SCREENING AND CHARACTERIZATION OF *ESCHERICHIA COLI*-SPECIFIC BACTERIOPHAGE ISOLATED FROM SEWAGE WATER

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Juthamas Chumsen

Researcher

บทคัดย่อ

เรื่อง : การแยกและการศึกษาคุณสมบัติของแบคเทอริโอฟาจจากแหล่งน้ำเสียที่จำเพาะต่อเชื้อ *Escherichia coli*

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งานวิจัยนี้มีวัตถุประสงค์เพื่อแยกแบคเทอริโอฟาจจากแหล่งน้ำเสีย และเพื่อศึกษาคุณสมบัติของแบคเทอริโอฟาจที่จำเพาะต่อเชื้อเอสเชอริเชีย โคไล ผลการศึกษาพบว่าสามารถแยกแบคเทอริโอฟาจจากตัวอย่างน้ำเสียจำนวน 3 ตัวอย่าง ได้แก่ น้ำเสียบริเวณกุดปลาขาว (bacteriophage JC01) น้ำจากบ่อบำบัดน้ำเสียโรงพยาบาลสรรพสิทธิประสงค์ (bacteriophage JC02) และน้ำจากบ่อบำบัดน้ำเสียโรงพยาบาลโขงเจียม (bacteriophage JC03) การศึกษาคุณสมบัติของแบคเทอริโอฟาจต่อการยับยั้งเชื้อเอสเชอริเชีย โคไล โดยวิธี spot test และ plaque assay พบว่าแบคเทอริโอฟาจสามารถยับยั้งเชื้อเอสเชอริเชีย โคไล ได้อย่างจำเพาะ ผลการทดสอบความสามารถในการยับยั้งแบคทีเรียก่อโรคชนิดอื่น ๆ พบว่าแบคเทอริโอฟาจทั้ง 3 ชนิด สามารถยับยั้งเฉพาะเชื้อเอสเชอริเชีย โคไล เท่านั้น ผลการทดสอบประสิทธิภาพของแบคเทอริโอฟาจในการยับยั้งเชื้อเอสเชอริเชีย โคไล ที่ดื้อต่อยาปฏิชีวนะ 3 กลุ่มขึ้นไป พบว่าแบคเทอริฟาจ JC01 แบคเทอริฟาจ JC02 และแบคเทอริฟาจ JC03 มีประสิทธิภาพในการยับยั้งเชื้อเอสเชอริเชีย โคไล ร้อยละ 51.7 (138/267), 52.4 (140/267) และ 28.5 (76/267) ตามลำดับ ผลการทดสอบประสิทธิภาพของแบคเทอริโอฟาจในการทนต่อสารเคมีและอุณหภูมิพบว่าแบคเทอริโอฟาจทั้งสามสามารถทนต่อ distilled water และ 0.85% normal saline ได้นานกว่า 40 นาที แต่ไม่สามารถทนต่อสารละลาย 10% ethanol และ 1% hydrogen peroxide ผลการทดสอบความทนอุณหภูมิพบว่า แบคเทอริโอฟาจ JC01 สามารถทนต่ออุณหภูมิ 60 องศาเซลเซียส ได้นานถึง 60 นาที แต่แบคเทอริโอฟาจ JC02 และแบคเทอริโอฟาจ JC03 สามารถทนต่ออุณหภูมิ 60 องศาเซลเซียส ได้เพียง 45 นาที ผลการศึกษาคุณสมบัติของสารพันธุกรรมของแบคเทอริโอฟาจ ทั้ง 3 ชนิด พบว่าสารพันธุกรรมของแบคเทอริโอฟาจ JC01 และ JC02 ถูกตัดด้วยเอนไซม์ *Hin*dIII และ DNase สารพันธุกรรมของแบคเทอริโอฟาจ JC03 ถูกตัดด้วยเอนไซม์ *Nco*I *Hin*dIII และ DNase แต่สารพันธุกรรมของแบคเทอริโอฟาจทั้ง 3 ชนิด ไม่ถูกย่อยด้วยเอนไซม์ RNase A ซึ่งสรุปได้ว่าแบคเทอริโอฟาจทั้ง 3 ชนิด มีสารพันธุกรรมเป็น double-stranded DNA การศึกษารูปร่างของแบคเทอริโอฟาจด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่านพบว่า แบคเทอริโอฟาจทั้ง 3 ชนิด มีส่วนหัวเป็นรูปหกเหลี่ยม มีหางยาว มีขนาดอนุภาคประมาณ 200 นาโนเมตร เมื่อพิจารณา

รูปร่างลักษณะและชนิดสารพันธุกรรมของแบคเทอริโอฟาจทั้ง 3 ชนิด พบว่าจัดอยู่ในวงศ์ *Myoviridae* ออร์เดอร์ *Caudovirales* ดังนั้น แบคเทอริโอฟาจที่แยกได้ในงานวิจัยครั้งนี้จึงน่าสนใจในการนำไปศึกษาต่อในขั้นสูงต่อไป

ABSTRACT

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KEYWORDS : BACTERIOPHAGE, *ESCHERICHIA COLI*, PHAGE THERAPHY

The objectives of this study were to isolate and to characterize bacteriophage specific to *Escherichia coli* from different sources of waste waters. Based on spot test and plaque assay, the result showed that bacteriophages could be isolated from waste water treatment plant Kudprakhow (bacteriophage named JC01), Sappasitthiprasong hospital (bacteriophage named JC02), and Khongjiam hospital (bacteriophage named JC03). Host range determination of bacteriophage revealed that all bacteriophage types had high specific host range only for *E. coli*. Inhibition of clinical isolates *E. coli* with multidrug resistant property showed that bacteriophage JC01, JC02, and JC03 inhibited the growth of *E. coli* at 51.7% (138/267) 52.4% (140/267), and 28.5% (76/267), respectively. Bacteriophage stability in different solutions and heat at different time points demonstrated that all bacteriophages could tolerate 0.85% normal saline and distilled water for more than 40 minutes but could not tolerate 10% ethanol and 1% hydrogen peroxide at every time point. Heat stability showed that bacteriophage JC01 had resisted at 60 °C after 60 minutes of incubation. Bacteriophage JC02 and JC03 showed the ability to resist the temperature of 60 °C after 45 minutes of incubation. The result of bacteriophage classification by genome analysis demonstrated that the extracted DNA of JC01 and JC02 could be digested with *Hind*III and DNase but not for RNase. For bacteriophage JC03, the extracted genome could be digested with *Nco*I, *Hind*III and DNase, but not for RNase. This

result indicated that the bacteriophage JC01, JC02, and JC03 genome was a DNA virus and their genome was a double-stranded DNA (dsDNA). In addition to viral genome analysis, determination of viral particle morphology by transmission electron microscope can also be used to classify bacteriophage group. The result found that all bacteriophages had the viral particle which composed of a head with a hexagonal shape and long tails with contractile. The size from head to tail was approximately 200 nm. Based on Intraclass correlation coefficient (ICC) classification of prokaryotic (bacterial and archaeal), bacteriophage JC01, JC02, and JC03 could be classified in Family *Myoviridae*, Order *Caudovirales*. Therefore, the bacteriophages derived from this study could be used to study their potential use in further advanced steps.

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LIST OF ABBREVIATIONS

CHARACTER	MEANING
CFU	Colony forming unit
°C	Degree Celsius
DNA	Deoxyribonucleic acid
g	Gram
h	Hours
Kb	Kilobase pairs
µg	Microgram
µL	Microliter
µm	Micrometer
mL	Milliliter
mm	Millimeter
min	Minute
PFU	Plaque forming unit
/	Per
%	Percent
rpm	Revolutions per minute
RNA	Ribonucleic acid
TEM	Transmission electron microscope
×*g*	Times gravily
SDS	Sodium dodecyl sulfate
EDTA	Ethylenediaminetetraacetic acid
TAE	Tris-acetate

CHAPTER 1
INTRODUCTION

1.1 Background and rational of the study

Escherichia coli, or *E. coli*, is a gram negative bacteria. The rod shape does not produce spores as facultative anaerobes. Can be grown in an oxygenated and oxygenless in environment. In the genus *Enterobacteriaceae.* The grouped in the coliform is a coliform that is found in human feces and warm-blooded animals. It is used as an indicator of the hygiene of food and water. It exists naturally in the large intestine of animals and humans. This type of bacteria causes diarrhea most often. Both in children and adults. Make a liquid defecation or water, but the symptoms are not severe. For both children And adults often have immunity because they get infected in small doses but get infected constantly. The infection is often contaminated with food, water or hand of the cook. Normally these infections may be present in the faeces, even if there is no symptoms. Originating in Southeast Asia such as Burma, Thailand, Laos, Cambodia, Indonesia, etc. (McLaughlin, Balaa, Sims, & King, 2006)

E. coli is a normal flora found in the intestines of humans and warm-blooded animals.Normally, it will not harm or cause serious disease. When in the intestines will help digest the food we eat, but if *E. coli* into the body system. The body will also cause serious infections such as urinary tract infections. Meningitis Infection with blood and so on, and some *E. coli* strains that cause diarrhea.By the contamination of the virus. In food or drinking water, *E. coli* can cause diarrhea. (Diarrheagenic *E. coli*) has a pathogenic mechanism and can Toxins produced in different species, such as enterotoxigenic *E. coli*, which form enterotoxin enterotoxins, cause acute diarrhea. Enterotoxigenic *E. coli*, a species that produces enterotoxin, produces acute diarrhea. Liquid feces or Enterohaemorrhagic *E. coli* that cause Shiga poisoning cause severe diarrhea. Bloody stools cause hemophilia. And acute kidney failure.

E. coli in the laboratory of the Institute of Public Health Sciences from the past 7 years (2007 - 2013) found *E. coli* causes diarrhea. All of them accounted for 10.1 percent. The results of 10 antimicrobial susceptibility test is Ampicillin, Amoxicillin, Cefotaxime, Ceftazidime, Cefuroxime, Cephalothin, Co-trimoxazole, Gentamicin, Norfloxacin and Tetracycline.

Bacteriophages or phages are virus of bacteria which can be found enormously in nature together with their specific hosts. There are two forms of life cycle which are lytic (virulent phage) that causes bacterial lysis after complete the phage propagation to release the progeny and lysogenic (temperate phage) that integrate phage genome into bacterial genome without causing cell lysis. Bacterial resistance is a very important problem worldwide. The use of phages is an effective alternative that has been developed for medical use in both Europe and America (Biswas et al., 2002) Phages virus that live in cells each bacterium has a high host specificity. Can be selected and separated from the environment such as sewage, waterspout, soil. Phages can destroy pathogenic bacteria that are resistant to antibiotics and prevent infection and can be used with antibiotics. Phages that are specific to pathogenic bacteria are safe for humans and animals because the virus is specific to the host bacterium. It has the specificity to kill the pathogenic bacteria (Chhibber & Kumari, 2012); (Van Twest & Kropinski, 2009)

For the reasons mentioned above, it is the source of this research. In this study, this research is to screening bacteriophage specific to *E. coli* and characterization this bacteriophage.

Therefore, bacteriophage screening in this research has good properties. It is interesting to continue to study in advanced. Particularly, the ability of bacteriophage to inhibit bacteria in cultured cells and in experimental animals.

1.2 Objectives

1.2.1 To isolate bacteriophage specific to *Escherichia coli* bacteria from sewage Water

1.2.2 To study the properties of bacteriophage specific to *Escherichia coli* isolated from waste water sources.

1.3 Scope and limitation of research

1.3.1 Isolate bacteriophage specific to *Escherichia coli* bacteria from sewage water in Ubon Ratchathani

1.3.2 Isolate bacteriophage specific to *Escherichia coli* bacteria from sewage water in Ubon Ratchathani

1.4 Anticipated outcomes

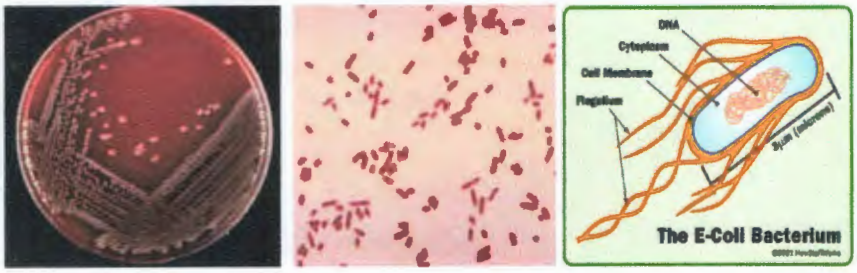
Know the properties of bacteriophage isolated from waste water sources in Ubon Ratchathani Province.

CHAPTER 2
LITERATURE REVIEWS

2.1 Bacteriology of *Escherichia coli*

2.1.1 General characteristics of *E. coli*

Escherichia coli is an abbreviation for *E. coli*, a gram-negative bacterium that is not rodent. Cell walls are quite thick and hard because they contain more protein than carbohydrates. The small cellular area called the pili is protruding from the bacterial cell, which is used to bind to a particular area of the host's skin. The flagellate is a flagella. Or a slender longitudinal line. Facultative anaerobes are able to grow in both oxygenated and non-oxygenated environments. Family *Enterobacteriaceae* The coliform is a coliform that is found in human feces and warm-blooded animals. It is used to index the hygiene of food and water. (Guentzel, 1996)



A B C

Figure 2.1 (A) Characterization of *E. coli* colonies on blood agar
(B) Characteristics of *E. coli* under microscope Gram-negative rods
(C) Structural characteristics of *Escherichia coli* bacteria

2.1.2 Classification of diseases caused by *E. coli*

Escherichia coli or *E.coli* is a common bacterial pathogen in humans and animals. There are 8 pathovars that can be divided into pathovars. There are 8 types of pathovars that can be divided into two groups.

2.1.2.1 Extraintestinal *E.coli* (ExPEC) refers to *E. coli* that cause gastrointestinal and intestinal diseases.

1) Uropathogenic *E.coli* (UPEC) is an *E. coli* that causes urinary tract infections. Most *E.coli* infections are caused by *E.coli*. *E.coli* is most often contaminated by the gastrointestinal tract. When entering the urinary tract, the infection is caught in the cell. Lining Bacterial communities are called intracellular bacterial communities (IBCs). When the bacteria in the IBC come out of the cell, they can enter the next layer of the urinary tract. And if not treated, it may cause kidney infection.

2) Neonatal meningitis *E.coli* (NMEC) is an *E.coli* that causes cortex in newborn babies. It is believed that *E.coli* is derived from the maternal digestive tract that is contaminated at birth. The infection passes through the intestinal mucosa into the bloodstream. And the virus enters the brain through a blood brain barrier (BBB), which can pass through the brain into the brain and nervous system and cause inflammation in the cortex.

2.1.2.2 Diarrhoeagenic *E.coli* refers to *E. coli* that causes intestinal adherence. There are 6 types.

1) Enteropathogenic *E.coli* (EPEC) is an *E. coli* that causes diarrhea in young children. When the infection attaches to the intestinal wall, it releases the substance into the cell. This will interfere with the absorption of water and minerals. Cause diarrhea. Enteropathogenic *E. coli*, abbreviated as EPEC This species, although it causes food poisoning. But not the result of Entropyxin. According to studies, it has been found that Bacteria cause disease by localized localization mechanisms to the tissue shell. As a result, the mucus in the mucus. Gut The bacteria then grow and multiply in the intestinal mucosa. Then the protein is inhibited. In general, EPEC causes diarrhea under the age of one year.

2) Enterohaemorrhagic *E.coli* (EHEC) is an *E. coli* that causes bloody diarrhea in both children and adults. It is contaminated with beef. Toxin toxin called Shiga toxin (Stx) or verocytotoxin Shiga toxin-producing *E.coli* (STEC) is sometimes called the toxin. This inhibits protein production and destroys lymphatic tissue and toxins. It can enter the bloodstream and produce pathogens that cause diarrhea with blood. EHEC This breed produces toxic substances that are similar to

Shiga like toxins and toxic substances. Verotoxin, Verocytotoxin, is a toxin that kills Vero cells in the laboratory. Vertex cells from the kidneys of African green monkeys. One species There are two types of EHECs: STL-I (VT-I) and SLT-II (VT-II). The new terminology is Stx1 and Stx2, respectively. This is different at the chemical element. The examples of *E. coli* in this group are *E. coli* O157: H7

3) Enterotoxic *E.coli* (ETEC) is an *E.coli* that causes diarrhea in travelers (diarrhea). Toxin that is made from toxin is heat-stable toxin (heat-stable enterotoxin: ST) and heat-labile enterotoxin (LT) interfere with the absorption of minerals in the intestinal mucosa, resulting in diarrhea. (Enterotoxigenic *E. coli*). ETEC These two types of steroids are heat stable toxins (ST), which are classified as STA or ST-I and STB or ST-II. Two types of heat-labile toxins (LT) and LTB are similar to those of cholera. Food poisoning from ETEC begins with eating foods that contain about 10^6 living bacteria.-10^{10} cfu /g into bacteria can increase the number in the small intestine. With the poison out. Diarrhea is a watery diarrhea similar to cholera. The symptoms are less severe. Feces are not bloody. Diarrhea is a result. Adenylate cyclase from the intestinal wall, which results in cAMP (Cyclic 3 ', 5'-adenosine monophosphate), increases the amount of fluid secreted in the gastrointestinal tract. Toxic substances Hot stimulates the secretion of cGMP (Cyclic guanosine monophosphate) in the mucous membranes. Causes loss of fluid and electrolyte of the body. (Perez et al., 2014)

4) Enteroinvasive *E.coli* (EIEC) is *E.coli* with pathogenicity similar to Shigella is a disease and bloody diarrhea. Both flea-like and non-follicle-like viruses are responsible for the transmission of infection. The infection is transmitted from one cell to another. It can destroy cells in the deeper layers of the intestinal mucosa. Enteroaggregative *E.coli* (EAEC) is a common cause of traveler's diarrhea. After ETEC, watery diarrhea is present, but some people may have bloody diarrhea. Can be found in both the small intestine and colon. The bacteria are combined into a biofilm, causing the disease to pass through the mucous membranes covering the intestinal mucosa, causing it to attach to the intestinal mucosa and releasing substances that interfere with the cell's absorption process, resulting in diarrhea.(Enteroinvasive *E. coli*). EIEC This species does not produce endothelium. But destroy the host cell. Bacteria penetrate into the epithelial cell and spread to neighboring cells, similar to the

behavior of the virus. Bacteria in this group like in the intestines. Causes of diarrhea are both bloody and bloodless. Born with babies and old people Bacteria take time.
EIEC is the first *E. coli* strain that has been found to cause foodborne illness, with outbreaks occurring in England in 1947. The outbreak occurred in the United Kingdom in 1947. One school consumes salmon, although it proves that the outbreak is from food. But it is known that EIEC can spread from one person to another.One species has previously been isolated from stools of diarrheal travelers and is commonly found in children's stools.

5) Diffusely adherent *E.coli* (DAEC) is an *E. coli* that causes diarrhea in children between the ages of 18 months and 5 years. It also causes urinary tract infection in adults. This group differs from other groups in that it generates a number of cytotoxic agents, and toxin (secreted autotransporter toxin: Sat) breaks down the cell's tight junctions. Abnormal Diarrhea.

6) Groups that cause intestinal cell lining. (Enteroaggregative *E. coli*), an abbreviation for EAggEC. For EAggEC is a newly discovered species that does not appear violent.

2.1.3 Guidelines for the treatment of *E. coli* infections

Treatment of mineral salts, to replace what the body loses and treats the symptoms only. No need for antibiotics to treat. Since most *E. coli* diarrhea is caused by the toxin. Taking antibiotics can cause more toxins and make them worse. Antibiotics may be used in some cases, such as diarrhea. Combined with high fever To help reduce the severity of the disease. However The decision to use antibiotics should be at the discretion of the treating physician. So, the pivotal choice is the type of antibiotic to treat. Use the drug resistance information in the laboratory. To know the trend of resistance and prevention of the epidemic of resistance.Treatment is usually symptomatic, but most are treated with antimicrobial or antibiotics. Beta-lactams, neomycin, gentamycin, amoxycillin, sulphonamide, flouroquinolones and tetracyclines are among the most susceptible. Currently, *E. coli* has a high rate of resistance and is resistant to many groups of drugs. *E. coli* produces extended-spectrum beta-lactamase (ESBL) enzymes. The higher the problem, the more healing with up. (Bugarel, Beutin, Martin, Gill, & Fach, 2010)

2.1.4 *E. coli*-ESBL and treatment problems

Extended Spectrum β-Lactamases: ESBLs refers to the class A enzyme β-Lactamases that can destroy drugs and make resistant bacteria. Both cephalosporins, cefotaxime, ceftazidime and ceftriaxone ESs Enzymes are as follows. The TEM and SHV derivatives are found in Enterobacteriaceae. There are over 150 types of CTX-M types present. There are more than 100 types. Minor types are less common enzymes such as VEB and PER. However, not only ESBLs are available. The second and third generation of oxyimino-cephalosporins, but also other mechanisms, such as Enterobacteriaceae, Enterobacteriapecies, Plasmid-mediated AmpC β-Lactamases such as CMY-type enzymes in Klebsiellaspecies and E.coli K1 chromosomal β-Lactamases, such as K.oxytoca Metallo (IMP, VIM, NDM), and non metallo (KPC and OXA-48 enzymes). Efflux-mediated resistance carbapenemases in P.aeruginosa. Carbapenemases in Acinetobacter. species 4(Haruki, Hagiya, Haruki, & Sugiyama, 2018)

2.1.5 The main mechanism of resistance in *Enterobacteriaceae*.

Bacteria develop more resistant to antimicrobial agents. The use of new antimicrobials Bacterial antimicrobial resistance is caused by the mutation of the bacteria, which may occur at the chromosome. The large chromosomes contain genes that are expressed in various ways. Many infections, including resistance. Antimicrobial resistance may be caused by plasmids. The genetic material outside the chromosome. (Extrachromosome). Plasmid is smaller than chromosome. The gene that controls the resistance of one of the bacterial plasmids can be easily transmitted to other bacteria, resulting in rapid spread of resistance to one another. Naked DNA, phage or plasmids, which are responsible for the development of resistance and resistance to pathogens, have been identified. One drug may be resistant to many drugs, so there are fewer drugs available.

2.1.5.1 Resistance

The resistance of bacteria can occur in two ways.

1) Natural selection. Each bacterium has antibiotic-resistant bacteria already in the mix. But a small number. It does not involve antibacterial drugs. But the problem is when the bacteria are exposed to antibacterial and much longer. The drug

will destroy the drug is not resistant to it. The resistance to the drug is increased, and is expressed as resistant bacteria.

2) Induction by the use of antibacterial drugs this approach says. Each bacterium The former is sensitive to antimicrobial agents. When to contact antimicrobial agents? In particular, the size and timing of unsuitable germs to destroy the infection. The pathogenesis of genetic mutations is likely to be resistant to drug destruction. These resistance may be due to the gene or DNA that is resistant to other organisms.

2.1.5.2 Transformation

It is a gene transfer process. Naked DNA, in which the DNA is transported without an inducer, does not have a high molecular weight. Only one or two resistant genes will be transported at once. Transformation occurs in both Gram-positive and Gram-negative bacteria.

2.1.5.3 Transduction

It is a process of transferring genes from the virus to the transgenes. The phage-resistant gene is expressed in this manner. The DNA that is transcribed is not very large and is usually fixed. Because of the limited scope of the virus envelope, only one or two resistant genes will be transmitted at once. This process is found in Gram-positive and Gram-negative bacteria. Most are found in Grams. Like the drug's resistance *Staphylococcus aureus*.

2.1.5.4 Conjugation (Mating)

In general, this process involves transferring the gene from the host to the host via pili as a link between the two. The plasmid or R-plasmid type Conjugative plasmid is the type that is transmitted by this process. There is a gene that mimics r-determinant resistance. It also has a gene that mimics the transmission of drug resistance (RTF), which can induce the cell to produce sex pili, thereby passing the plasmid to another pili-bound cell. This is noteworthy that both the donor and the receptor are resistant. While the first two processes this method of conjugation can transfer 1-10 or more genes at the same time.

2.1.5.5 The main mechanism of resistance found in *Enterobacteriaceae*

1) Resistance to beta-lactams (Eckert-Boulet et al., 2004)

Beta-lactams resistance in *Enterobacteriaceae* There are many ways to create enzymes, destroy drugs, reduce drug imports, etc. The main mechanism of drug-induced enzymatic damage (Drug inactivation) is beta-lactamases The enzyme that destroys the ring of beta-lactams in the drug can not destroy the bacteria. This enzyme has many kinds. The amino acids are classified into 4 classes.

1.1) Class A enzyme has a wide variety of enzymes. Penicillinase and cephalosporinase such as extended spectrum beta-lactamase (ESBLs), inhibitor resistant beta-lactamase (IRT)

1.1.1) Properties of ESBLs

1.1.2) ESBLs are enzymes of β-lactamases, (Eckert et al., 2004) that can break down penicillin, first-, second-, third-generation cephalosporins and aztreonem (but not resistant to cephamicins or carbapenems) by hydrolysis. These microorganisms. In addition, it is inhibited by β-lactamases inhibitors such as clavulanic acid. ESBLs are enzyme groups. There are several species (Philippon, Labia, & Jacoby, 1989).

1.1.3) SHV-type ESBLs (Ullah et al., 2016) are common in isolates from patients. It is different from SHV-1 by replacing glycine at position 238. Serine is found in *Enterobacteriaceae* and outbreaks of *Pseudomonas aeruginosa* and *Acinetobacter* spp.

1.1.4) TEM-type ESBLs developed from TEM-1 and TEM-2 have enzymes developed from TEM that can hydrolyse third-generation cephalosporins but not ESBLs because they are not inhibited by clavulanic acid. These enzymes are complex mutants of TEM. CMT)

1.1.5) CTX- M and Toho β-lactamases can destroy cefotaxime and cefepime very well and the enzyme CTX-M has a high incidence and transgenic resistance gene (Minarini, Poirel, Trevisani, Darini, & Nordmann, 2009).

1.1.6) OXA-type ESBLs, such as OXA-18, developed from OXA-13 found in Pseudomonas aeruginosa

1.1.7) PER-type ESBLs were first found in Pseudomonas aeruginosa and Acinetobacter spp.

1.1.8) VEB-1, BES-1 and other ESBLs such as GES, BES, and IBC are examples of ESBLs that are not TEM or SHV.

1.2) Class B enzyme is metallo beta-lactamase

1.3) Class C enzyme has chromosomal cephalosporinase properties such as AmpC Properties of Amp C (Black, Moland, & Thomson, 2005)

AmpC is resistant to cephamycins such as cefoxitin. In high doses, false positives can be detected. In ESBLs, hydrolysis of 3rd cepholosporins may be found but not inhibited by clavulanic acid.

1.4) Class D enzyme is oxacillinase.

Enzyme formation beta-lactamases Frequent in the infection. Enterobacteriaceae are Class A and Class C enzymes, both narrow spectrum and broad spectrum enzymes.

2) Resistance in the group Aminoglycosides

2.1) Reduce uptake or decreased celll permeability as a mechanism of chromosomal mediated resistance. Resistance to this drug has been reported in both groups .(Sauvage, Kerff, Terrak, Ayala, & Charlier, 2008)

2.2) Altered Ribosomal Binding Sites is a rare mechanism of drug resistance. Enzymatic modification is the most common mechanism and gene that controls plasmid resistance.

2.3) Resistance in the group. Trimethoprim-sulfamethoxazole

2.4) Resistance to Quinolones

Is the mechanism of resistance. The mutation of the DNA gyrase gene, which is the target of the drug. (Singh & Gupta, 2017)

3) Monitoring of *E. coli* resistance in the laboratory of the Institute of Public Health Sciences From the past 7 years (2007 - 2013), *E. coli* causes diarrhea. All of them were 10.1 percent. Ampicillin, Amoxicillin, Cefotaxime, Ceftazidime, Cefuroxime, Cephalothin, Co-trimoxazole, Gentamicin, Norfloxacin and Tetracycline Amoxicillin, Tetracycline, Co-trimoxazole, Cephalothin and Gentamicin were 80.5%, 69.7, 69.0, 23.7 and 15.6 respectively. Enteroaggregative *E. coli* resistant to Ampicillin, Tetracycline, Co ¾ Enterotoxigenic *E. coli* resistant to Ampicillin, Tetracycline, Co-trimoxazole and Cephalothin 44.6%, 54.2%, 31.7% and 16.7%, respectively. Ampicillin, Tetracycline and Co-trimoxazole 80.0%. Shiga toxin-

producing *E. coli* resistant to Ampicillin, Tetracycline and Co-trimoxazole. This drug also found resistance in the group. extended-spectrum cephalosporins By the enzyme. Extendedspectrum β-lactamase (ESBL) of *E. coli* causes diarrhea 3.0% (Torkar & Bedenić, 2018)

4) Resistance Report in Thailand

The first KPCs were detected in *Escherichia coli* from urine specimens from patients in the hospital's surgical ward. First time at King Prajadhipok's hospital from car accident, he was transferred to another hospital. The cause of such an accident is about a month and a half. Then moved back to the treatment. At King Prajadhipok's Hospital By the time the move. Back to treatment at King Prajadhipok's Hospital was found infected with *E.coli* infection KPCs. Urine from the garden where the patient has a catheter.During treatment with *E. coli*, resistant to Cefazolin, Ampicillin, Trimethoprim / Sulfamethoxazole, Gentamicin, Cefotaxime, Ampicillin / Clavulanic acid, Norfloxacin, Ciprofloxacin, Ceftazidime, Ampicillin / Sulbactam, Ertapenem and Meropenem, have intermediate effects on Imipenem. Specificity in the test Amikacin and Piperacillin / Tazobactam from the disc diffusion method, as well as positive effects.Modified Hodge Test (MHT) Test 9. Subsequently, *E.coli* was tested for the Minimal Inhibitory Concentration (MIC) test at the Institute of Public Health Sciences Department of Medical Sciences to test drug resistance has a drug resistant effect. Ertapentin (MIC> 32 ug / ml) and Meropenem (MIC> 4 ug / ml) did not cause Carbapenems. Hemoculture, Sputum culture and Pus culture are not available. Found the drug. It can not be identified as infection. Such as pathogenic or simply colonization (Thaicharuen). Of 3,004 gram-negative bacilli collected from intra-abdominal infections in the Asia-Pacific region during 2007, 42.2% and 35.8% of *E. coli* and *Klebsiella* spp., respectively, were extended-spectrum β-lactamase (ESBL) positive. Moreover ESBL rates in India for *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were 79.0%, 69.4%, and 100%, respectively. ESBL-positive *E. coli* rates were also relatively high in China (55.0%) and Thailand (50.8%). Ertapenem and imipenem were the most active over 90% of all species, including drugs tested, inhibitESing BL-positive isolates with the exception of *Pseudomonas aeruginosa* isolates (<90% susceptible to all study drugs) and ESBL-positive *Klebsiella pneumoniae* isolates (<90% susceptible to all study drugs except

imipenem). Quinolones achieved 90% inhibition levels only against ESBL-negative *K. pneumoniae* and ESBL-negative K. oxytoca. A decline in ampicillin-sulbactam activity was noted, with only 34.5% of all *Enterobacteriaceae* inhibited in this study (Hawser et al., 2009). A cross-sectional pilot study was conducted in Chiang Mai, Thailand, to determine the prevalence of *Salmonella* and *Escherichia coli* in swine, broiler chickens and human workers from farms and abattoirs in northern Thailand, and compare their antimicrobial resistance profiles. Fecal samples and cloacal swabs were collected from 150 swine and 150 chickens at the farm. Fecal samples from swine, cloacal swabs from chickens, and carcass swabs from both animals were collected from 100 swine and 100 chickens at the abattoir. Stool samples were collected from 15 swine farm workers and seven chicken farm workers. Primary isolation and identification of *Salmonella* and *E. coli* were conducted using standard methods. In vitro susceptibility testing of *Salmonella* and *E. coli* was conducted using the broth microdilution method, based on the United States National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The prevalence of *Salmonella* from swine and chicken samples ranged from 2% to 25%. The prevalence of *E. coli* in chickens and swine ranged from 36.8% to 47.6%. In humans, the prevalence of *Salmonella* was 15%, and the prevalence of *E. coli* ranged from 51% to 53%. Resistance in *Salmonella* was found for tetracycline (84.7%), nalidixic acid (27.1%), florfenicol (18.6%), ampicillin (13.6%), and ceftiofur (3.4%), and in *E. coli* for tetracycline (91.5%), nalidixic acid (67.4%), ampicillin (61.6%), florfenicol (51.8%), enrofloxacin (28.7%), ciprofloxacin (12.5%), ceftiofur (4.9%) and ceftriaxone (1.5%) (Hanson, Kaneene, Padungtod, Hirokawa, & Zeno, 2003).

2.2 Bacteriophage

A bacteriophage or phage is a bacterial virus that can be used as a host to increase the number of obligate intracellular parasites. Many bacteriophages are found in nature. Common in feces, soil and even sea water. It was found that the number of phage and host are uncertain. Can change by season Also found Marine phage also has a food web (food web) that destroys host cells to break out, causing nutrients to be released. Or change to another form that is beneficial to other organisms anymore. (Withey, Cartmell, Avery, & Stephenson, 2005)

Bacteriophages are generally highly specific to the host bacteria. Each bacteriophage is specific to only one bacterium. Only two or three types of bacteria. Bacteriophage can destroy bacteria cells. Bacteriophage creates enzymes that destroy certain cells. Prior to the release of the newly born recombinant bacteriophage particles inside the cell, Based on the specificity of bacteriophage against bacteria. Bacteriophages are widely used in a wide range of applications, including phage typing, biocontrol, and phage therapy . (H. W. Ackermann & Krisch, 1997) (Loessner, 2005)

2.2.1 Structure and composition of bacteriophage

There are three basic structure of phages (Figure 2.2) Bacteriophages are generally structurally important . (H. W. Ackermann, 2003a)

2.2.1.1 Head consists of capsid, a protein that has many subunits together into a structure called capsomer, which is responsible for encapsulating the genome. Shape of bacteriophage The head of the bacteriophage is usually composed of a polyhedral or icosahedral symmetry, with the exception of some filamentous phage capsids, which are helical symmetry.

2.2.1.2 Tail is a hollow tube covered by sheath. Some bacteriophages have a sheath that is stretched and contracted. It is called contractile tail. The bacteriophages use the tail tip to attach to the receptor on the host's surface and inject the genome into the host. Head into the host cell of the genome. At the tail pipe to pass through cell walls into the cells eventually. Some bacteriophages have a long tail. Some species have short tail and some have no tail.

2.2.1.3 Genome is the genetic material of bacteriophage. Included in the header of the bacteriophage. Genetic material may be either DNA or RNA. Genotypes and shapes can be used as an important criterion for the identification of bacteriophages . (H. W. Ackermann, 2003b)

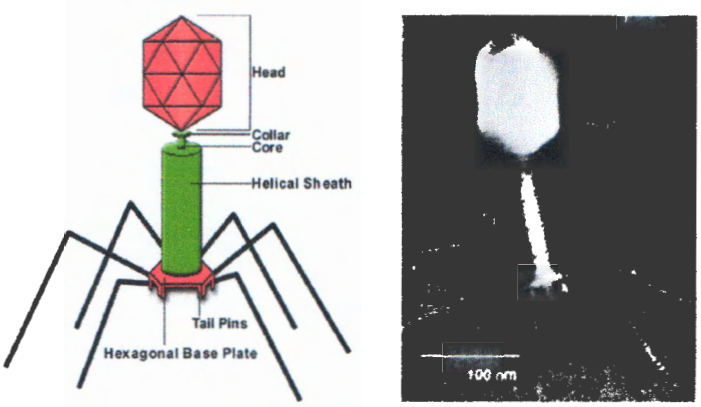


Figure 2.2 Structure and composition of bacteriophage

Source: Khakhum, Yordpratum, & Wongratanacheewin (2010)

2.2.2 Classification of bacteriophage

All bacteriophages contain a genome that is encapsulated by the capsid protein. Double-stranded DNA, single-stranded RNA, or single-stranded RNA, have linear and circular capsids of various shapes, such as hexagonal The filamentous or complex shape consists of the head and the tail (Figure 2.4). Currently, the International Committee for Taxonomy of Viruses (ICTV) classifies bacteriophages into 1 order 13 families and 30 genera. Nature of nucleic acid and particle morphology by many bacteriophages. Over 96 percent are tailed phage and most of them have dsDNA genome (H.-W. Ackermann, 2009) (Zafar, Mazumder, & Seto, 2002)

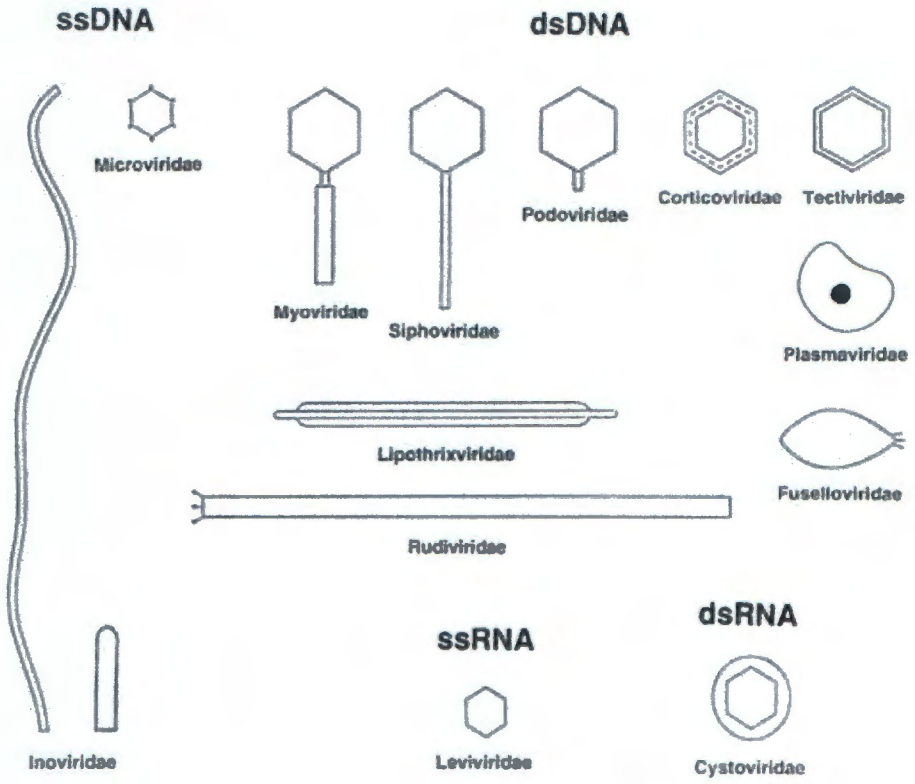


Figure 2.3 The families shapes of major phage groups

Source: Khakhum et al. (2010)

2.2.3 Bacteriophage life cycle

The survival of bacteriophages in bacterial cells is divided into two types, depending on the type of bacteriophage (Figure 2.6): (1) lytic life is found in lytic phage virulent phage and (2) lysogenic cycle living in lysogenic phage or temperate phage. (Jassim & Limoges, 2014)

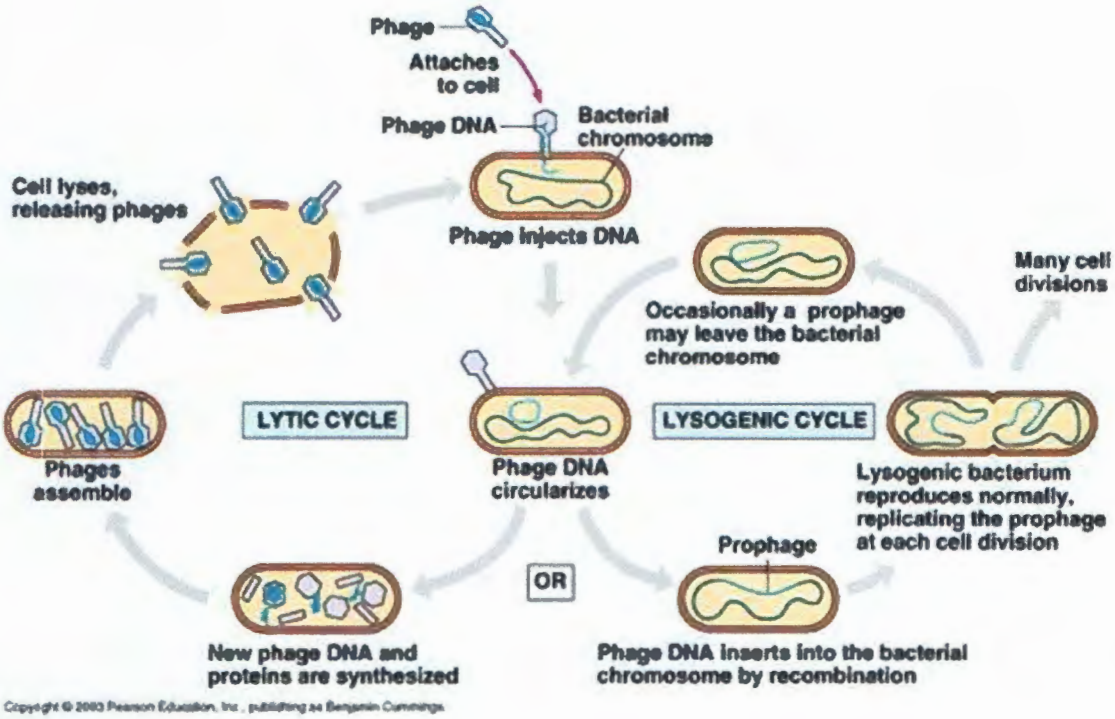


Figure 2.4 Living of lytic cycle and lysogenic cycle.

Source: Khakhum et al. (2010)

2.2.3.1 Lytic cycle when entering into bacterial cells. Bacteriophage can be increased in the cell. Using the substance. From the host to create the genetic material, the capsid protein and the various components are formed within the cell before. Then the components. These are made up of progeny phages or recombinant bacteriophages. It looks like a bacteriophage that starts infecting bacterial cells. Finally, bacteriophage creates an enzyme that destroys the bacterial cell wall. The cells break down (lysis) to release the phage progeny and enter the next cell. This lytic cycle of living if cultured.

The bacteriophages are mixed with the agar medium with a percentage of agar and then poured onto the surface of the agar plate. The bacterium is destroyed by a clear spot called plaque. Figure 2.5)

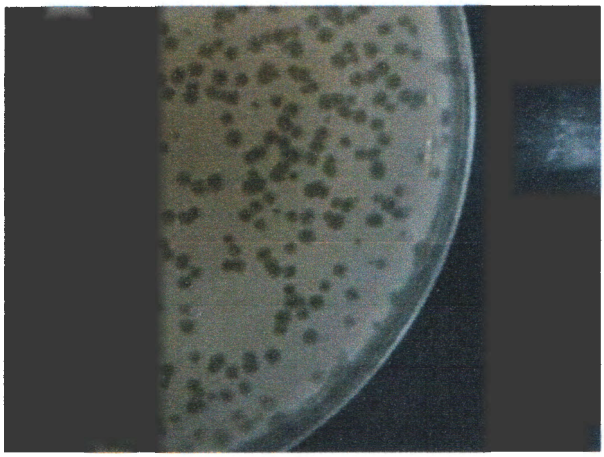


Figure 2.5 Plaque caused by lytic phage (ST70s) when cultured with host bacteria. (*Burkholderia pseudomallei*) on the skin surface.
Source: Khakhum et al. (2010)

2.3.1.2 Lysogenic cycle when bacteriophages enter the bacteria. No progeny phage is produced, but the bacteriophage genome is inserted into the chromosome of the bacterium by genetic recombination. This is called prophage. When the bacteria divide the cells to increase binary fission and reproduce the chromosome of the bacteria, the new prophage is still inserted into every new chromosome created. Part of the chromosome of bacteria So, the new bacterial cells that occur are therefore prophage. lysogenization

Bacteria with this latent prophage are called lysogen or lysogenic bacteria. The coexistence between host and lysogenic phage can lead to co-evolution. The coevolution is a mobile genetic element that moves the gene between living organisms through lateral or horizontal gene transfer. This phenomenon results in many host changes such as changing non-pathogenic bacteria. For example, *Salmonella* spp. when lysogenized with bacteriophage A3 or bacteriophage A4 will result in somatic O antigen changes in the cell wall. *Corynebacterium diphtheriae*, which produces diphtheria toxin that causes diphtheria, was found to be resistant to antibodies. Bacteria can produce toxins because they contain corynebacteriophage, which lysogenize within *C. diphtheriae*. The non-lysogenic species with corynebacteriophage does not produce toxins and does not cause the disease.

Clostridium botulinum, which causes botulism, is caused by *C. botulinum*. Was lysogenized with clostridialphage As well, it makes the bacteria create toxins and disease. Also found *Streptococcus pyogenes* , which causes scarlet fever, can infect only strains that have been invaded by phage T12 (Kutter et al., 2004).

2.2.4 Mechanisms of lytic phage in the destruction of bacteria

The destruction of the lytic phage bacterial cell wall is based on two endolysin (or lysine) and holin (endolysin) proteins, which in turn act as enzymes. peptidoglycan This is because the endolysin lacks some of the properties that make it impossible to move through the cell membrane and go on to act or destroy it. Therefore, endolysin is required to live or work with holin, which is a protein that is inserted into the cell membrane. Then the endolysin is created and inside the cell, it can pass out to the layer. The bacterial cell walls are destroyed and broken down so that the inner progeny phage is released into the extracellular space (Fischetti, 2005).

2.2.5 Use of bacteriophage for the treatment of infectious diseases

In the past, bacteriophages have been used to destroy bacteria. But when antibiotics are found to be more disinfectant. The interest in using the bacteriophage decreases. In Russia, the use of bacteriophage has been continuously developed. Currently, the medical side has adopted pure endolysin as a therapeutic agent, which may be used alone. Or use with antibiotics to destroy resistant bacteria. Antibiotic-resistant bacteria or biofilm-producing bacteria are difficult to remove because of their high efficiency and specificity in pathogenic bacteria (Brussow, Canchaya, & Hardt, 2004) .

The use of bacteriophage for the treatment of infectious diseases called phage therapy is based on the properties of lytic phage bacteriophages: (1) the ability to destroy bacteria or host to break down or die. And (2) the specificity between the bacteriophage and the host. And with these two important properties, bacteriophages can be used to replace antibiotics. In theory, bacteriophages also have superior advantages over antibiotics. In many ways, such as not destroy the normal flora bacteria that live in the body. Can increase the number on the host specific. No need for high doses or volume to enter the body. It is also reported that bacteriophages give better results in the treatment of infections than antibiotics in humans and in animals. For example, one report found that when bacteriophages were used to treat high doses

of *E. coli*, mice could die. The survival rate was 92% when treated with bacteriophage. However, there was a 33% survival rate with antibiotic therapy (Levin, 1996). Another report found that the use of *S. aureus*-specific bacteriophage to treat infected patients. Pus and pleurisy. The patients were divided into two groups, treated with bacteriophage and another treated with antibiotics. It was found that treatment with bacteriophage was associated with a high recovery rate of 82%, while antibiotic treatment had a median rate of recovery of only 64%. In addition, bacteriophage There are no side effects to the patient. If given intravenously, the rate of hepatotoxicity can be 95% (Sulakvelidze, Alavidze, & Morris, 2001) (Lin, Koskella, & Lin, 2017).

Table 2.1 Comparison of bacteriophage and antibiotic properties for use in the treatment of infectious diseases.

Bacteriophages	Antibiotics
1. Highly specific (highly specific) to bacteria.Targeted (bacterial host or target bacteria)	1. Non-destructive non-Specific or broad spectrum can destroy both. Pathogenic bacteria and Bacteria (normal flora)
2. Can increase the number of infected places. The concentration or amount enough to disinfect.	2. The disease is eliminated by the metabolic process of the body.The remaining concentration in the body is not enough. Sterilization at the infected site.
3. No side effect.	3. There are many side effects (side effects). Or sometimes it causes allergies.
4. Bacteriophage resistance of bacteria is limited.Just target bacteria.	4. Resistance to antibiotics of bacteria is not limited to. Target bacteria but can spread to Other bacteria
5. Search for new bacteriophages. To breakBacteria resistant to bacteriophage (phage-resistant bacteria. It is not long and easy).	5. The development or invention of new antibiotics to destroy.Antibiotic resistant to antibiotics (antibiotic-resistant bacteria). It takes a long time and is difficult.

Source: Parichat Phumkhachorn (2009)

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Study Design

The research design of this study was divided into two parts as follows:

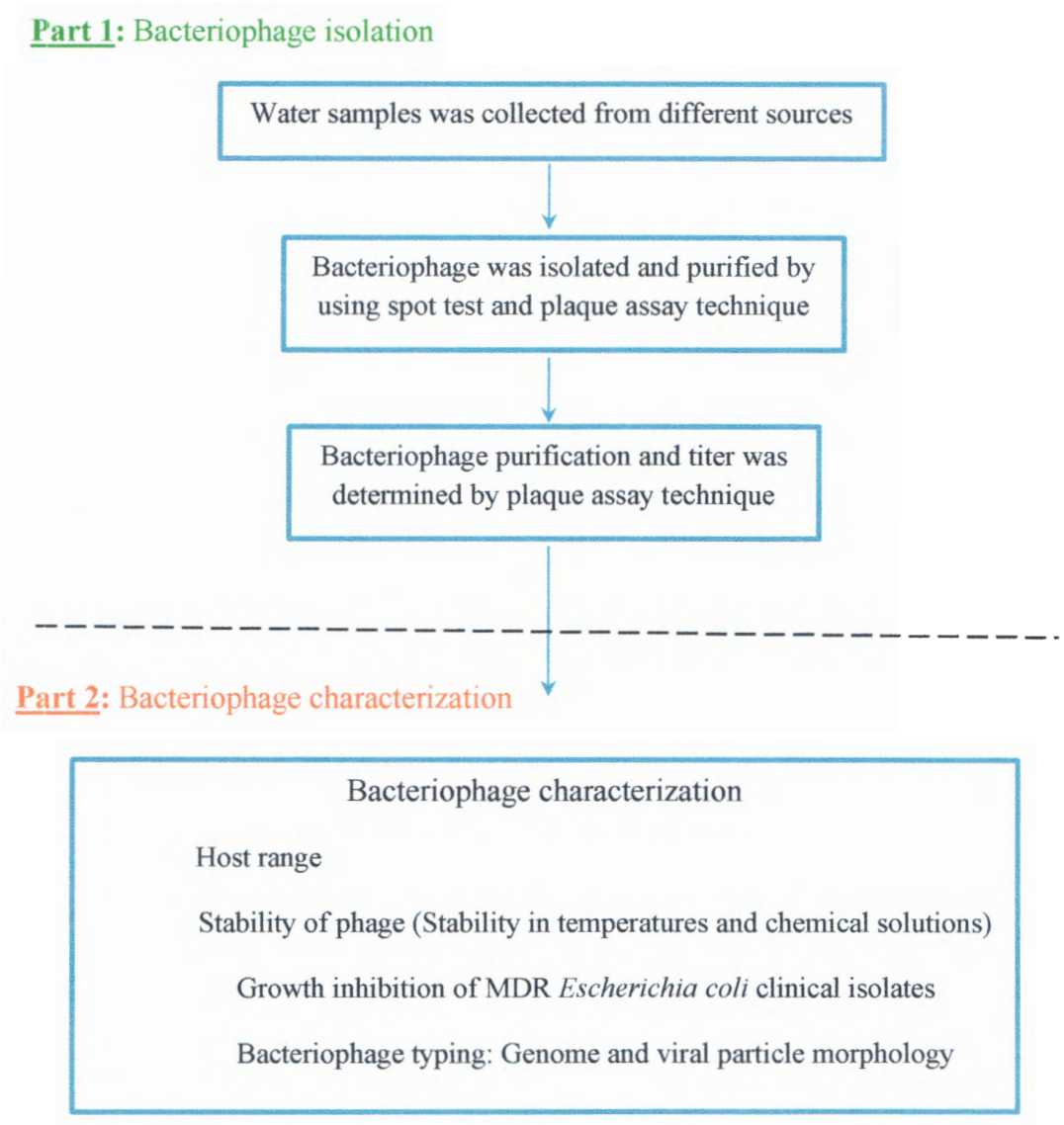


Figure 3.1 The work plan of this study

3.2 Bacterial strains and growth conditions

Bacteria strains used in this study were *Escherichia coli* (*E. coli*), multidrug resistant strains that were isolated from the admitted patients in Sapphasitthiprasong Hospital, Muang District, Ubon Ratchathani Province, Thailand. *E. coli* strains were grown in nutrient broth (NB) media and incubated at 37 °C for 18-24 h in aerobic condition. Agarified media was prepared by adding bacteriological-graded agar into the corresponding media.

3.3 Antibiotic susceptibility test

E. coli strains were cultured in NB media and incubated at 37 °C for 18-24 h in aerobic condition. After incubation, bacterial cell density was adjusted to McFarland no. 0.5 which was represented to 1.5 x 10^8 CFU/mL. McFarland is the standard turbidity which can be used to estimate the number of bacterial cells in the solution. One ml of adjusted bacterial solution was swabbed onto NA plate. The paper disc containing antibiotic was put onto the same NA plate. The plate was further incubated at 37 °C for 24 h. The interpretation of antibiotic susceptibility test results was based on the description of Clinical and Laboratory Standards Institute (CLSI) (Putthanachote, Homkrai, & Sarakarn). The antibiotic used in this study is shown in table 3.1

Table 3.1 Antibiotics with their disc concentration used in this study.

No.	Antibiotic	Abbreviation	Concentration (μg)
1.	Ampicillin	AMP	10
2.	Amikacin	AK	30
3.	Auqmentin	AMC	30
4.	Cefoxitin	FOX	30
5.	Cefotaxime	CTX	30
6.	Ceftazidime	CAZ	30
7.	Ceftriaxone	CRO	30
8.	Cefuroxime	CXM	30
9.	Co-trimoxazole	SXT	25
10.	Ciprofloxacin	CIP	5
11.	Ertapenem	ETP	10
12.	Gentamicin	CN	10
13.	Imipenem	IPM	10
14.	Meropenem	MEM	10
15.	Netilmicin	NET	30
16.	Sulperazone	SCF	105

3.4 Bacteriophage isolation and amplification

All water samples were collected from nine sites in Ubon Ratchathani province. First, wastewater treatment Kudprakhow plant Warin Chamrap. Second, wastewater treatment plant of Sapprasitthiprasong Hospital Mueang Ubon Ratchathani. Third, Moon river at waterfront of Wat Supatnaram-worawihan Mueang Ubon Ratchathani. Fourth, Moon River at Kaeng Saphue, Phibunmangsahan district. Fifth, Moon River at waterfront Wat Don That Phibunmangsahan. Sixth, wastewater treatment plant of Khong Chiam Hospital Khong Chiam district. Seventh, waterspout at Khong Chiam Hospital. Eighth, Khong River (clear water) Khong Chiam. The last sample was obtained from Khong River (thick water) Khong Chiam. Ten ml of water sample was centrifuged at 3000 rpm for 5 min. The supernatant was collected and further filtered with a 0.45 μm-pore size membrane filter, designated as filtrate_1. Five mL of filtrate_1 was added to 5 mL of a double strength nutrient (2xNB) supplemented with

100 µL of *E. coli* No.40, a strain used as bacterial host for bacteriophage isolation. The suspension was then incubated at 37 °C for 18-24 h. After centrifuge at 3000 rpm for 5 min, the culture was filtered with a 0.45 µm-pore size membrane filter and then collected through the filter paper, designated as filtrated_2, which was further used for bacteriophage detection

3.5 Bacteriophage detection

3.5.1 Spot test

A single colony of *E. coli* grew on NA agar was selected and incubated in NB media. After incubation at 18 to 24 h, bacterial cell suspension was adjusted the concentration of 0.5 McFarland ($1x10^8$ CFU /mL). The culture was swabbed on a NA agar. The filtrate_2 derived from 3.4 was dropped on the center of the NA agar plate. The plate was further incubated at 37 °C for 18 to 24 h. After incubation the cultured plate was checked for the presence of clear zone. Once the plate showed the clear zone at the area of filtrate_2 was dropped, indicating that the presence of specific bacteriophage.

3.5.2 Plaque assay

The filtrate_2 was prepared by ten-fold serial dilution method. A 100 µL of diluted filtrate_2 was added to 3 mL of semisolid Brain Heart Infusion (BHI) media, which was warmed at 60 °C. The 100 µL of *E. coli* was added into filtrated_2-BHI mixer was incubated at 37 °C for 18-24 h. The number of clear zone, hereafter called plaque, presented on agar plates was counted and recorded. The quantity of bacteriophage as Plaque-forming unit/ml (PFU/mL) was calculated as the following equation:

$$\text{Plaque-forming unit/mL (PFU/mL)} = \text{Plaque number} \times 10 \times \text{dilution factor} \qquad (3.1)$$

3.6 Purification of Bacteriophage

The bacteriophage was purified by using sterile micropipette tips (Yordpratum, Tattawasart, Wongratanacheewin, & Sermswan, 2011). The sterile micropipette tip was pressed into the plaque area to collect the bacteriophage in NA media. The agar containing bacteriophage was then added into NB media, incubated by shaking for 1 h

and was centrifuged at 3000 rpm for 20 min at 4 °C. Then, the solution was filtered by using 0.22 μm-pore size membrane filter. Then filtered solution was further used for plaque assay as previously described in 3.5.2. The experiment step was repeated three times to purify the bacteriophage.

3.7 Host range determination

The method used to determine the host range of bacteriophage was performed by spot test as previously described in 3.5.1. Bacteria used in the test are shown in table 3.2.

Table 3.2 Bacteria used in host range determination

Pathogen	Properties
1. *Klebsiella pneumoniae*	Not know
2. *Bacillus subtilis*	Not know
3. *Enterococcus faecalis*	Quality control of these materials is only performed to demonstrate that the material distributed by BEI Resources is identical to the deposited material.
4. *Escherichia coli* ATCC 25922	This organism is a CLSI control strain for antimicrobial susceptibility testing.
5. *Escherichia coli* No. 37	Not know
6. *Escherichia coli* No. 38	Not know
7. *Escherichia coli* No. 39	Not know
8. *Escherichia coli* No. 40	Not know
9. *Salmonella* spp.	*Salmonella* spp. are a group of bacteria which reside in the intestinal tract of human beings and warm blooded animals and are capable of causing disease.
10. *Shigella dysenteriae*	*Shigella* spp. are bacteria that cause shigellosis, also known as bacillary dysentery. They are a highly infectious organism, with foodborne outbreaks often involving infected food handlers.
11. *Staphylococcus aureus* ATCC 29213	Quality control strain for API, BBL, bioMerieux Vitek, Micro-Media, MicroScan™, and Sensititre products. Standard strain for CLSI antimicrobial susceptibility testing.

Table 3.2 Bacteria used in host range determination (Continued)

Pathogen	Properties
12. *Staphylococcus aureus* No.MU50	This strain is Methicillin resistant (MRSA). Resistant to Oxacillin and shows reduced Vancomycin susceptibility. Genome sequenced strain.
13. *Vibrio* spp.	Vibrio cholerae (V. cholerae), strain Nanking 32/124 was deposited at ATCC® in 1962 by Dr. Kenneth J. Steel, National Collection of Type Cultures, Central Public Health Laboratory, London, England. This strain showed no agglutination in O group I antiserum prior to deposition.
14. *Pseudomonas aeruginosa* Control	Not know
15. *Pseudomonas aeruginosa* No.910	Not know
16. *Lactobacillus* casei TISTR1341	Not know
17. *Lactobacillus* No.906	Not know

3.8 Growth inhibition of MDR *Escherichia coli* clinical isolates

E.coli strains with multidrug resistance (MDR) were derived from Sapphasitthiprasong Hospital. *E.coli* taken from the hospital was put on a Mueller-Hinton agar (MHA) plate by streak plate method and was incubated at 37 °C for 18-24 h. The method used to determine the inhibition MDR *E.coli* clinical isolates host was performed by spot test as previously described in 3.5.1.

3.9 Bacterial inhibition by bacteriophage cocktail

The filtrate_2 of bacteriophage (code JC01, JC02, JC03) was used to determine the cross bacteriophage inhibition. Nine *E. coli* strains received from Sapphasitthiprasong hospital was selected for this test. Nine bacterial host was cultured in NB media at 37 °C for 18-24 h. The filterate_2 of bacteriophage code JC01, code JC02, code JC03, code JC01 plus JC02, code JC02 plus JC03, was dropped down onto the center of NA plate. Then culture plate was incubated at 37 °C for 18-24 h.

3.10 Stability of bacteriophage

The chemical solution of 0.85% normal saline, 10% ethanol, and 1% hydrogen peroxide was used this study. Distilled water was used as the control. An 100 µL of bacteriophage solution was added to 900 µL of each chemical solution which included 0.85% normal saline, an 10% ethanol, and 1% hydrogen peroxide. The solutions were then incubated at 37 °C for 10, 20, 30 and 40 min. The sample used for calculation of the survival of the bacteriophage was collected at the those time points. The calculation was done according to the following formula:

$$\frac{\text{Bacteriophage titer in chemical treatment}}{\text{Bacteriophage titer in control}} \times 100 \qquad (3.2)$$

3.11 Heat stability

The test in this step is to test the heat resistance of the bacteriophage. The NB media was pre-incubated at 5, 10, 15, 25, 30, 35, 40, 45, 50, 55, and 60 °C for 15 min. After incubation, an 100 µL of bacteriophage solution was added into the 900 µL pre-warmed NB in the microcentrifuge tube. The solution was further incubated at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 °C for 60 min. After incubation, the solution was used to test by spot test technique as previously described in 3.5.1.

3.12 Growth inhibition of bacteriophage

Bacterial host was cultured in 3 mL of NB broth and incubated at 37 °C. The overnight culture was adjusted to 0.5 McFarland ($1x10^8$ CFU/mL). The filtrated_2 solution was added to bacterial host. The incubated solution was collected at 2, 4, and 6 h. After incubation, the solution was used to prepare ten-fold dilution. The diluted solution of 10^4, 10^5, and 10^6 was spreaded onto the plate and then incubated at 37 °C for 18-24 h. After incubation, the number of colonies forming unit was counted and calculated.

3.13 Bacteriophage genome analysis

A 100 µL of bacteriophage (10^8-10^9 PFU/mL) was mixed by 10% sodium dodecyl sulfate and incubated at 65 °C for 15 min. Equal volume of Phenol: Chloroform: Isoamyl Alcohol (1:1:24) was added and mixed by inversion. The upper phase of supernatant of the mixture was collected by centrifugation at 13,000 rpm for 10 min at 4°C. The suspension was added with 300 µL of 3 M sodium acetate (pH 4.8) and mixed by inversion. Equal volume of isopropanol was added to the suspension and incubated at -20 °C for 1 h. The genomic DNA was collected by centrifugation at 13,000 rpm for 10 min at 4°C. Finally, the DNA pellet was rinsed with 1 mL of 70 % ethanol followed by air-dried and suspended in 50 µL of sterile distilled water or TE (pH 8.0). The genomic DNA was kept at -20°C for further analysis.

3.14 Genome analysis by restriction enzyme digestion

The purified phage genome was digested by restriction enzyme *Eco*RI, *Nco*l, *Pae*l, *Hin*dIII. RNase A and DNase were also used to digest purified phage genome.

3.15 Agarose gel electrophoresis

The molecular size of DNA was determined by gel electrophoresis as previously described by Sambrook (Sambrook, Russell, & Russell, 2001) Briefly, the DNA was mixed with the 6X gel-loading buffer in a ratio (1:2). The mixture was then loaded into the wells covered by electrophoresis buffers. The electrophoresis was carried through 50 voltage (V) for 4 h. The agarose gel was stained with ethidium bromide for about 15 min. Finally, the gel was visualized under UV transillumination. The molecular size of DNA was determined by comparing its bands with standard size DNA.

3.16 Bacteriophage morphology

Ten µL of the filtrate_2 was transferred to a copper grid and incubated for about 5 min to allow the copper plate absorbing the bacteriophage. Then, 2% phosphotungstic acid (pH 7.0) was dropped onto copper grid and incubated for 15 min. The dye was dropped onto copper grid and incubated for 15 min. The copper grid

was further incubated for 2 h. The electron microscope (JEOL Ltd.) was operated at 80 kV.

3.17 Site of conducting experiments

All experiments were performed at the College of Medicine and Public Health, Ubon Ratchathani University.

CHAPTER 4
RESULTS

4.1 Antibiotic susceptibility test for *Escherichia coli* no.40

In this study, *Escherichia coli* strain no.40 was firstly used as a bacterium host for bacteriophage isolation, because it has been tested as multidrug resistant (MDR) stain. The *E. coli* no.40 was kindly provided from Sapphasitthiprasong hospital. To ensure that *E. coli* no.40 was the real MDR strain, antibiotic susceptibility test was performed by using antimicrobial disk diffusion method. As shown in Table 4.1 it was found that *E. coli* no.40 showed the resistance to seven antibiotics which include ampicillin, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, cefuroxime, and gentamicin. Of these antibiotics, ampicillin, cefotaxime, ceftazidime, ceftriaxone, and cefuroxime are cell wall synthesis inhibitors, ciprofloxacin are nucleic acid synthesis inhibitors, and gentamicin are protein synthesis inhibitors. The results indicated that *E. coli* no.40 is MDR strain.

Table 4.1 Determination of antibiotic susceptibility *Escherichia coli* no.40

No.	Antimicrobial disk	Inhibition zone (mm.)	Interpretation
1.	Ampicillin (AMP)	0	R
2.	Amikacin (AK)	2	S
3.	Auqmentin (AMC)	18.5	I
4.	Cefoxitin (FOX)	29	S
5.	Cefotaxime (CTX)	0	R
6.	Ceftazidime (CAZ)	0	R
7.	Ceftriaxone (CRO)	0	R
8.	Cefuroxime (CXM)	0	R
9.	Co-trimoxazole (SXT)	25	S
10.	Ciprofloxacin (CIP)	0	R
11.	Ertapenem (ETP)	30.5	S
12.	Gentamicin (CN)	0	R
13.	Imipenem (IPM)	30	S
14.	Meropenem (MEM)	31	S
15.	Netilmicin (NET)	28	S
16.	Sulperazone (SCR)	22.5	S

Remark: R = Resistant, S = Susceptible, I = Intermediate, mm. = Millimetre

4.2 Bacteriophage isolation

The purpose of this test was to isolate bacteriophage from different wastewaters. The supernatant of filtrate_2 was dropped on nutrient agar (NA) supplemented with E. coli No.40. After incubation for 24 h, the inhibition zone was observed and recorded. It was found that the filtrate_2 derived from three wastewater samples of Kudprakhow swamp, Sapprasitthiprasong hospital, and treatment plant of Khong Chiam hospital, demonstrated the inhibition zone on NA plate supplemented with E. coli No.40. The result indicated the present of bacteriophage in those of wastewater samples (Table 4.2 and Figure 4.1). The bacteriophage of wastewater treatment kudprakhow plant, Sapprasitthiprasong hospital, and Khong Chiam hospital, was designated as JC01, JC02, and JC03, respectively.

Table 4.2 Bacteriophage isolated from wastewater by spot test

Wastewater sample	Inhibition zone
1.Kudprakhow plant Warin Chamrap, Ubon Ratchathani.	+
2. Sapprasitthiprasong Hospital Mueang Ubon Ratchathani, Ubon Ratchathani	+
3. Moon River at waterfront Watsupatnaram-worawihan Mueang Ubon Ratchathani, Ubon Ratchathani	-
4. Moon River at Kaeng Saphue Phibunmangsahan, Ubon Ratchathani	-
5. Moon River at waterfront Wat Don That Phibunmangsahan, Ubon Ratchathani	-
6. Wastewater treatment plant of Khong Chiam Hospital Khong Chiam, Ubon Ratchathani	+
7. Waterspout at Khong Chiam Hospital Khong Chiam, Ubon Ratchathani	-
8. Khong River (clear water) Khong Chiam, Ubon Ratchathani	-
9. Khong River (thick water) Khong Chiam, Ubon Ratchathani	-

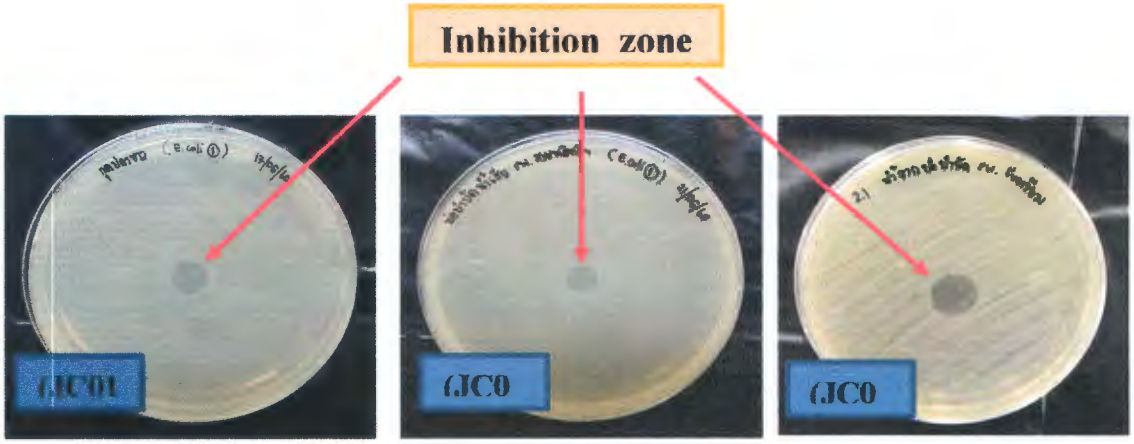


Figure 4.1 Bacteriophage isolated from wastewater by spot test

4.3 Determination of bacteriophage titer

To determine of bacteriophage titer in the unit of plaque-forming unit in one ml (PFU/mL), plaque assay was performed. As shown in Figure 4.2 and Table 4.3, the result demonstarted that bacteriophage titer of JC01, JC02, and JC03 was 2.66×10^8, 6.98×10^7, and 2.23×10^7 PFU/mL, respectively.

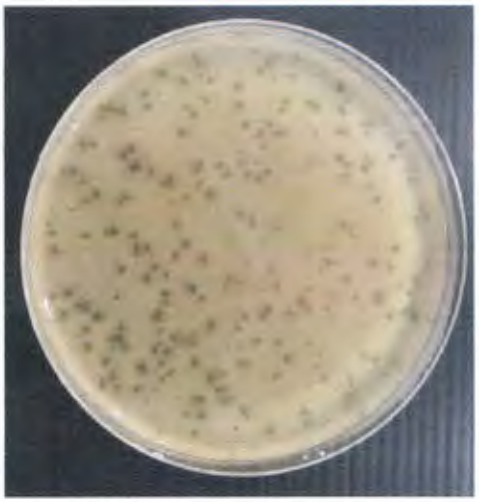


Figure 4.2 Bacteriophage detection by plaque assay

Table 4.3 Bacteriophage titer by plaque assay

Sample (bacteriophage name)	Bacteriophage titer
1.Wastewater treatment Kudprakhow plant Warin Chamrap, Ubon Ratchathani (JC01)	2.66 x10^8 PFU/mL
2. Wastewater treatment plant of Sapprasitthiprasong Hospital Mueang Ubon Ratchathani, Ubon Ratchathani (JC02)	2.98 x10^7 PFU/mL
3. Wastewater treatment plant of Khong Chiam Hospital, Khong Chiam, Ubon Ratchathani (JC03)	2.23 x10^7 PFU/mL

4.4 Determination of bacteriophage host range

To determine the bacterial host range of bacteriophage JC01, JC02, and JC03, all these three bacteriophages were used to test against seventeen pathogenic bacterial stains by using spot test method. As shown in Table 4.4, all 3 bacteriophages showed the inhibition of *E. coli* ATCC 25922 and *E. coli* No.40, but did not inhibit for other bacteria. In addition, bacteriophage JC01 and JC02 inhibited *E. coli* No.38 and *E. coli* No.39, bacteriophage JC01 and JC03 inhibited *E. coli* No. 37. These result suggested that bacteriophage JC01, JC02, and JC03 had highly specific and narrow host ranges for *E. coli*.

Table 4.4 Bacteriophage host range determination by spot test method

Pathogenic bacteria	JC01	JC02	JC03
1. *Klebsiella pneumoniae*	-	-	-
2. *Bacillus subtilis*	-	-	-
3. *Enterococcus faecalis*	-	-	-
4. *Escherichia coli* ATCC 25922	+	+	+
5. *Escherichia coli* No. 37	+	-	+
6. *Escherichia coli* No. 38	+	+	-
7. *Escherichia coli* No. 39	+	+	-

Table 4.4 Bacteriophage host range determination by spot test method (continued)

Pathogenic bacteria	JC01	JC02	JC03
8. *Escherichia coli* No. 40	+	+	+
9. *Salmonella* spp.	-	-	-
10. *Shigella dysenteriae*	-	-	-
11. *Staphylococcus aureus* ATCC 29213	-	-	-
12. *Staphylococcus aureus* No.MU50	-	-	-
13. *Vibrio* spp.	-	-	-
14. *Pseudomonas aeruginosa* Contral	-	-	-
15. *Pseudomonas aeruginosa* No.910	-	-	-
16. *Lactobacillus* casei TISTR1341	-	-	-

Remark: JC01=Bacteriophage JC01, JC02 = Bacteriophage JC02, and JC03 = Bacteriophage JC03 (-) = Don't have inhibition zone.
(+) = Have inhibition zone

4.5 Inhibition of clinical isolates *Escherichia coli*

In addition to test the specific hosts range of bacteriophage, the potential for inhibition of clinical isolates *E. coli* have been investigated. All 267 clinical *E. coli* isolates with multidrug resistant property were included in this study. As shown in Table 4.5 and based on spot test method, it was found that bacteriophage JC01, JC02, and JC03 showed the inhibition of clinical isolates *E. coli* at the percentage of 51.7 (138/267), 52.4 (140/267), and 28.5 (76/267). The results are in the appendix.

4.6 Bacterial inhibition by using bacteriophage cocktail approach

To investigate the use of bacteriophage cocktail, a suspension containing more than one bacteriophage type, for inhibition of *E. coli* strains. The combination of bacteriophage JC01 + JC03, and JC02 + JC03 were used to test inhibit different *E. coli* strains. As shown in Table 4.6, use of the single type of bacteriophage including JC01 or JC02 could not inhibit *E. coli* No.100, 129, 156, 162 and 165, but when using of bacteriophage cocktail JC01+JC03 and JC02 + JC03, it could inhibit all *E. coli* strains *coli* No. 100, 129, 156, 162 and 165 strain.

Table 4.5 Bacterial inhibition by bacteriophage cocktail

No.	No.	Bacteriophage				
		JC01	JC02	JC03	JC01+JC03	JC02+JC03
100.	1.	-	-	+	+	+
129.	2.	-	-	+	+	+
156.	3.	-	-	+	+	+
162.	4.	-	-	+	+	+
165.	5.	-	-	+	+	+
168.	6.	+	+	+	+	+
169.	7.	+	+	+	+	+
171.	8.	+	+	+	+	+
175.	9.	+	+	+	+	+

Remark: JC01 = Bacteriophage JC01, JC02=Bacteriophage JC02,
JC03 = Bacteriophage JC03 (-) = Don't have inhibition zone
(+) = Have inhibition zone.

4.7 Stability of bacteriophage

To determine the stability of bacteriophage in different solutions at the different time points (10, 20, 30 and 40 min), the solution of ethanol, hydrogen peroxide, normal saline and sterile distill water were used. Based on spot test method, all 3 types of bacteriophage were tolerated to normal saline and distilled water for up to 40 min after incubation, but they cannot resist to ethanol and hydrogen peroxide at every time point, even at 0 h of incubation (Table 4.7). To determine the quantity of survival of bacteriophages, plaque assay was performed. As shown in Table 4.8, survival of bacteriophages revealed that all 3 types of bacteriophage could survive in normal saline and distilled water at more than %90, but cannot survive to ethanol and hydrogen peroxide at every time point.

Table 4.6 Stability of bacteriophage by spot test method

Bacteriophage / Chemical	Distilled water (Control)				0.85% Normal saline				10% Ethanol				1% Hydrogen peroxide			
	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
JC01	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
JC02	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
JC03	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

Remark: 10 = 10 minute, 20 = 20minute, 30 = 30minute, 40 = 40minute.

JC01= Bacteriophage JC01, JC02=BacteriophageJC02,

JC03=Bacteriophage JC03.

(-) = Don't have inhibition zone. (+) = Have inhibition zone.

Table 4.7 Bacteriophage survival stability of bacteriophage to chemical

Chemical	% bacteriophage survival											
	JC01				JC02				JC03			
	10	20	30	40	10	20	30	40	10	20	30	40
1. Distilled water (Control)	99.8	98.6	96.5	96.0	98.2	97.5	95.7	92.3	97.4	96.1	95.5	93.3
2. 0.85 % Normal saline	98.4	97.1	95.2	94.5	98.2	98.0	97.3	96.8	98.9	98.2	96.8	94.9
3. 10% Ethanol	0				0				0			
4. 1% Hydrogen peroxide	0				0				0			

Remark: 10 = 10 minute, 20 = 20 minute, 30 = 30 minute, 40 = 40 minute.

JC01= Bacteriophage JC01, JC02 = Bacteriophage JC02,

JC03 = Bacteriophage JC03

4.8 Heat stability of bacteriophage

As shown in Table 4.9, it was found that bacteriophage JC01 showed the ability to resist the temperature of 60 °C after 60 min of incubation. Bacteriophage JC02 and JC03 showed the ability to resist the temperature of 60 °C after 45 min of incubation.

Table 4.8 Heat stability of Bacteriophage

Time	Degree Celsius (Phage/Temp.)											
	JC01				JC02				JC03			
	30	40	50	60	30	40	50	60	30	40	50	60
5 min	+	+	+	+	+	+	+	+	+	+	+	+
10 min	+	+	+	+	+	+	+	+	+	+	+	+
15 min	+	+	+	+	+	+	+	+	+	+	+	+
20 min	+	+	+	+	+	+	+	+	+	+	+	+
25 min	+	+	+	+	+	+	+	+	+	+	+	+
30 min	+	+	+	+	+	+	+	+	+	+	+	+
35 min	+	+	+	+	+	+	+	+	+	+	+	+
40 min	+	+	+	+	+	+	+	+	+	+	+	+
45 min	+	+	+	+	+	+	+	+	+	+	+	+
50 min	+	+	+	+	+	+	+	-	+	+	+	-
55 min	+	+	+	+	+	+	+	-	+	+	+	-
60 min	+	+	+	+	+	+	+	-	+	+	+	-

Remark: JC01=BacteriophageJC01, JC02=BacteriophageJC02,
JC03 = BacteriophageJC03 (-) = Don't have inhibition zone.
(+) = Have inhibition zone.

4.9 Bacterial growth inhibition by bacteriophage

To determine the growth inhibition of *E. coli* by bacteriophage, the bacteriophage suspension at different volume, 100 µl, 200 µl, 300 µl, and 400 µl were used to incubate with *E. coli* cells. After of incubation, the survival of *E. coli* cells was calculated as CFU/ml. As shown in Table 4.10, it was found that bacteriophage JC02 showed a higher percentage of inhibition against bacterial pathogens than other bacteriophages. At this stage, the bacteriophages were tested for growth inhibition of bacteria. It was found that, when tested twice, the average % survival of bacteria as shown in Table 4.11

Table 4.9 Survival of bacteria at different volume of acteriophage suspension

Time.	replicate	Survival of bacteria at different volume of bacteriophage suspension (CFU/mL)															
		100µl				200µl				300µl				400µl			
		10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
0 h	1	$1.1x10^5$	$3x10^5$	0	0	$1x10^4$	0	0	0	0	0	0	0	0	0	0	0
	2	$2.3x10^5$	$1x10^5$	0	0	$1x10^4$	0	0	0	0	0	0	0	0	0	0	0
	3	$3.3x10^5$	$1.8x10^6$	$3x10^6$	0	$2x10^4$	0	0	0	$1x10^4$	0	0	0	0	0	0	0
3 h	1	$2x10^4$	$1x10^5$	0	0	0	0	0	0	0	0	0	0	$1x10^4$	0	0	0
	2	$7x10^4$	0	0	0	$5x10^5$	0	0	0	0	0	0	0	0	0	0	0
	3	$4x10^4$	$2x10^5$	0	0	$1x10^5$	0	0	0	0	0	0	0	0	0	0	0
6 h	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	$3x10^5$	$3x10^5$	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	$7x10^4$	0	0	0	0	0	0	0	$1x10^4$	0	0	0	0	0	0	0

Remark* 100 µl, 200 µl, 300 µl, and 400 µl are volume of bacteriophage suspension 10^3, 10^4 , 10^5, 10^6 = Dilution point of bacteria

4.10 Bacteriophage genome analysis

To classify the bacteriophage group, the genome of bacteriophage was analyzed by genome extraction and enzyme digestion. The extracted DNA was digested with different enzymes 6-bases cutter endonuclease, RNase and DNase.

For bacteriophage JC01, the extracted DNA was digested with 6-bases cutter endonuclease *Hind*III, *Eco*RI, *Nco*I, and *Pae*I. In addition, RNase and DNase were also used. As shows in Figure 4.3, gel electrophoresis revealed that only *Hind*III digested the bacteriophage JC01 genome. *Hind*III-digested bacteriophage JC01 genome showed the ladder DNA bands, indicating that the extracted DNA could be digested with *Hind*III and the bacteriophage JC01 genome could be predicted as double stranded-DNA (dsDNA), due to *Hind*III has AAGCTT/TTCGAA as specific recognition site. In addition, the bacteriophage JC1 genome was digested with DNase but not for RNase, indicating that the bacteriophage genome was DNA type. Thus bacteriophage JC01 could be dsDNA virus.

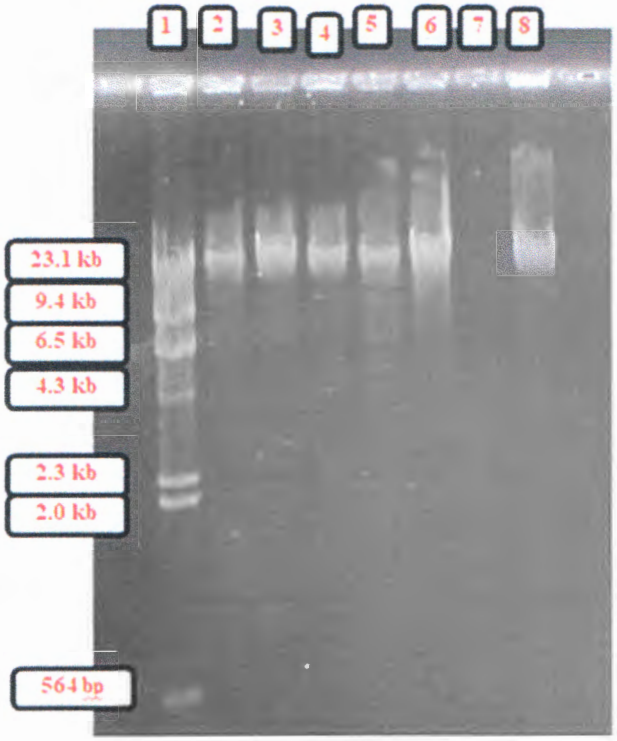


Figure4. 3 Ethidium bromide staining gel of restriction enzyme-digested of Bacteriophage JC01

Number 1 is HindIII marker (Thermo Fisher Scientific) was used as standard DNA ladder, Number 2 is the restriction enzyme EcoRI, Number 3 is the restriction enzyme NcoI, Number 4 is the restriction enzyme PaeI, Number 5 is the restriction enzyme HindIII, Number 6 is digested by RNase, Number 7 is digested by DNase And number 8 is Uncuted (that is, bacteriophage DNA alone).

For bacteriophage JC02, the extracted DNA was digested with enzyme with the same in case of bacteriophage JC02, it was found that bacteriophage JC02 genome was digested with *Hind*III and DNase, but not for RNase, indicating that the bacteriophage JC02 genome was DNA type, and thus bacteriophage JC02 could be dsDNA virus. As shows in Figure 4.4

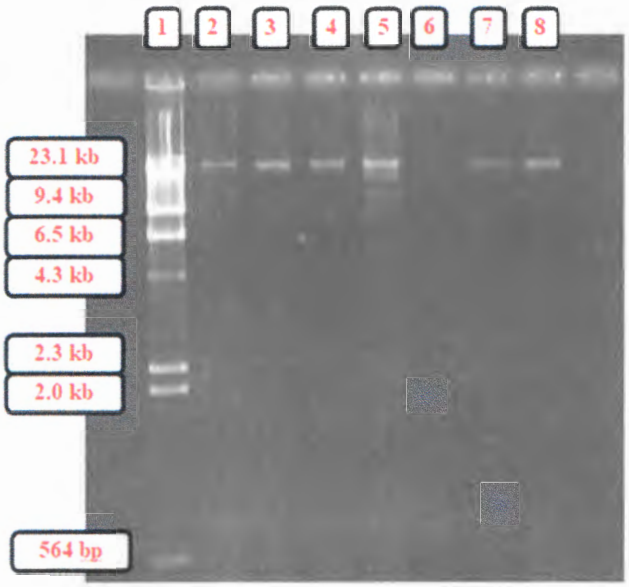


Figure 4.4 Ethidium bromide staining gel of restriction enzyme-digested of Bacteriophage JC02

Number 1 is *Hind*III marker (Thermo Fisher Scientific) was used as standard DNA ladder, Number 2 is the restriction enzyme *Eco*RI, Number 3 is the restriction enzyme N*col*, Number 4 is the restriction enzyme P*ael*, Number 5 is the restriction enzyme *Hind*III, Number 6 is digested by DNase, Number 7 is digested by RNase And number 8 is Uncuted (that is, bacteriophage DNA alone).

For bacteriophage JC03, the extracted DNA was digested with enzyme with the same with case of bacteriophage JC01 and JC02, it was found that bacteriophage JC02 genome was digested with *Nco*I, *Hind*III and DNase, but not for RNase. This result indicated that the bacteriophage JC02 genome was DNA type, and thus bacteriophage JC03 could be dsDNA virus. As shows in Figure 4.5

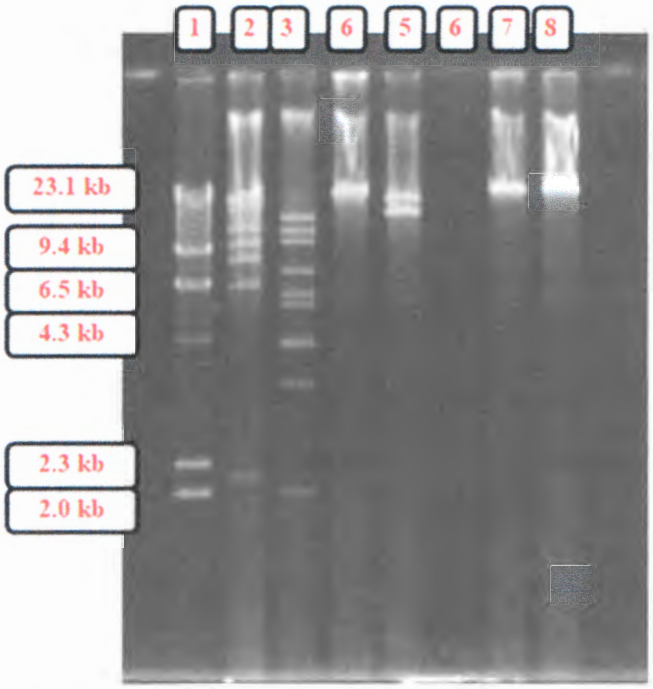


Figure 4.5 Ethidium bromide staining gel of restriction enzyme-digested of Bacteriophage JC03

Number 1 is HindIII marker (Thermo Fisher Scientific) was used as standard DNA ladder, Number 2 is the restriction enzyme EcoRI, Number 3 is the restriction enzyme NcoI, Number 4 is the restriction enzyme PaeI, Number 5 is the restriction enzyme HindIII, Number 6 is digested by DNase, Number 7 is digested by RNase And number 8 is Uncuted (that is, bacteriophage DNA alone).

4.11 Bacteriophage morphology

As shown in Figure 4.6-4.8, the particle of bacteriophages were successfully found under electron microscope. For bacteriophage JC01, the viral particle composed of head with hexagonal shape, long tail with contractile. The size from head to tail was about 155 nm. Based on International Committee on Taxonomy of Viruses (ICTV), bacteriophage JC01 could be classified in Family *Myoviridae*, Order *Caudovirales*.

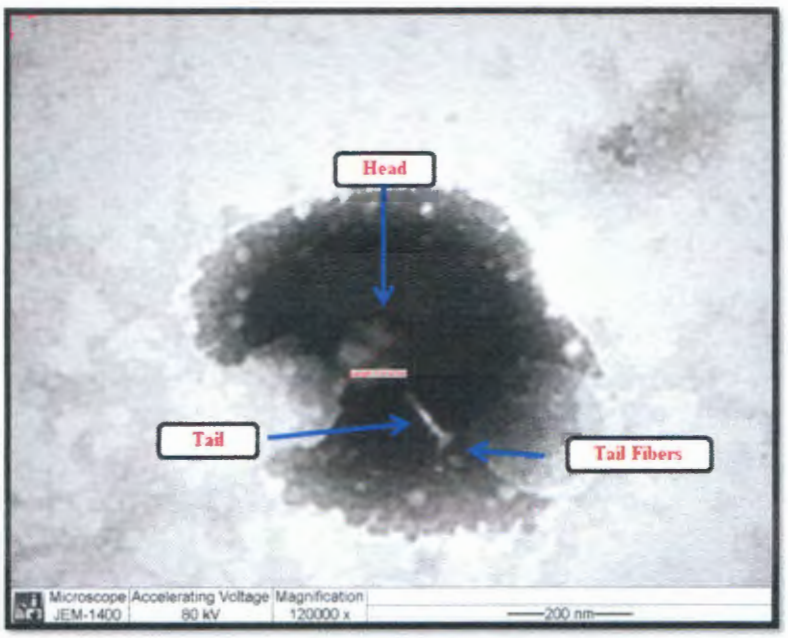


Figure4. 6 Bacteriophage morphology of JC01

For bacteriophage JC02, the viral particle composed of head with hexagonal shape, long tail with contractile. The size from head to tail was about 200 nm. Based on ICTV, bacteriophage JC02 could be classified in Family *Myoviridae*, Order *Caudovirales*.

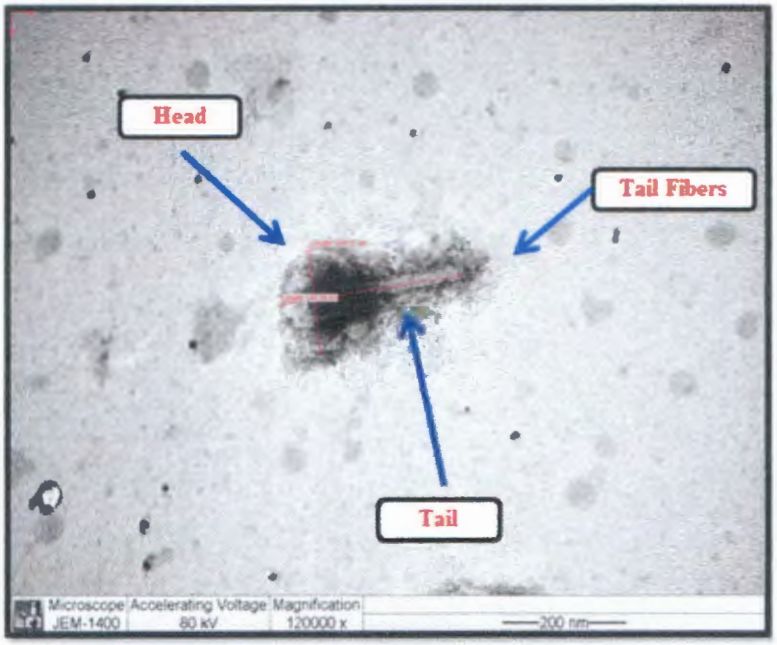


Figure 4.7 Bacteriophage morphology of JC02

For bacteriophage JC03, the viral particle composed of head with hexagonal shape, long tail with contractile. The size from head to tail was about 200 nm. Based on ICTV, bacteriophage JC03 could be classified in Family *Myoviridae*, Order *Caudovirales*.

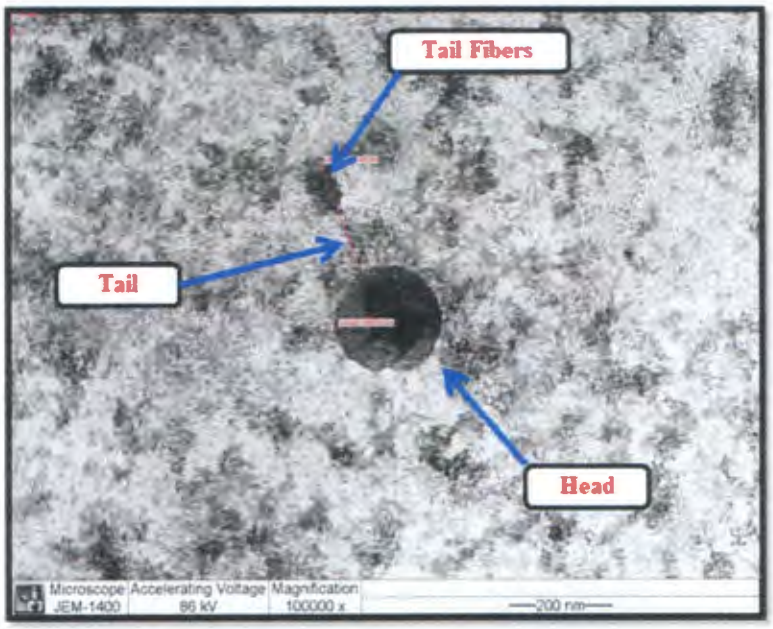


Figure 4.8 Bacteriophage morphology of JC03

CHAPTER 5
DISCUSSION AND CONCLUSION

Nowadays, effective treatment of infectious diseases caused by some microbes including bacteria can still be obtained by using antibiotics. However, it has been discovered the pathogenic bacteria that are able to resist any groups of antibiotic, called multidrug resistant bacteria or MDR bacteria. Most of reported MDR bacteria include ESKAPE, which refers to Gram-positive *Enterococcus faecium* and *Staphylococcus aureus*, and Gram-negative *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (Nataro & Kaper, 1998) (Rice, 2008). Recently *Escherichia coli* has been reported as a major public health concern due to this bacterium showed to resist antibiotics which is mediated by extended-spectrum β-lactamases, resulting in the serious diseases in patient (Alhashash et al., 2015; Karaiskos & Giamarellou, 2014) (Hadifar et al., 2017). For these reasons, the problem of untreatable of bacterial infectious disease by antibiotic could be became in the near future, and thus the alternative approach for treatment of bacterial infectious diseases are now being interested. One of the interesting approaches is bacteriophage therapy, the administration of phage (virus of bacteria) into patient with the aim of lysis of target bacterial cells. (Lin, Koskella, & Lin, 2017).

Escherichia coli is gram-negative bacterium that can be found in various environments, including in gastrointestinal tract of human (Guentzel, 1996). Actually, *E. coli* live in human as commensal and that do not cause any diseases in healthy people except in immunocompromised hosts or whose the normal gastrointestinal microbiota are imbalanced. Currently, there are six pathotypes of *E. coli*, a group of strains of a single species that cause a common disease in human using a common set of virulence factors. These include enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli*, (EIEC) and diffusely

adherent *E. coli* (DAEC) (Nataro & Kaper, 1998). The efficacy and safety of using bacteriophage therapy for treatment of *E. coli* infection has been reported in clinical human trials. It was demonstrated that use of bacteriophage in human was safe and showed to reduce the illness (Sarker et al., 2012).

Bacteriophage is a type of virus that has bacteria cell as host for propagation. Bacteriophage has been isolated from many environments including soil, water sewage and human feces (Naghavi, Golgoljam, & Akbari, 2013). In general bacteriophage is usually presented in the environment where bacterial host live. In this study, there were three types of bacteriophage specific to MDR E. coli no.40 could be isolated from waste water treatment plants, these included bacteriophage JC 01from Kudprakhow, bacteriophage JC 02from Sapprasitthiprasong hospital, and bacteriophage JC 03from Khong Chiam Hospital. The result supported the presence of bacteriophage in waste water especially in the hospital where the potential pathogenic bacteria are daily released into the environment.

Bacteriophage titer revealed that the concentration of bacteriophage JC01, JC02, and JC03 was 2.66 x 108, 298. x 107, and 2.23x 107CFU/mL, respectively, indicating the high viral titer which is sufficient for further analysis and thus for any applications. Bacterial host range determination demonstrated that all bacteriophages JC01, JC02, and JC03 had highly specific to E. coli strains. This property leads to the possibility for apply bacteriophage in human. In addition, the growth inhibition of MDR E. coli clinical isolates was determined. The result found that bacteriophage showed the inhibition of clinical isolates E. coli at the percentage of 51.7 (138/267), 52.4 (140/267), and 28.5 (76/267), respectively to JC01, JC02, and JC03.

Resistant to chemical substances of the three types of bacteriophage, all 3 types of bacteriophage were tolerated to normal saline and distilled water for up to 40 min but cannot resist to ethanol and hydrogen peroxide. Heat stability showed that bacteriophage had resisted at 60 °C after 60 min of incubation for JC01 and 60 °C after 45 min for JC02 and JC03.

Bacteriophage classification was determined by genome analysis and phage morphology analysis. The results demonstrared that the bacteriophage JC01, JC02, and JC03 genome was DNA virus and their genome was double-stranded DNA (dsDNA). Due to extracted DNA of all bacteriophage was digested with endonuclease,

and enzyme cut only double stranded DNA type, such as the genome of JC01 and JC02 could be digested with HindIII and DNase but not for RNase. For bacteriophage JC03, the extracted genome could be digested with NcoI, HindIII and DNase, but not for RNase. Based on International Committee on Taxonomy of Viruses (ICTV), bacteriophage JC01, JC02, and JC03 could be classified in Family Myoviridae, Order Caudovirales. Therefore, the bacteriophages derived from this study can be used to study their potential use in further advanced step.

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APPENDICE

APPENDICE A

Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
1.	AMP				CN		NOR,CIP		+	+	+
2.	AMP,AMX	CXM,CTX	FOX					SXT	+	+	-
3.	AMP, AMX	CTX,CAZ, CXM,CRO, Cefazolin[12]			CN,NET		NOR,CIP		-	-	-
4.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]		IPM,MEM, ETP			NOR,CIP		-	+	-
5.	AMP,AXM	CXM,CTX, CRO,CAZ			CN,NET		CIP	SXT	-	-	-
6.	AMP	CXM,CTX, CRO			CN				+	+	-
7.	AMP,AMX	Cefazolin[12], CXM,CRO, CAZ, Cefoperazone[17]		IPM,MEM, ETP			NOR,CIP	SXT	+	+	-
8.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ			CN		NOR,CIP		-	-	-

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates (Continued)

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
9.	AMP,AMX	CTX,CAZ	FOX		CN			SXT	+	+	-
10.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]		IPM	CN		CIP	SXT	+	+	-
11.	AMP,AMX	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+
12.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+
13.	AMP	CXM,CTX, CRO			CN		NOR,CIP		+	+	-
14.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+
15.	AMP,AMX	Cefazolin[12], CXN,CTX, CRO,CAZ			CN		NOR,CIP	SXT	-	-	-
16.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX					SXT	+	+	-

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	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
17.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	ETP	CN		CIP	SXT	-	-	-
18.	AMP	Cefazolin[12], CXM,CTX, CRO			CN		CIP		+	+	+
19.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	-
20.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP	SXT	+	+	-
21.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX	IPM,MEM, ETP	CN		NOR,CIP	SXT	+	+	-
22.	AMP	CXM.CTX, CRO,CAZ					NOR,CIP		+	+	-
23.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN		NOR,CIP		-	-	-
24.	AMP	CXM,CTX, CRO,CAZ			CN				+	+	+

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	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
25.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX				NOR,CIP		-	-	-
26.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	IPM,MEM, ETP	CN	TET	NOR,CIP		-	-	-
27.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	IPM,MEM, ETP	CN		CIP	SXT	-	-	-
28.	AMP	CXM,CTX, CAZ							+	+	+
29.	AMP	CXM,CTX, CRO					CIP	SXT	+	+	+
30.	AMP	CXM,CTX, CRO							+	+	-
31.	AMP	CXM,CTX, CRO,CAZ			CN		CIP	SXT	+	+	-
32.	AMP	Cefazolin[12], CTX,CRO	FOX					SXT	+	+	-

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	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
33.	AMP	CTX,CRO, CAZ	FOX						+	+	+
34.	AMP	CRO,CAZ	FOX						+	+	-
35.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ							+	+	+
36.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		CIP		-	-	-
37.	AMP	CXM,CTX, CRO,CAZ					CIP	SXT	+	+	+
38.	AMP	CXM,CTX, CRO,CAZ			CN				-	-	-
39.	AMP	CXM,CTX, CRO					CIP	SXT	+	+	-
40.	AMP	CXM,CTX, CRO,CAZ							+	+	+
41.	AMP	CTX,CXM CAZ,CRO			CN		CIP	SXT			

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	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
42.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		CIP	SXT	-	-	-
43.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ		IPM,MEM, ETP	CN		CIP	SXT	-	-	-
44.	AMP	CXM,CTX, CRO,CAZ					CIP	SXT	+	+	+
45.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP	SXT	-	-	-
46.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX	IPM,MEM, ETP	CN		NOR,CIP	SXT	-	-	-
47.	AMP	CXM,CTX, CRO,CAZ			CN		NOR	SXT	-	-	-

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	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
48.	AMP,AMX	Cefazolin12, CXM,CTX, CAZ,CRO	FOX	IPM,MEM, ETP	CN		NOR,CIP	SXT	-	-	-
49.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN,NET	TET	NOR,CIP		-	-	-
50.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN,NET	TET	NOR,CIP		-	-	-
51.	AMP,AMX	CXM,CTX, CRO			CN	TET	NOR,CIP		+	+	+
52.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN,NET		NOR,CIP		-	-	-
53.	AMP	Cefazolin[12], CXM,CTX, CRO			CN		NOR,CIP	SXT	+	+	+
54.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX					SXT	+	+	-

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55.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]		IPM,MEM, ETP	CN		NOR,CIP	SXT	-	-	-
56.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ			CN		NOR,CIP		+	+	+
57.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+
58.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP		-	-	-
59.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP	SXT	-	+	-
60.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+

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No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
61.	AMP	CXM,CTX, CRO			CN			SXT	+	+	+
62.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP	SXT	+	+	+
63.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+
64.	AMP	Cefazolin[12], CXM,CTX, CRO			CN		NOR,CIP	SXT	+	+	+
65.	AMP	CXM,CTX, CRO					NOR,CIP		+	+	+
66.	AMP	CXM,CTX, CRO						SXT	+	+	+
67.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN		CIP	SXT	+	+	+
68.	AMP	CXM,CTX, CRO						SXT	-	-	-

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
69.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN		CIP	SXT	-	-	-
70.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					CIP	SXT	+	+	+
71.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					CIP	SXT	+	+	-
72.	AMP,AMX	CXM,CTX, CRO,CAZ					CIP	SXT	+	+	+
73.	AMP	CXM,CTX, CRO,CAZ					CIP	SXT	-	-	-
74.	AMP	Cefazolin[12], CXM,CTX, CRO			CN		CIP		+	+	+
75.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ					CIP	SXT	-	-	-

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
76.	AMP	CXM,CTX, CRO					CIP	SXT	-	-	-
77.	AMP	CXM,CTX, CRO,CAZ			CN			SXT	-	-	-
78.	AMP,AMX	CXM,CTX, CRO,CAZ			CN		CIP	SXT	+	+	+
79.	AMP	CXM,CTX, CRO,CAZ			CN		CIP	SXT	+	+	+
80.	AMP,AMX				CN		CIP	SXT	-	-	-
81.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX				NOR,CIP	SXT	-	-	-
82.	AMP	CXM,CTX, CRO			CN		CIP	SXT	+	+	-
83.	AMP	CXM,CTX, CRO, Cefoperazone[17]	FOX		CN		NOR,CIP	SXT	-	+	+
84.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
85.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP		+	+	-
86.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP		-	-	-
87.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP	STX	-	-	-
88.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+
89.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	IPM,MEM, ETP	CN		NOR,CIP	SXT	-	-	-
90.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	MEM,ETP	CN		NOR,CIP	SXT	-	-	-
91.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP	SXT	-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
92.	AMP	CXM,CTX, CRO	FOX		CN		NOR,CIP	SXT	+	+	+
93.	AMP	CXM,CTX, CRO					NOR,CIP	SXT	+	+	+
94.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP		+	+	-
95.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP		+	+	-
96.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+
97.	AMP	CXM,CTX, CRO			CN		NOR,CIP	SXT	+	+	-
98.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP	SXT	+	+	+
99.	AMP	CXM,CTX, CRO			CN		NOR,CIP		+	+	-
100.	AMP	CXM,CTX, CRO,CAZ							-	-	+

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
101.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	MEM,ETP	CN		NOR,CIP	SXT	+	+	-
102.	AMP	Cefazolin[12], CXM,CTX, CRO						SXT	+	+	-
103.	AMP	Cefazolin[12], CXM,CTX, CRO					NOR,CIP	SXT	-	-	-
104.	AMP	Cefazolin[12], CXM,CTX, CRO					NOR,CIP	SXT	+	+	-
105.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP	SXT	+	+	+
106.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ			CN		NOR,CIP		-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
107.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP	SXT	+	+	-
108.	AMP	Cefazolin[12], CXM,CTX, CRO					NOR,CIP		-	-	-
109.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	-
110.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	-
111.	AMP	Cefazolin[12], CXM,CTX, CRO			CN		NOR,CIP	SXT	+	+	-
112.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	MEM,ETP	CN		NOR,CIP	SXT	-	-	-
113.	AMP,AMX	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+
114.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
115.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+
116.	AMP,AMX	CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+
117.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	MEM,ETP	CN		NOR,CIP	SXT	-	+	-
118.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	MEM,ETP	CN		NOR,CIP	SXT	-	-	-
119.	AMP	CXM,CTX, CRO			CN		NOR,CIP	SXT	+	+	+
120.	AMP	CXM,CTX, CRO					NOR,CIP		-	-	-
121.	AMP,AMX	Cefazolin12, CXM,CTX, CRO,CAZ,			CN		NOR,CIP	SXT	-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
122.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	+	+	+
123.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP		+	+	+
124.	AMP	Cefazolin12, CXM,CTX, CRO					NOR	SXT	-	-	-
125.	AMP	CTX,CRO							-	-	-
126.	AMP	Cefazolin[12], CXM,CTX, CRO					NOR,CIP	SXT	+	-	+
127.	AMP,AMX	CXM	FOX				NOR,CIP		-	-	-
128.	AMP						NOR,CIP	SXT	+	+	-
129.	AMP,AMX	Cefazolin[12], CXM,CRO, CAZ			CN		NOR,CIP	SXT	-	-	+
130.	AMP	CXM,CRO, CAZ			CN				-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
131.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	MEM,ETP	CN		NOR,CIP	SXT	-	-	-
132.	AMP	CXM,CTX, CRO,CAZ			CN		CIP	SXT	+	+	+
133.	AMP	CXM,CTX, CRO,CAZ			CN		CIP	SXT	-	-	-
134.	AMP	Cefazolin[12], CXM,CRO					NOR,CIP	SXT	+	+	+
135.	AMP	CXM,CTX, CRO,CAZ					CIP	SXT	-	+	-
136.	AMP	CXM,CTX, CRO,CAZ			CN		CIP	SXT	-	-	-
137.	AMP	CXM,CTX, CRO,CAZ			CN,NET		NOR,CIP	SXT	-	-	-
138.	AMP	CXM,CTX, CRO,CAZ					CIP	SXT	-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
139.	AMP	CXM,CTX, CRO,CAZ			CN		NOR,CIP	SXT	-	-	-
140.	AMP	Cefazolin[12], CXM,CTX, CRO			CN			SXT	+	+	+
141.	AMP	CTX,CRO			CN		NOR,CIP		+	-	+
142.	AMP	CXM,CTX, CRO					NOR,CIP	SXT	+	+	-
143.	AMP	CXM,CTX, CRO,CAZ					NOR,CIP	SXT	-	-	-
144.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP	SXT	-	-	-
145.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP	SXT	-	-	-
146.	AMP	Cefazolin12, CXM,CTX, CRO,CAZ							-	-	-

Remark: AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
147.	AMP	Cefazolin12, CXM,CTX, CRO					CIP		+	+	+
148.	AMP	CXM,CRO, CAZ	FOX		CN		NOR,CIP	SXT	+	-	-
149.	AMP	Cefazolin[12], CXM,CRO	FOX		CN		NOR	SXT	+	-	+
150.	AMP	CXM,CTX, CRO,CAZ			CN		CIP	SXT	+	+	+
151.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ					NOR,CIP		-	-	-
152.	AMP	Cefazolin[12], CXM,CTX			CN		NOR,CIP	SXT	-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
153.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX		CN		NOR,CIP	SXT	-	-	-
154.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN		NOR,CIP		-	-	-
155.	AMP	CXM,CTX, CRO	FOX				CIP	SXT	-	-	-
156.	AMP	CXM,CRO					CIP	STX	-	-	+
157.	AMP,AMX	CXM,CRO, CAZ, Cefoperazone[17]	FOX	IPM,MEM, ETP	CN		CIP	SXT	-	-	-
158.	AMP,AMX	CXM,CRO, CAZ					CIP		+	+	-
159.	AMP,AMX	Cefazolin[12], CXM,CTX, CRO,CAZ, Cefoperazone[17]							-	-	-
160.	AMP	CXM,CTX, CRO					CIP	SXT	+	+	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
161.	AMP	Cefazolin[12], CXM,CTX, CRO					CIP	SXT	+	+	-
162.	AMP	CXM,CTX, CRO						SXT	-	-	+
163.	AMP	CXM,CTX, CRO,CAZ					CIP		+	-	-
164.	AMP	Cefazolin[12], CXM,CTX, CRO					CIP	SXT	+	+	-
165.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		CIP		-	-	+
166.	AMP,AMX	Cefazolin12, CTX,CRO, CAZ	FOX		CN				-	-	-
167.	AMP,AMX	CXM,CTX, CRO,CAZ	FOX		CN		CIP		-	-	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
168.	AMP	Cefazolin[12], CXM,CTX, CRO					CIP		-	-	+
169.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ			CN,NET		CIP		-	-	+
170.	AMP	Cefazolin[12], CXM,CTX, CRO,CAZ	FOX		CN		CIP		-	-	-
171.	AMP	CXM,CTX, CRO			CN		CIP	SXT	-	-	+
172.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN		CIP	SXT	-	-	-
173.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX		CN		CIP	SXT	-	-	-
174.	AMP	CXM,CRO, CAZ			CN			SXT	-	-	-
175.	AMP	CXM,CTX, CRO							-	-	+

Remark: AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
176.	AMP	CXM,CTX					CIP	SXT	+	+	+
177.	AMP	CXM,CTX			CN			SXT	-	-	-
178.	AMP	CXM,CTX, CRO					CIP	SXT	-	-	-
179.	AMP	CXM,CTX, CRO					CIP	SXT	-	-	-
180.	AMP	CXM,CTX, CRO						SXT	-	-	-
181.	AMP,AMX	CXM,CTX, CRO, Cefoperazone[17]	FOX		CN		CIP	SXT	-	-	-
182.	AMP,AMX	CXM,CTX, CRO,CAZ, Cefoperazone[17]	FOX	IPM,MEM, ETP	CN		CIP	SXT	-	-	-
183.	AMP,AMX	CXM,CTX, CRO,CAZ,	FOX		CN		CIP		-	-	-
184.	AMP	CXM,CTX, CRO					CIP	SXT	-	-	-

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

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	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
185.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
186.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
187.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
188.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
189.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-
190.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-
191.	AMP	CXM	FOX,SCR		CN			SXT	-	+	-
192.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-
193.	AMP	CXM	FOX,SCR		CN			SXT	+	+	-
194.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
195.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
196.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-

Remark: AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

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No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
197.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
198.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
199.	AMP	CXM	FOX,SCR		CN			SXT	+	+	+
200.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
201.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
202.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
203.	AMC	CRO,CTX			AK,NET		CIP		-	+	+
204.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
205.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
206.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
207.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
208.	AMC	CRO,CTX			AK,NET		CIP		-	-	-

Remark: AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
209.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
210.	AMC	CRO,CTX			AK,NET		CIP		+	-	-
211.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
212.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
213.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
214.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
215.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
216.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
217.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
218.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
219.	AMC	CRO,CTX			AK,NET		CIP		-	-	-

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates .

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
220.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
221.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
222.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
223.	AMC	CRO,CTX			AK,NET		CIP			-	-
224.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
225.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
226.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
227.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
228.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
229.	AMC	CRO,CTX			AK,NET		CIP		+	+	+

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
230.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
231.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
232.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
233.	AMP	CXM	FOX,SCR		CN			SXT	+	+	+
234.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
235.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
236.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
237.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
238.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
239.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
240.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
241	AMC	CRO,CTX			AK,NET		CIP		+	+	-

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
242	AMC	CRO,CTX			AK,NET		CIP		+	+	+
243	AMC	CRO,CTX			AK,NET		CIP		+	+	+
244.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
245.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
246.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
247.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
248.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
249.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
250.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
251.	AMC	CRO,CTX			AK,NET		CIP		+	+	-
252.	AMC	CRO,CTX			AK,NET		CIP		-	+	-
253.	AMP	CRO,CTX			AK,NET		CIP		+	+	+

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
254.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
255.	AMP	CXM	FOX,SCR		CN			SXT	+	+	-
256.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-
257.	AMP	CXM	FOX,SCR		CN			SXT	+	+	-
258.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-
259.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
260.	AMC	CRO,CTX			AK,NET		CIP		+	+	+
261.	AMC	CRO,CTX			AK,NET		CIP		+	+	-

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1. Table of Inhibition of multidrug resistant *Escherichia coli*: clinical isolates

No.	Antibiotics								Spot Test		
	Cell wall synthesis inhibitors				Protein synthesis inhibitors		Nucleic acid synthesis inhibitors	Antimetabolites	Bacteriophage		
	β-lactam	Cephalosporin	Cephamycins	Carbaphenems	Aminoglycosides	Tetracyclines	Quinolones	Sulfonamides	JC01	JC02	JC03
262.	AMP	CXM	FOX,SCR		CN			SXT	-	-	-
263.	AMC	CRO,CTX			AK,NET		CIP		-	-	-
264.	AMP	CXM	FOX,SCR		CN			SXT	+	+	+
265.	AMP	CXM	FOX,SCR		CN			SXT	+	+	+
266.	AMP	CXM	FOX,SCR		CN			SXT	+	+	-
267.	AMP	CXM	FOX,SCR		CN			SXT	+	+	+
Total.									**138**	**140**	**76**
Percentage.									**51.7%**	**52.4%**	**28.5%**

Remark:AMP=Ampicillin,AMX=Amoxicillin,AMC=Augmentin,CXM=Cefuroxime,CTX=Cefotaxime,CAZ=Ceftazidine,CRO=Ceftriaxone,Cefazolin[12]=Cefazolin,Cefoperazone[17]=Cefoperazone,FOX=Cefoxitin,SCR=Sulperazone,IPM=Imipenem,MEM=Meropenem,ETP=Ertapenem, CN=Gentamicin,AK=Amikacin,NET=Netilmicin,TET=Tetracycline,NOR=Norfloxacin,CIP=Ciprofloxacin,SXT=Co-trimoxazole

APPENDICE B

List of chemicals

2. List of chemicals

No.	Chemicals and Reagents	Company
1	Absolute ethanol	AMRESCO®
2	Agarose	AMRESCO®
3	Amikacin (AK)	HIMEDIA®
4	Ampicillin (AMP)	HIMEDIA®
5	Augmentin (AMC)	HIMEDIA®
6	Cefoxitin (FOX)	HIMEDIA®
7	Cefotaxime (CTX)	HIMEDIA®
8	Ceftriaxone (CRO)	HIMEDIA®
9	Cefuroxime (CXM)	HIMEDIA®
10	Co-trimoxazole (SXT)	HIMEDIA®
11	Ertapenem (ETP)	HIMEDIA®
12	Gentamicin (CN)	HIMEDIA®
13	Imipenem (IPM)	HIMEDIA®
14	Meropenem (MEM)	HIMEDIA®
15	Netilmicin (NET)	HIMEDIA®
16	Sulperazone (SCR)	HIMEDIA®
17	DNAse	PanReacAppliChem
18	Ethidime Bromide	AMRESCO®
19	Ceftazidime (CAZ)	HIMEDIA®
20	Ciprofloxacin (CIP)	HIMEDIA®
21	phenol: chloroform: isoamyl alcohol (1:1:24)	Invitrogen™
22	Isoamyl alcohol	BDH PROLABO®
23	*Eco*RI, *Hind*III, P*ae*I and *Nco*I restriction enzyme	Thermo Scientific®
24	RNAse	PanReac AppliChem

APPENDICE C

Media preparation

C. Media preparation

2.1 Nutrient Agar (NA)

Nutrient Agar (NA)	13 g
Agar	15 g
Distilled water	1,000 mL

Autoclave and store at 4 °C

2.2 Nutrient broth (NB)

Nutrient broth (NB)	13 g
Distilled water	1,000 mL

Autoclave and store at 4 °C

2.3 Double strange NB (2xNB)

Nutrient broth (NB)	26 g
Distilled water	1,000 mL

2.4 Brain Heart Infusion (BHI) agar

Brain Heart Infusion (BHI)	52 g
Agar powder	15 g
Distilled water	1,000 mL

Autoclave and store at 4 °C

2.5 Brain Heart Infusion (BHI) soft agar

Brain Heart Infusion (BHI)	52 g
Agar powder	3 g
Distilled water	1,000 mL

Autoclave and store at 4 °C

2.6 Mueller Hinton agar (MHA)

Mueller Hinton agar	38 g
Distilled water	1,000 mL

Autoclave and store at 4 °C

APPENDICE D

Reagents preparations

D. Reagents preparations

3.1 0.7% Agarose gel

Agarose gel	0.7 g
Distilled water	100mL

Melt it in microwave and let it warm until use.

3.2 2% Phosphotungstic acid

Phosphotungstic acid	0.2 g
Deionized water	10 mL

Adjust to pH 7.0 with NaOH and adjust volume to 10 mL with distilled water, Store at 4°C

3.3 10% SDS

SDS	10 g
Distilled water	100 mL

Adjust volume to 1,000 mL with distilled water

3.4 70% Alcohol

Absolute ethanol	70 mL
Distilled water	30 mL

Store at 4°C

3.5 Tris-EDTA (TE) buffer

To prepare 1 M Tris-HCl

Tris base	121.1 g
Distilled water	800 mL

Adjust to pH 7.2 with HCl and adjust volume to 1,000 mL with distilled water, autoclave and store at room temperature.

To prepare 0.5 M EDTA pH 8.0

$Na_2 \cdot EDTA \cdot 2H_2O$	186.1 g
Distilled water	800 mL

Adjust to pH 8.0 with NaOH and adjust volume to 1,000 mL with distilled water, autoclave and store at room temperature.

To prepare Tris-EDTA (TE) buffer

1 M Tris-HCl	10 mL
0.5 EDTA pH 8.0	2 mL
Distilled water	980.8 mL

Autoclave and store at room temperature.

APPENDICE E

List of instruments

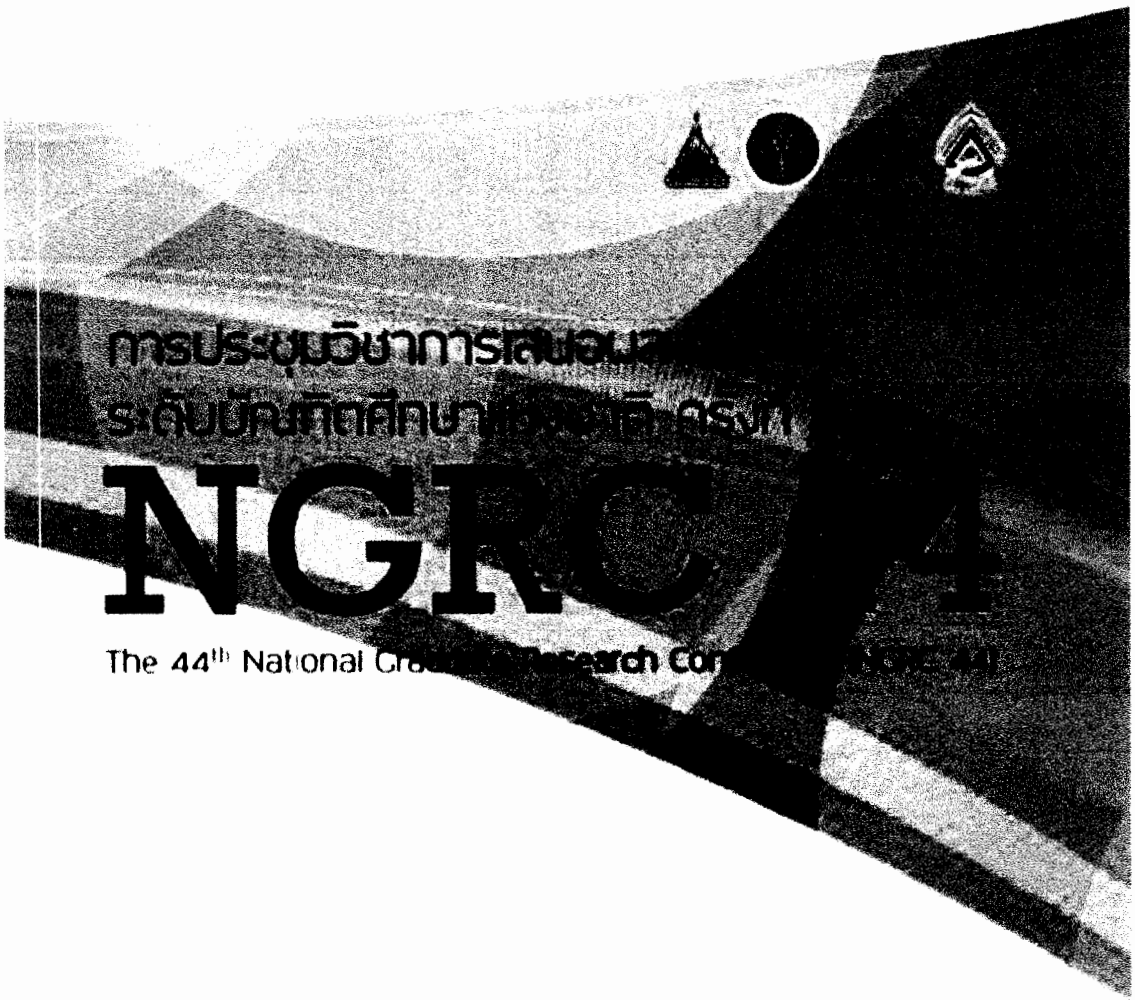
E. List of instruments

No.	Instruments	Company
1	Class II, Biological Safety Cabinets	Bio-Clean Air Devices & Services
2	MPW-380R refrigerated laboratory centrifuge	MPW MED. INSTRUMENTS
3	Digital dry bath	Labnet International Inc.
4	UVP's ChemiDoc-ItTS2 Imagers	Analytik Jena
5	Heating / drying ovens	MEMMERT
6	High-pressure steam sterilizer	WiseClave - autoclave
7	Incubator	Contherm digital series
8	pH Meter	SI Analytics
9	PowerPac™ Basic Power Supply	Mupid®-One
10	Transmission Electron Microscope	JEOL Ltd.

APPENDICE F

List of instruments

F. Publication



สารบัญ

The 44th National Graduate Research Conference (NGRC 44)

การแยกและการศึกษาคุณสมบัติของแบคเทอริโอฟาจจากแหล่งน้ำเสียที่จำเพาะต่อเชื้อ ESCHERICHIA COLI.

[illegible]

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อคัดแยกแบคทีริโอฟาจ (bacteriophage) จากแหล่งน้ำเสีย และเพื่อศึกษาคุณสมบัติของแบคทีริโอฟาจที่จำเพาะต่อเชื้อเอสเชอริเชีย โคไล (Escherichia coli) โดยตัวอย่างน้ำที่นำมาศึกษา ได้แก่ น้ำเสียบริเวณชุมชนประตูน้ำ และน้ำจากบ่อบำบัดน้ำเสียโรงพยาบาลสวนพลิทยประสงค์ ปริมาตร 10 มิลลิลิตร และทำการศึกษาพบว่าตัวอย่างน้ำทั้ง 2 แหล่ง มีแบคทีริโอฟาจปนอยู่ การศึกษาคุณสมบัติของแบคทีริโอฟาจต่อการยับยั้งเชื้อเอสเชอริเชีย โคไล พบว่า แบคทีริโอฟาจสามารถยับยั้งเอสเชอริเชีย โคไล ได้ด้วยวิธีแพร่ โดยให้ผลการทำ spot test และ Plaque assay เป็นบวก โดยในขั้นตอนการทดสอบด้วยวิธี Plaque assay พบว่า plaque มีหลายลักษณะแต่ที่คือ กลมที่มีขนาดเล็กมาก ไปจนถึงกลมขนาดใหญ่ ซึ่งขนาดของ plaque ที่พบคือ 0.2-4 มิลลิเมตร ดังนั้นแบคทีริโอฟาจที่แยกได้ในงานวิจัยครั้งนี้จึงเป็นจุดที่น่าสนใจในการนำไปศึกษาต่อในขั้นตอนต่อไป โดยเฉพาะอย่างยิ่งการทดสอบความสามารถของแบคทีริโอฟาจในการยับยั้งแบคทีเรียในเซลล์เพาะเลี้ยงและในสัตว์ทดลองต่อไป

คำสำคัญ แบคทีริโอฟาจ, เอสเชอริเชีย โคไล, ฟาจเทอราปี

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รางวัลชมเชยอันดับหนึ่ง ประเภท การนำเสนอผลงานแบบบรรยายดีเด่น NGRC Oral presentation เรื่อง การแยกและการศึกษาคุณสมบัติของแบคทีเรียที่ผลิตเอนไซม์ยับยั้งเชื้อดื้อยาต่อเชื้อ Escherichia coli

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