thermal Stability test

A total of 64 nutrient agar base plates were prepared and allowed to stay overnight for sterility test. For the 4 phages isolated, each has 16 plates for the 4 temperature (45, 55, 65, and 75^{o}C). Into sterile test-tubes, 90ul of SM buffer was added. They were heated to temperatures; 45, 55, 65 and 75^{o}C in a water bath and maintained at ± 0.05^{o}C. Water level was maintained 2cm above the treatment with a stirrer. Aliquot of 100ul of the phages for each test-tube of the respective temperature was added to the 900ul and boiled for 60 minutes. A serial dilution to 10^{-8} was prepared with a sterile micro-titer plate which was added 90ul of SM buffer. For every 15 minutes, 10ul of the sample or treatment phages was pipetted and added into 90ul in micro-titer plate and diluted.

Overlay already seeded with the host bacteria and poured onto nutrient agar plate was spotted with the diluent to 10^{-8}. It was allowed to dry and was incubated by inverting at 37^{o}C for overnight. This procedure was repeated for the individual phages.

pH Stability Test

A total of 24 agar plates were prepared and incubated overnight for sterility test. Nutrient agar over-lay was prepared with 1M NaOH and HCL to yield a pH of 1, 2, 5, 7, 9, 11 using SM buffer and were standardized. 900ul of the individual pH and 100ul of the phages were prepared and incubated at 37^{o}C for 24h. From there, a serial dilution was made (10 folds) i.e. 90ul of SM buffer to 10ul of phage lysate. 10ul of the individual diluents were inoculated into an already solidified overlay agar and incubated at 37^{o}C for 24h.

Salinity Stability

SM buffer containing NaCl of 0.5%, 5%, 10% and 15% was prepared while using SM buffer that has no salt as a negative control. A total of 20 agar base plates were prepared for the 4 phages each for different percentage. A 10 folds serial dilution was prepared as described above. 90ul SM buffer + NaCl and 10ul of the individual phages were incubated at room temperature for 24h. An overlay seeded with test organism was prepared, allowed to dry. 10ul of the diluents were spotted on them and allowed to dry, incubated by inverting at 37^{o}C for 24h.

Chloroform Stability Test

Eight base plates were prepared. Four base plates for the four phages treated with chloroform and the other four served as a negative control for each of the phages treated without the chloroform. An overlay was prepared, 24h overnight *E. coli* O157:H7 prepared and 10%v/v of the chloroform using SM buffer prepared. Equal aliquot of 500ul of the lysate and 500ul of the chloroform was mixed in a sterile test-tube and was allowed to stand for 60minutes with slight shaking at intervals. They were centrifuged at 10,000g for 10minutes. A serial dilution to 10^{-8} as described earlier was carried out. Overlay seeded with the test organism was prepared and allowed to solidify. Individual diluents were spotted (10ul) and allowed to dry under aseptic condition and were incubated by inverting for 24h at 37^{o}C.

HOST RANGE

Nutrient agar base plates were prepared. Overlay agar seeded with the individual organisms were dispensed against the base agar and allowed to gel. 10ul of the 4 phages were spotted against the overlay. They were allowed to dry, inverted and incubated at 37^{o}C for 24h (Ross *et al*., 2016)

PRESERVATION/STORAGE OF PHAGES

The phages were stored in 50% glycerol at -80^{o}C.

STATISTICAL ANALYSIS

The mean values of the three replicates were determined by repeating the experiments three times. Populations of the surviving phage obtained in each study, were converted to $\log_{10}$ PFU mL^{-1}. SPSS software version 21 was used to carry out the statistical analysis. To determine the analysis of variance (ANOVA) using Duncan's multiple range test (JMP v.12 software; SAS Inst., Cary, NC, USA), data were analyzed. P-value less than 0.05 ($P < 0.05$) were estimated to determine the significant differences.

Results

Figure 1. shows that there is a significant difference in the mean microbial load of the environmental isolates where Angwa Rukuba_1 having a mean value of 7.69 ± 8.89 is significantly different from Angwa Rukuba_2 (7.57 ± 3.61) and Student Village Hostel_1 (7.52 ± 2.65). There is no significant difference between the mean values of Student Village Hostel_2 (7.64 ± 6.35) and Old JUTH_1 (7.64 ± 5.20) while Old JUTH_2 having a mean value of 7.63 ± 3.79 has a slight significant difference between Student Village Hostel_2 (7.64 ± 6.35) and Old JUTH_1 (7.64 ± 5.20).

Table 1. shows that the isolates were differentiated on the basis of their morphological, microscopic and biochemical characteristics. It illustrates that the isolates varies in their Gram reaction, cell morphology, motility, haemolysis, lactose fermentation, citrate test, urease, catalase and triple sugar iron agar test. Three isolates EIA_18 (*Escherichia coli*), EIA_19 (*Bacillus* spp) and EIA_26 (*Pseudomonas* spp) were observed to be permissive host for the phages.

Table 2. shows the confirmation of the environmental isolates identified in Table 4 using **MALDI-TOF mass spectrometry.** The first and second isolation was based on phenotype on CHROM agar and their probability determined.

Figure 2 shows that *Bacillus* spp has the highest number of occurrence 30%, followed by *Escherichia coli* with 28% occurrence and *Pseudomonas aeruginosa* with 26% occurrence. *Proteus* spp and *Staphylococcus aureus* has the least number of occurrence, followed by *Klebsiella* spp.

Table 3. shows **antibiotics susceptibility profile of the test organism and environmental isolates. The result shows that all the isolates were resistant to Ceftazidime, having less resistance to** Cefuroxime, Ampicillin and Augmentin while the isolates showed a high susceptibility to Gentamicin, Ciprofloxacin, Nitrofurantoin and Ofloxacin.

Table 4. shows that out the six sample locations, only four; Student Village Hostel_1, Student Village Hostel_2, Old JUTH_1 and Old JUTH_2 showed the presence of bacteriophages coded ECPA_1, ECPA_2, ECPA_3, ECPA_4 respectively.

Figure 3. shows the mean viral load of the phages. It illustrates the there is a significant difference in the viral load of phage ECPA_1 and ECPA_4 having 11.58 ± 2.65 and 10.04 ± 35.64 respectively while ECPA_2 and ECPA_3 having a viral load of 11.85 ± 13.45 and 9.80 ± 38.55 respectively are not significantly different.

Figure 4. shows the thermal stability of the at phages at temperature of 45^{o}C, 55^{o}C, 65^{o}C and 75^{o}C while varying the time (minutes) at 15, 30, 45 and 60 minutes respectively. At 45^{o}C, phage ECPA_1 show a sharp decrease in titer from the working concentration of 12.58 ± 1.00 $\log_{10}$ PFU/ml to 10.11 ± 1.00 $\log_{10}$ PFU/ml, 9.11 ± 1.00 $\log_{10}$ PFU/ml and 4.11 ± 1.00 $\log_{10}$ PFU/ml as the time increases from 15, 30 and 45min. respectively while at 60min., there is a sharp increase in the titer of the phage, having 9.20 ± 3.00 $\log_{10}$ PFU/ml. Phage ECPA_2 show a slight decrease in titer from the working concentration of 12.58 $\log_{10}$ PFU/ml to 9.23 ± 2.00 $\log_{10}$ PFU/ml, 8.15 ± 1.00 $\log_{10}$ PFU/ml and 9.04 ± 1.00 $\log_{10}$ PFU/ml at 15, 30 and 45min. respectively while at 60mins, there is no

visible plaque detected. Phage ECPA_3 showed a sharp decrease in the in the titer from the working concentration of 12.58 log_{10} PFU/ml to 9.08 ± 2.00 log_{10} PFU/ml, 8.28 ± 2.65 log_{10} PFU/ml and 8.20 ± 3.00 log_{10} PFU/ml at 15, 30 and 45mins respectively while at 60 mins there is no visible plaque recorded. Phage ECPA_4 shows a decrease in the phage titer as the time increases from the working concentration of 12.58 log_{10} PFU/ml to 9.23 ± 1.00 log_{10} PFU/ml, 10.26 ± 2.00 log_{10} PFU/ml and 7.04 ± 1.00 log_{10} PFU/ml at 15, 30 and 45mins respectively while at 60 mins, there is no visible plaque observed.

At 55^{o}C ECPA_1 shows a sharp decrease in the titer from the working concentration of 12.58 log_{10} PFU/ml to 8.04 ± 1.00 log_{10} PFU/ml, 8.18 ± 1.00 log_{10} PFU/ml and 4.30 ± 5.00 log_{10} PFU/ml at 15, 45 and 60 mins respectively, having no visible plaque at 30 mins. ECPA_2 show a decrease in the phage titer from the working concentration of 12.58 log_{10} PFU/ml to 9.15 ± 1.00 log_{10} PFU/ml, 10.15 ± 1.00 log_{10} PFU/ml and 7.23 ± 2.00 log_{10} PFU/ml at 15, 30, and 45 minutes respectively, while at 60 minutes, there is no visible plaque observed. ECPA_3 and ECPA_4 shows no visible plaque at 15, 30, 45 and 60 minutes.

At 65^{o}C and 75^{o}C, there is no visible plaques observed for phages ECPA_1, ECPA_2, ECPA_3 and ECPA_4 at 15, 30, 45, and 60 minutes.

Figure 5. shows pH stability of the phages. ECPA_1 shows no visible plaque at pH 1, 2 and 11. There is a sharp decrease in the phage titer from the working concentration of 10.79 log_{10} PFU/ml to 5.40 ± 3.00 log_{10} PFU/ml, 5.64 ± 1.00 log_{10} PFU/ml and 3.447158 ± 8.89 log_{10} PFU/ml at pH of 5, 7 and 9 respectively. ECPA_2 shows a decrease in from the working concentration of 10.79 log_{10} PFU/ml to 5.16 ± 2.00 log_{10} PFU/ml at pH of 7 while there is no visible plaque at pH of 1, 2, 5, 9, 11. ECPA_3 shows a sharp decrease in titer from the working concentration of 10.79 log_{10} PFU/ml to 5.51 ± 6.56 log_{10} PFU/ml and 2.30 ± 1.00 log_{10} PFU/ml at pH of 5 and 7 respectively while there is no visible growth observed at pH of 1, 2, 9 and 11. ECPA_4 decrease from the working concentration of 10.79 log_{10} PFU/ml to 3.43 ± 4.36 log_{10} PFU/ml and 5.41 ± 2.00 log_{10} PFU/ml at pH of 2 and 5 respectively while there is no visible plaque at pH of 1, 7, 9, 11.

Figure 6. shows the stability of the phages at different NaCl concentrations. ECPA_1 shows decrease from the working concentration of 10.51 log_{10} PFU/ml to 10.51 ± 1.00 log_{10} PFU/ml, 10.40 ± 1.00 log_{10} PFU/ml, 10.18 ± 3.00 log_{10} PFU/ml, 8.08 ± 2.65 log_{10} PFU/ml at NaCl concentration of 0.5, 5.0, 10.0, and 15.0 respectively. ECPA_2 shows a decrease in the phage titer from the working concentration of 9.08 log_{10} PFU/ml to 9.08 ± 2.65 log_{10} PFU/ml, 10.20 ± 1.73 log_{10} PFU/ml, 10.256 ± 1.00 log_{10} PFU/ml and 8.00 ± 2.00 log_{10} PFU/ml at NaCl concentration of 0.5, 5.0, 10.0, and 15.0 respectively. ECPA_3 show a decrease from the working concentration of 10.26 log_{10} PFU/ml to 10.18 ± 4.00 log_{10} PFU/ml, 10.30 ± 1.00 log_{10} PFU/ml, 10.18 ± 3.00 log_{10} PFU/ml, 6.41 ± 2.65 log_{10} PFU/ml at NaCl concentration of 0.5, 5.0, 10.0, and 15.0 respectively. ECPA_4 show a slight increase from the working concentration of 8.08 log_{10} PFU/ml to 10.11 ± 1.00 log_{10} PFU/ml, 10.20 ± 1.00 log_{10} PFU/ml, 9.11 ± 3.00 log_{10} PFU/ml at NaCl concentration of 0.5, 5.0, and 10.0 respectively while there is no visible plaque at NaCl concentration of 15.00.

Figure 7. shows the stability of the phages at 10% v/v chloroform concentration. The phages show a decrease from working concentration of 12.80 log_{10} PFU/ml to 9.76 ± 2.51 log_{10} PFU/ml, 9.79 ± 1.00 log_{10} PFU/ml, 9.75 ± 6.56 log_{10} PFU/ml and 9.78 ± 4.36 log_{10} PFU/ml for the phages ECPA_1, ECPA_2, ECPA_3, ECPA_4 respectively.

Table 5. shows that ECPA_1 has a high activity against the pathogenic organisms except *Salmonella typhi_2, Salmonella pullorum_2, Staphylococcus capitis, Pseudomonas aeruginosa_2 and Escherichia coli 026*, with a slight activity against *Pseudomonas aeruginosa_1*. ECPA_2 has a high activity against *Salmonella typhi_1, Salmonella typhi_3, Salmonella pullorum_1, Klebsiella pneumonia, Staphylococcus aureus and Escherichia coli O157:H7* with no activity against *Salmonella typhi_2, Salmonella pullorum_2, Salmonella pullorum_1, Staphylococcus capitis, Pseudomonas aeruginosa_2 and Escherichia coli 026* and a slight activity against *Pseudomonas aeruginosa_1.* ECPA_3 has a moderate activity against *Salmonella pullorum_1* and a high activity against *Salmonella typhi_3, Klebsiella pneumonia, Staphylococcus aureus* and *Escherichia coli O157:H7* with no activity against *Salmonella typhi_1, Salmonella typhi_2, Salmonella pullorum_2, Pseudomonas aeruginosa_1, Pseudomonas aeruginosa_2* and *Escherichia coli 026*. ECPA_4 has a high activity against *Salmonella typhi*_1, *Salmonella typhi_3, Salmonella pullorum_1, Klebsiella pneumonia, Staphylococcus aureus*, and *Escherichia coli O157:H7*, with no activity against *Salmonella typhi_2, Salmonella pullorum*_2, *Staphylococcus capitis, Pseudomonas aeruginosa*_2, *Escherichia coli 026* and a slight activity against *Pseudomonas aeruginosa*_1.

Table 1
Cultural identification of environmental bacteria isolates and screening for permissive host

S/n	Isolate ID	Gram reaction	Cell Morphology	Motility Test	Haemolysis	Lactose Fermentation	Citrate test	Urease Test	Catalase test	TSI test				Organ
										Slope	Butt	H_2S	Gas	
1	EIA_1	+	Large Bacilli	Non motile	++									*Bacil*
2	EIA_2	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
3	EIA_3	+	Large Bacilli	Non motile	++									*Bacil*
4	EIA_4	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
5	EIA_5	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
6	EIA_6	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
7	EIA_7	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
8	EIA_8	-	Bacilli	Non motile	-	+	+	+		A	A	-	-	*Klebs*
9	EIA_9	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
10	EIA_10	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
11	EIA_11	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
12	EIA_12	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
13	EIA_13	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
14	EIA_14	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
15	EIA_15	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
16	EIA_16	-	Bacilli	Motile	-	-	+	+		K	A	+	+	*Prote*
17	EIA_17	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
18	EIA_18	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
19	EIA_19	+	Large Bacilli	Non motile	++									*Bacil*
20	EIA_20	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
21	EIA_21	+	Cocci	Non motile	-	+			+					*Staph* spp
22	EIA_22	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
23	EIA_23	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
24	EIA_24	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
25	EIA_25	-	Bacilli	Non motile	-	+	+	+		A	A	+	-	*Klebs*
26	EIA_26	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp

S/n	Isolate ID	Gram reaction	Cell Morphology	Motility Test	Haemolysis	Lactose Fermentation	Citrate test	Urease Test	Catalase test	TSI test				Organ
										Slope	Butt	H_2S	Gas	
27	EIA_27	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
28	EIA_28	+	Large Bacilli	Non motile	++									*Bacil*
29	EIA_29	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
30	EIA_30	-	Bacilli	Non motile	-	+	+	+		A	A	+	-	*Klebs*
31	EIA_31	+	Large Bacilli	Non motile	++									*Bacil*
32	EIA_32	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
33	EIA_33	-	Bacilli	Very motile	-	++	-	-		A	A	+	-	*Esch*
34	EIA_34	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
35	EIA_35	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
36	EIA_36	+	Large Bacilli	Non motile	++									*Bacil*
37	EIA_37	+	Large Bacilli	Non motile	++									*Bacil*
38	EIA_38	+	Large Bacilli	Non motile	++									*Bacil*
39	EIA_39	+	Large Bacilli	Non motile	++									*Bacil*
40	EIA_40	+	Large Bacilli	Non motile	++									*Bacil*
41	EIA_41	+	Cocci	Non motile	-	+	-		+					*Staph* spp
42	EIA_42	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
43	EIA_43	+	Large Bacilli	Non motile	++									*Bacil*
44	EIA_44	+	Large Bacilli	Non motile	++									*Bacil*
45	EIA_45	+	Large Bacilli	Non motile	++									*Bacil*
46	EIA_46	+	Large Bacilli	Non motile	++									*Bacil*
47	EIA_47	-	Bacilli	Motile	-	-	+	+		K	A	+	+	*Prote*
48	EIA_48	-	Bacilli	Non motile	-	+	+	+		A	A	-	-	*Klebs*
49	EIA_49	-	Bacilli	Motile	+	-	-			K	K	-	-	*Pseu* spp
50	EIA_50	+	Large Bacilli	Non motile	++									*Bacil*

Keys; - = Negative

+ = Positve

A = Acidic

K = Alkaline

Table 2
Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) Identification

S/N	CODE	ISOLATE TYPE	1ST ISOLATION	2ND ISOLATION	BRUKER	COMMENTS	TEST ORGANISM NAME	ACCESSION NAME #	TOP PROBABILITY	SCORE
1	ECA_1	*E. coli* O157:H7								
2	ECA_2	*E. coli* O157:H7	ok	ok	ok		*Escherichia coil*	ECA_2	2.43	+++
3	ECA_3	*E. coli* O157:H7	re-isolate	ok	ok		*Escherichia coil*	ECA_3	2.33	+++
4	EIA_1	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_1	2.37	+++
5	EIA_2	*Pseudomonas sp*	re-isolate	OK	ok		*Pseudomonas aeruginosa*	EIA_2	2.29	++
6	EIA_3	*E. coli*								
7	EIA_4	*E. coli*								
8	EIA_5	*E. coli*	re-isolate	OK	ok		*Escherichia coil*	EIA_5	2.39	+++
9	EIA_6	*Klebsiella* sp								
10	EIA_7	*E. coli*	re-isolate	OK	ok		*Escherichia coil*	EIA_7	2.48	+++
11	EIA_8	*Pseudomonas sp*	re-isolate	OK	ok		*Pseudomonas aeruginosa*	EIA_8	2.13	++
12	EIA_9	*Pseudomonas sp*	re-isolate	OK	ok		*Pseudomonas aeruginosa*	EIA_9	2.08	++
13	EIA_10	*Pseudomonas sp*								
14	EIA_11	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_11	2.29	++
15	EIA_12	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_12	2.49	+++
16	EIA_13	*Pseudomonas sp*	re-isolate	ok	ok		*Pseudomonas aeruginosa*	EIA_13	2.26	++
17	EIA_14	*Proteus sp*								
18	EIA_15	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_15	2.41	+++
19	EIA_16	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_16	2.49	+++
20	EIA_17	*Pseudomonas sp*	re-isolate	ok			*Unspecified*	EIA_17	1.49	-
21	EIA_18	*Staphylococcus sp*								
22	EIA_19	*E. coli*	re-isolate	ok	pending		*Escherichia coil*	EIA_19	2.46	+++
23	EIA_20	*Pseudomonas sp*								
24	EIA_21	*E. coli*								
25	EIA_22	*Klebsiella* sp								
26	EIA_23	*Pseudomonas sp*	re-isolate	ok		didn't grow				
27	EIA_24	*E. coli*								
28	EIA_25	*Pseudomonas sp*	re-isolate	ok		didn't grow				
29	EIA_26	*Klebsiella* sp								
30	EIA_27	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_27	2.39	+++
31	EIA_28	*E. coli*	re-isolate	ok	ok		*Escherichia coil*	EIA_28	2.49	+++
32	EIA_29	*Pseudomonas sp*	re-isolate	ok	ok		*Pseudomonas aeruginosa*	EIA_29	2.13	++
33	EIA_30	*Pseudomonas sp*	re-isolate	ok	ok		*Pseudomonas aeruginosa*	EIA_30	2.26	++
34	EIA_31	*Staphylococcus sp*	re-isolate	ok	ok		*Staphylococcus species*	EIA_31	1.85	+

S/N	CODE	ISOLATE TYPE	1ST ISOLATION	2ND ISOLATION	BRUKER	COMMENTS	TEST ORGANISM NAME	ACCESSION NAME #	TOP PROBABILITY	SCORE
35	EIA_32	*Pseudomonas sp*	re-isolate	ok	ok		*Pseudomonas species*	EIA_32	1.83	+
36	EIA_33	*Proteus sp*	re-isolate	ok			*Lysinibacillus*	EIA_33	2.21	++
37	EIA_34	*Klebsiella* sp	re-isolate	ok			*Enterobacter species*	EIA_34	2.17	++
38	EIA_35	*Pseudomonas sp*	re-isolate	ok	wrong but keep	Keep it	*Staphylococcus aureus*	EIA_35	2.35	+++

Keys; - = not okay

+ = slight okay

++ = Moderate okay

+++ = Highly okay

Table 3
Antibiotics Susceptibility Profile of the test organism and Environmental Isolates

S/n	Isolate ID	Organism identified	ZONES OF INHIBITION (mm)							
			Caz	Crx	Gen	Cpr	Nit	Amp	Ofl	Aug
1	EIA_1	*Bacillus* spp	0	0	32	0	32	0	30	22
2	EIA_2	*Escherichia coli*	0	0	23	20	17	0	19	0
3	EIA_3	*Bacillus* spp	0	38	42	0	30	0	36	24
4	EIA_4	*Pseudomonas* spp	0	0	8	24	0	0	23	0
5	EIA_5	*Escherichia coli*	0	0	14	13	9	0	9	0
6	EIA_6	*Escherichia coli*	0	12	10	22	35	0	24	0
7	EIA_7	*Escherichia coli*	0	0	22	24	9	0	22	0
8	EIA_8	*Klebsiella* spp	0	0	23	0	14	0	0	0
9	EIA_9	*Escherichia coli*	0	0	22	19	0	0	17	0
10	EIA_10	*Pseudomonas* spp	0	0	24	24	18	0	20	0
11	EIA_11	*Pseudomonas* spp	0	0	24	33	22	0	31	0
12	EIA_12	*Pseudomonas* spp	0	0	20	18	16	0	18	0
13	EIA_13	*Escherichia coli*	0	0	23	14	12	0	0	0
14	EIA_14	*Escherichia coli*	0	0	13	21	0	0	10	0
15	EIA_15	*Pseudomonas* spp	0	0	9	6	24	0	0	0
16	EIA_16	*Proteus* spp	0	34	32	23	34	10	13	11
17	EIA_17	*Escherichia coli*	0	0	24	21	26	0	24	0
18	EIA_18	*Escherichia coli*	0	0	20	17	23	0	12	0
19	EIA_19	*Bacillus* spp	0	13	25	0	0	0	28	0
20	EIA_20	*Pseudomonas* spp	0	0	0	21	23	0	0	0
21	EIA_21	*Staphylococcus* spp	12	0	22	28	33	0	31	0
22	EIA_22	*Escherichia coli*	0	0	9	0	22	0	0	0
23	EIA_23	*Pseudomonas* spp	0	0	9	11	0	0	9	0
24	EIA_24	*Escherichia coli*	0	0	24	22	0	0	12	0
25	EIA_25	*Klebsiella* spp	0	0	31	0	20	0	0	0
26	EIA_26	*Pseudomonas* spp	0	0	24	11	0	0	14	0
27	EIA_27	*Escherichia coli*	0	0	21	23	0	0	21	0
28	EIA_28	*Bacillus* spp	0	0	42	6	35	0	35	20
29	EIA_29	*Pseudomonas* spp	21	20	0	0	0	0	0	0
30	EIA_30	*Klebsiella* spp	0	0	23	13	9	0	11	0
31	EIA_31	*Bacillus* spp	0	0	30	0	28	18	25	22
32	EIA_32	*Escherichia coli*	0	0	0	25	28	0	31	0
33	EIA_33	*Escherichia coli*	0	0	8	24	0	0	23	0
34	EIA_34	*Pseudomonas* spp	0	0	23	20	17	0	19	0
35	EIA_35	*Pseudomonas* spp	0	0	14	21	0	0	0	0
36	EIA_36	*Bacillus* spp	0	0	15	0	30	0	0	0
37	EIA_37	*Bacillus* spp	0	25	27	0	30	0	28	26
38	EIA_38	*Bacillus* spp	0	0	18	0	24	0	27	23
39	EIA_39	*Bacillus* spp	0	16	30	0	0	0	25	34
40	EIA_40	*Bacillus* spp	0	28	30	0	28	0	30	25

S/n	Isolate ID	Organism identified	ZONES OF INHIBITION (mm)							
			Caz	Crx	Gen	Cpr	Nit	Amp	Ofl	Aug
41	EIA_41	*Staphylococcus* spp	0	0	20	0	0	0	22	0
42	EIA_42	*Pseudomonas* spp	0	0	19	21	0	0	19	0
43	EIA_43	*Bacillus* spp	0	12	30	0	46	0	35	27
44	EIA_44	*Bacillus* spp	0	0	32	0	30	0	0	35
45	EIA_45	*Bacillus* spp	0	0	41	12	37	0	35	33
46	EIA_46	*Bacillus* spp	0	0	24	0	18	0	28	12
47	EIA_47	*Proteus* spp	0	0	22	21	26	0	18	0
48	EIA_48	*Klebsiella* spp	0	0	22	12	24	0	10	0
49	EIA_49	*Pseudomonas* spp	0	0	12	8	0	0	15	0
50	EIA_50	*Bacillus* spp	0	0	27	0	34	0	28	20
51	Test organism	E. coli **O157:H7**	0	0	22	19	0	0	17	0

Key; Caz – Ceftazidime Crx – Cefuroxime Gen – Gentamicin Cpr – Ciprofloxacin

Nit– Nitrofurantoin Amp – Ampicillin Ofl – Ofloxacin Aug – Augmentin

Table 4
Distribution of Bacteriophages in Relation to Sample Location

S/N	Sample location	Code	Presence of Phage plaque	Size	Distinct Plaque Appearance
1	Student Village Hostel_1	ECPA_1	+	1mm	Round, smooth edges, entire and transparent
2	Student Village Hostel_2	ECPA_2	+	2mm	Round, smooth edges, entire and transparent
3	Old JUTH_1	ECPA_3	+	3mm	Round, smooth edges, entire and transparent
4	Old JUTH_2	ECPA_4	+	4mm	Round, smooth edges, entire and transparent
5	Angwa Rukuba_1	-	-	-	-
6	Angwa Rukuba_2	-	-	-	-

Key; - = Absent

+ = Present

Working Conc. = 12.58 $\log_{10}$ PFU/ml

Working Concentration = 12.58 $\log_{10}$ PFU/ml

Working Concentration = 10.79 $\log_{10}$ PFU/ml

Working concentrations; ECPA_1 = 10.51 $\log_{10}$ PFU/ml, ECPA_2 = 9.08 $\log_{10}$ PFU/ml, ECPA_3 = 10.26 $\log_{10}$ PFU/ml, ECPA_4 = 8.08 $\log_{10}$ PFU/ml

Working concentration = 12.80 $\log_{10}$ PFU/ml

Figure 7. **Chloroform Stability Test**

Table 5
HOST RANGE OF THE TEST ORGANISM AND SOME SELECTED PATHOGENIC ORGANISMS

S/N	PATHOGENIC ORGANISMS	ECPA_1	ECPA_2	ECPA_3	ECPA_4
1	*Salmonella typhi_1*	+++	+++	-	+++
2	*Salmonella typhi_2*	-	-	-	-
3	*Salmonella typhi_3*	+++	+++	+++	+++
4	*Salmonella pullorum_1*	+++	-	++	+++
5	*Salmonella pullorum_2*	-	-	-	-
6	*Klebsiella pneumonia*	+++	+++	+++	+++
7	*Staphylococcus capitis*	-	-	-	-
8	*Staphylococcus aureus*	+++	+++	+++	+++
9	*Pseudomonas aeruginosa_1*	+	+	-	++
10	*Pseudomonas aeruginosa_2*	-	-	-	-
11	*Escherichia coli 026*	-	-	-	-
12	*Escherichia coli O157:H7*	+++	+++	+++	+++
Key; - = No activity					

+ = Slight activity

++ = Moderate activity

+++ = High activity

Discussion

The results obtained in this study indicates that the bacteriophages isolated from effluent water and soil has a lytic activity against *E. coli* O157:H7 and a large community of bacteria. A motivation for this study was the numerous reports about the occurrence of *E. coli* O157:H7 which has predominantly emerged in recent years as the causative agent of hemorrhagic colitis in humans (Ateba and Akindolire, 2019). This illness, with characteristic symptoms of bloody diarrhea and abdominal cramps, can progress into a more severe, life-threatening complication known as hemolytic uremic syndrome (HUS) (Sibanyoni *et al*., 2017). In current scenario, serious medical and social problem results from the increasing antibiotic resistance of bacterial strains (WHO, 2014; CDC, 2014; Ayodele *et al*., 2020). Due to unprofitability of the venture and the risks of development of resistance in bacteria, pharmaceutical industries are withdrawing from research and development on new antibiotics (Clarke, 2003), which has generated interest over conventional and current system of microbial control for alternatives to antibiotics therapy (Basdew and Laing, 2015). Lytic phages are the possible replacement for antibiotics to treat bacterial infections not responding to conventional antibiotic therapy (Wang *et al*., 2017; Ssekatawa *et al*., 2021).

Total viable count for environmental isolates

A high bacterial count was observed in Angwa Rukuba_1, followed by Old JUTH_1, Student Village Hostel_2 and Old JUTH_2 while a low count of the bacterial load was observed in Angwa Rukuba_2 and Student Village Hostel_1. This confirms that pathogenic bacteria can be isolated from the environment which supports the findings of Obayiuwana *et al*., 2018 carried out in wastewaters obtained from pharmaceutical facilities in Lagos and Ogun States, Nigeria, where a large community of bacteria was isolated. This observation also agrees with the work of Mulamattathil *et al*., 2014 carried out in Mafikeng, North West Province, South Africa which indicated the incidence of water contamination as some of these species are indicators of faecal contamination as demonstrated in the occurrence of heterotrophic bacteria, total coliforms, faecal coliforms, and *Aeromonas* and *Pseudomonas* in water samples analysed.

Identification of environmental isolates

From the cultural identification of environmental isolates, a total of 50 bacteria isolates were identified, where *Bacillus* spp has the highest number of percentage occurrence, followed by *Escherichia coli* and *Pseudomonas aeruginosa*. *Proteus* spp and *Staphylococcus aureus* has the least number of occurrence, followed by *Klebsiella* spp. This agrees with the research findings of Egbere *et al*., 2020 in the area of the isolates but disagrees with the percentage occurrence of the isolates where *Proteus* spp has the highest number of occurrence, followed by *P. aeruginosa*, E. *coli*, *Salmonella* spp, S. *aureus* and S. *faecalis* having the least percentage occurrence from hand washed samples of Internally Displaced Persons in Jos and environs. It also supports the research work of Bolaji *et al*., 2011 on antibiotic resistance pattern of bacteria spp isolated from hospital waste water in Ede South Western, Nigeria, where the following organisms; *Enterobacter aerogenes, Pseudomonas putida, Pseudomonas fluorescens, Klebsiella edwardsii, Proteus mirabilis, Pseudomonas aeruginosa, Shigella spp* and *Flavobacterium meningosepticum* are isolated from waste.

Antibiotics Susceptibility Profile of the test organism and Environmental Isolates

The environmental isolates show a low level of antibiotic resistance to Gentamicin, Ciprofloxacin, Nitrofurantoin and Ofloxacin. A high level of antibiotic resistance to Cefuroxime, Ampicillin and Augmentin was observed with a total resistance of all the isolates to Ceftazidime except a strain of *Pseudomonas aeruginosa.* This supports the work of Adewale *et al*., 2020 on antibiotic sensitivity of bacteria associated with selected waste dumpsites in Akure, Nigeria, where *Bacillus subtilis*, *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Streptococcus faecalis, Shigella dysenteriae, Escherichia coli, Serratia marcescens, Clostridium botulinum* are susceptible to antibiotics except *Micrococcus luteus* which was resistant to all the antibiotics used, thereby, posing to the local inhabitant of the area a potential threat. The research findings of Egbere *et al*., 2020 on the assessment of the hand hygiene status of internally displaced persons in Jos and environs, Nigeria disagrees with this findings, where *Streptococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp, *Pseudomonas aeruginosa*, and *Proteus* spp were the most resistant organisms to the antibiotics followed by *P. aeruginosa*. The highly sensitive organisms were *S. aureus*, supporting this research findings and *S. faecalis* respectively.

Distribution of Phages in Relation to Location of Sample

A total of four phages ECPA_1, ECPA_2, ECPA_3 and ECPA_3 were isolated from six different locations of effluent water and soil within Jos metropolis, supporting the research work of Ezemokwe *et al*., 2021 on complete genome sequence of pseudomonas phage Zikora where Zikora is a temperate bacteriophage that was isolated from sewage water from a hospital environment in Jos, Plateau State, Nigeria. This also supports the research findings of Sundar *et al*., 2009 on isolation of host-specific bacteriophages from sewage against human pathogens, where a total three bacteriophages were isolated from sewage water sampled using sterile dark containers from the Sewage Treatment plant located at Jinke Park, Bangalore, India.

Stability test for the phages

Studies have shown that high temperature inactivates phages due to nucleic acid and protein denaturation (Yamaki *et al*., 2014; Wang *et al*., 2001). Yamaki *et al*., 2014 observed that Myoviridae phages drastically lost phage activity (3.5 logs PFU mL^{-1}) after 60 min of incubation at 60°C. However, in the current study, the four phages show stability at temperature of 45^{o}C to 60^{o}C while there is no visible plaque observed at temperature above 60^{o}C. This is in line with the research work of Litt and Jaroni, 2017 on the isolation and physiomorphological characterization of *Escherichia coli* O157: H7-infecting bacteriophages recovered from beef cattle operations, where the phages remained stable for 24 h at a temperature range (40–60°C) and for 90 days in cold storage.

At a wide pH range (2–9), phages in the current study were also stable compared to the phages in other studies (Niu *et al*., 2012; Northrop, 1964). Niu *et al*., 2012 revealed that at pH 3 the titer of *E. coli* O157:H7 phage, AKFV33, dropped by 1.9 logs PFU mL^{-1} after 15 min and was undetectable after 2 hours. In the current study, only phage ECPA_4 survived at pH 2 and phage ECPA_1 survived at pH 9. All the phages survived at pH 5 and 7, excluding phage ECPA_4 which do not survived at pH 7 after 24 hours. It is possible for phages to acquire nonreversible mutations at low pH (Nobrega *et al*., 2016), which could explain the survival of phages at low pH in the present study. Strack *et al*., 1964 showed a linear relationship between phage mutation rate and incubation at low pH, suggesting that phages can acquire mutation to survive acidic environment. In the current study, the phages were also stable at alkaline pH (7–9), and showed no loss in titer at pH 7 and a maximal loss in titer at pH 9.

In the current study, the four phages were stable at NaCl concentration of 0.5 to 15, except phage ECPA_4 that show no visible plaque at 15. This research finding is in line with the work of Duyvejonck *et al*., 2021, where all phages survived at NaCl concentration of 0.9 without any decrease in the phage titer. A high stability in low salt concentration (0.5–5%) were shown by the isolated phages. But increasing the concentration (10% -15%) led to a greater reduction in phage activity. With studies conducted by some researchers where high levels of salt, in some cases up to 5M did not affect the phage titer, this agrees with the observed saline stability (Lu and Breidt, 2015; Smolarska *et al*., 2018). The adverse effect of increasing salt concentration can be due to osmotic pressure exerted on phage capsids preventing the ejection of phage DNA (Evilevitch *et al*., 2003) or aggregating effect of high salt concentration on phage particles with concomitant reduction in bacterial binding sites (Ateba, and Akindolire, 2019).

Certain chemicals such chloroform are incorporated into the growth medium to enhance lysis and also included in the phage lysates to prevent bacterial contamination during the isolation, purification and preparation of phages. (Sambrook *et al*., 1989). The isolated phages showed a high level of stability to chloroform treatment after 24 hours. Thus the incorporation of 10% chloroform into the phage medium during isolation and storage is recommended for these phages. However, in this study, when the sensitivity to this agent was unknown, chloroform was not used. Varying results on the susceptibility of phages to chloroform have been reported by some authors, suggesting that each of the phage sensitivity to chloroform is confirmed prior to chloroform treatment.

Host Range of the Test Organism and Some Selected Pathogenic Organisms

All the isolated phages showed high lytic activity against the test organism *Escherichia coli O157:H7* and some of the selected pathogenic bacteria, suggesting that they have a broad host range. Phages specific to one *E. coli* O157:H7 strain as shown by studies can also infect other O157:H7 strains (Raya *et al*., 2006; Bach *et al*., 2003; Sheng *et al*., 2006). In a study by Raya *et al*., 2006, phage AR1 lysed all O157:H7 tested strains, while in another study (Goodridge *et al*., 2003), phage CEV1 infected 17 of the tested 19 strains of *E. coli* O157:H7. Results from the current study are similar to these studies, revealing that isolated phages with high target specificity are virulent against a wide range of *E. coli* O157:H7 isolates.

The isolated phages could therefore be applied as a control strategy against transmission and survival of *E. coli* O157:H7 in the food chain. The emergence of phage-resistant bacteria, particularly with application of phages in animals or on the farm is however, a major concern with this approach. Use of phage cocktails that are regularly updated with new or different phages could potentially address the issue, by maintaining selective pressure on bacterial host (O'flynn *et al*., 2004; Tanji *et al*., 2004).

Conclusion

In conclusion, four strong, stable and virulent lytic bacteriophages were isolated and characterized against *E. coli* O157:H7 in this study. They can be considered as promising candidates in the biocontrol of an emerging food-borne pathogen *E. coli* O157:H7. With the help of this information, phage-based

interventions application can be determined. Against *E. coli* O157:H7 isolates, bacteriophages isolated in this study showed a wide range of host specificity along with high lytic activity, NaCl stability, pH and thermal stability, Chloroform stability and therefore could be applied as biocontrol agents in the area of food industry.

Future research should be directed to sequencing of the whole genomes so as to ascertain their safety in biocontrol and to the optimization of cocktails concentration that can be used to cause complete inhibition of *E. coli* O157:H7 in real food systems and artificial media.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

CONFLICT OF INTEREST.

Anayochukwu C. Ngene, John O. Egbere, Isaac A. Onyimba, Chinedu G. Ohaegbu, Michael M. Dashen, Uzal Umar, Nnaemeka E. Nnadi, and John C. Aguiyideclare that they have no conflict of interest

COMPETING INTEREST

Anayochukwu C. Ngene, John O. Egbere, Isaac A. Onyimba, Chinedu G. Ohaegbu, Michael M. Dashen, Uzal Umar, Nnaemeka E. Nnadi, and John C. Aguiyideclare no competing interest.

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AUTHORS' CONTIBUTION

Anayochukwu C. Ngene: Investigation, Methodology, Formal analysis, Original draft, Software

John O. Egbere: Supervision, Review, Editing, Validation

Isaac A. Onyimba: Supervision, Review, Editing, Validation

Chinedu G. Ohaegbu: Editing, Software

Michael M. Dashen: Supervision, Review, Editing, Validation

Uzal Umar: Methodology, Investigation,Data curation, Formal analysis

Nnaemeka E. Nnadi: Methodology, Investigation,Data curation, Formal analysis

John C. Aguiyi: Supervision, Review, Editing, Validation

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AUTHORS' INFORMATION

Not applicable

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Figures

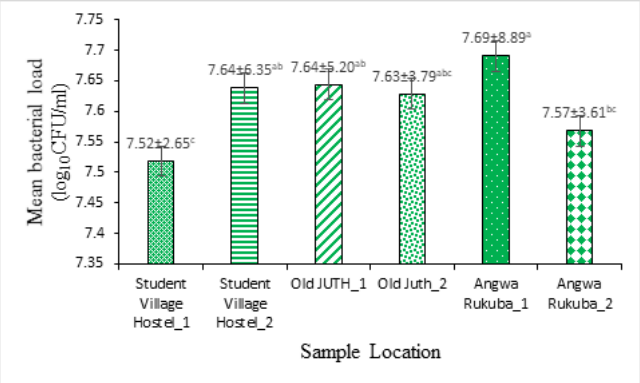


Figure 1

Total viable count for environmental isolates

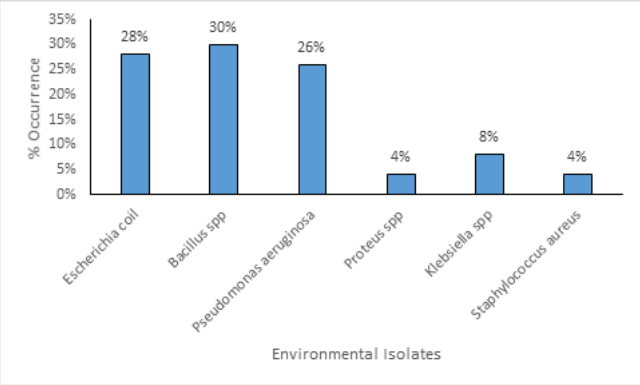


Figure 2

Percentage Occurrence of Environmental Isolates

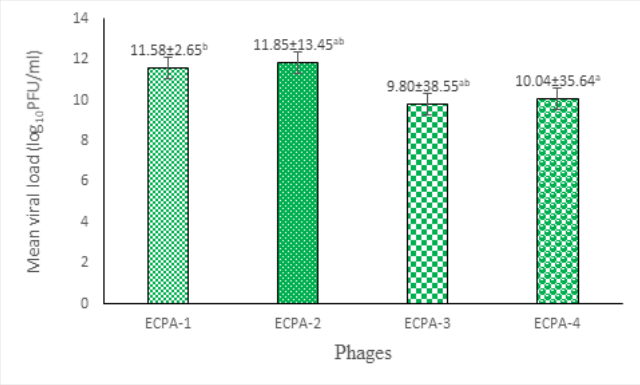
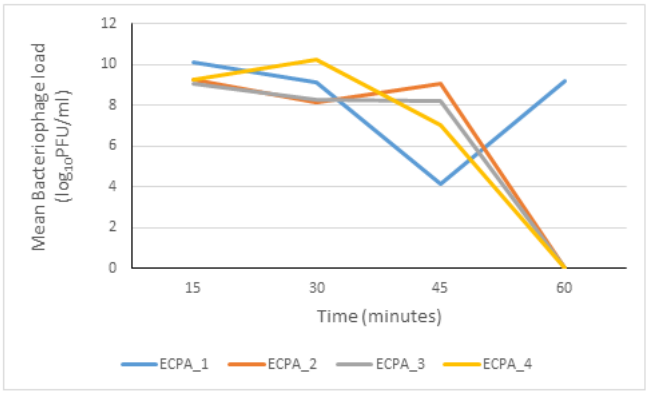
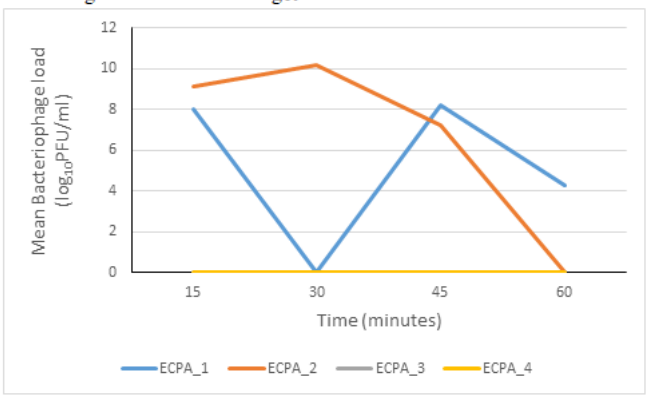


Figure 3

Bacteriophage viral load



Working Conc. = 12.58 log_{10} PFU/ml

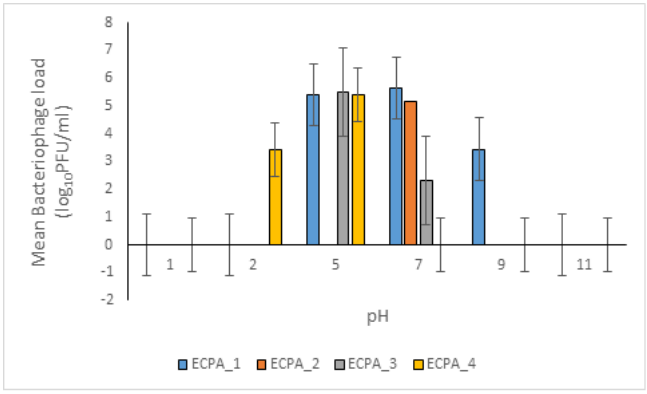


Working Concentration = 12.58 log_{10} PFU/ml

Figure 4

a. Thermal Stability of the phages at 45°C

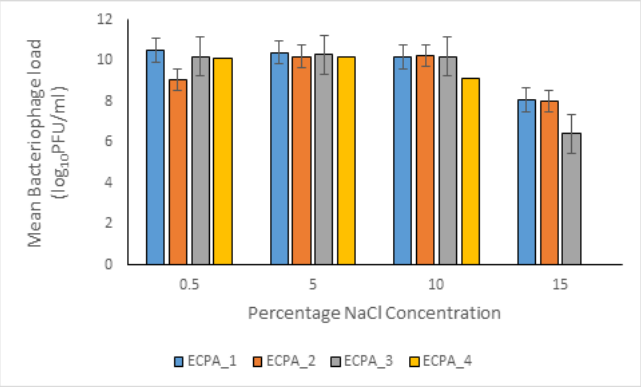
b. Thermal Stability of the phages at 55°C



Working Concentration = 10.79 log_{10} PFU/ml

Figure 5

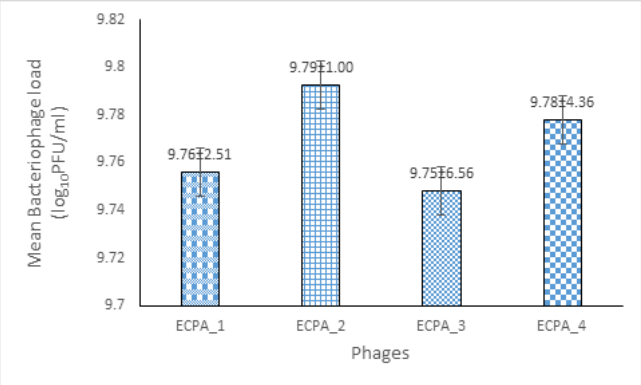
pH Stability Test



Working concentrations; ECPA_1 = 10.51 log_{10} PFU/ml, ECPA_2 = 9.08 log_{10} PFU/ml, ECPA_3 = 10.26 log_{10} PFU/ml, ECPA_4 = 8.08 log_{10} PFU/ml

Figure 6

Salinity Stability Test



Working concentration = 12.80 log_{10} PFU/ml

Figure 7

Chloroform Stability Test

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- APPENDIX.docx
- SupplementaryMaterials.docx