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Application of bacteriophages isolated from sewage to control urinary O157:H7 *Escherichia coli* and several bacterial uropathogens

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Abstract

Urinary tract infections (UTI) are global bacterial infections. Since the spread of antibiotic resistance, it is necessary to find alternative antimicrobial agents. The aim of this work was to investigate the effect of waste water bacteriophages on the growth of some multi-drug resistance *Escherichia coli* and other bacterial uropathogens. Urine samples were collected from 30 UTI patients at Minia University Hospital, Minia, Egypt. Twenty *Escherichia coli* strains were isolated from UTI patients. The Kirby-Bauer disc diffusion method was used to determine the isolates antibiotic susceptibility. The isolates showed resistance to ciprofloxacin and levofloxacin by 70%. Five *E. coli* bacteriophages were isolated from sewage water samples, tested for their host range and then examined by Transmission electron microscopy (TEM). The TEM examination revealed T4 like bacteriophages. The bacteriophages demonstrated lytic activities against the tested multidrug resistant clinical uropathogenic O157:H7 and non-O157 *E. coli* isolates, *E. coli* O157:H7 ATCC 43894, *E. coli* NRRL B-3008 and, *Pseudomonas aeruginosa* ATCC 27853 strain, but showed no activity against *Klebsiella pneumoniae* ATCC10031 and *Staphylococcus aureus* ATCC 6538. This study revealed that bacteriophages could act as effective alternatives of antibiotics especially against multidrug resistant bacteria; however, further *in-vivo* and shelf stability studies are needed.

Keywords: Urinary tract infection, Bacteriophages, Multidrug resistant bacteria, *Escherichia coli* O157:H7

1. Introduction

Urinary tract infections (UTIs) are one of the most prevalent bacterial infections either in the community or hospital acquired (Hryniewicz *et al*., 2001). A previous study conducted by Noor *et al*., (2013)

revealed that UTI is the infection of the kidneys, ureters, bladder and/ or urethra by bacterial invasion of the urinary tract that leads to an inflammatory response of the urothelium. Several studies of Ramesh *et al*., (2008); Beyene and Tsegaye, (2011) reported that *E. coli* has been and is still the most frequent etiological agent of UTIs, which accounts for more than 80% of community acquired, 50% of nosocomial and more than 80% of cases of uncomplicated pyelonephritis.

Bacteriophages host range can be classified into; narrower host range bacteriophages, which are able to infect few strains of the same species, whereas the others that can infect many strains of the same species are known to have a broad host range. Phages that can infect more than one species are known as polyvalent host range bacteriophages, as demonstrated by Carvalho *et al*., (2010); Gill and Hyman, (2010).

Determination of the host range of bacteriophages for their use in phage therapy is very important according to their use. As for infections caused by a single microorganism, it is desirable to use phages active against a single species to avoid killing of the other host's microbiome. On the other hand, Leibovici *et al*., (1998) documented that polyvalent and broad host range phages have the advantage of acting as broad spectrum antibiotics, which can infect many bacteria without the need for testing the sensitivity of bacteria to these bacteriophages.

Bacteriophages have many advantages over traditional antibiotics. The most critical one is that phages are not affected by antibiotic resistance mechanisms. In addition, they do not destroy the planktonic bacteria only, but have evolved the ability to penetrate the bacterial biofilms and lyse the cells in it, as reported in a previous study of Bjarnsholt, (2013). Unlike antibiotics, phages are significantly easier to modify to overcome the host resistance. According to the previous work conducted by Ho, (2001), culturing the phages with the newly resistant bacteria for several generations will be enough to develop a mutation in the phages that enables them to specifically target this resistant pathogen. The objectives of this study were to isolate and characterize bacteriophages from sewage water, and study the effect of these phages on the growth of *E. coli* and several bacterial uropathogens.

2. Material and methods

This study was approved by the Institutional Review Board (IRB) of Minia University.

2.1. Bacterial strains

A total of 30 urine samples were collected from patients suffering from urinary tract infection (UIT) at Minia University Hospital, Mini, Egypt. The urine samples were centrifuged, and then the supernatant was separated. Approximately, 100 µl of the supernatants were plated individually on Sorbitol MacConkey agar supplemented with 0.05 mg/ ml cefexime and 2.5 mg/ ml tellurite (CT-SMAC; Oxoid, UK), and then incubated for 24 h at 37◦C. Colorless colonies indicate the presence of Shiga toxin-producing *E. coli* (STEC) O157: H7, whereas pink colonies indicate non-O157 STEC, in reference to March and Ratnam, (1986).

Standard strains used in this study were *E. coli* O157:H7, *E. coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031 and *Staphylococcus aureus* ATCC 6538. These strains were provided by the Cairo Microbiological Resources Centre (Cairo MIRCEN), Ain Shams University, Cairo, Egypt.

2.2. Agglutination test

According to March and Ratnam, (1989), individual *E. coli* colonies that appeared on CT-SMAC were tested for the presence of O157, H7 *E. coli* antigens through agglutination with *E. coli* antisera latex reagent, for *in vitro* diagnostic use (Statens Serum Institute Diagnostica, Denmark). Each bacterial isolate was tested in duplicates.

2.3. Antimicrobial susceptibility assay

The antibiotic susceptibility pattern of the isolated *E. coli* against different antibiotics was determined by the Kirby-Bauer disc diffusion, using Muller Hinton agar (MHA) (LAB M, UK) as culture media, according to Hudzicki, (2009). The used antibiotics were purchased from Bioanalyse R, Turkey. Three independent replicates were used, and the assay was repeated twice. Isolates were recorded as sensitive, intermediate and resistant according to the zones of inhibition following the criteria of Clinical Laboratory Standards Institute (CLSI), in reference to (Wayne, (2018).

2.4. Bacteriophages isolation and lytic potential

Sewage water samples (100 ml each) obtained from Abokorkas waste water treatment plant, Minia, Egypt, were used for phages isolation. Briefly, a tube of 4.5 ml sewage water was used to inoculate a tube of 0.5 ml overnight cultured *E. coli* O157:H7 ATCC 43894 and another tube of 0.5 ml overnight cultured *E. coli* NRRL B-3008, followed by the addition of 0.5 ml 10x Trypticase soy broth (TSB, Difco) to each tube. The tubes were incubated for 48 h at 37°C. After incubation, the tubes were centrifuged at 2500 rpm for 10 min. The supernatants were finally filtered through 0.22-µm bacterial filters, as described by Harley, (2002).

The inhibitory effects of the isolated phages were examined using the spot assay method, at least 3 times. Twenty tested *E. coli* isolates, *E. coli* O157:H7 ATCC 43894 and *E. coli* NRRL B-3008 reference strains were spread on the surface of MHA plates. Plates were left to dry through incubation at 30°C for 40 min. Filtrates containing phages were spotted on the surface of the plates, and then incubated at 37°C for 24-48 h, as highlighted by Sheng *et al*., (2006).

Clear zones usually differ by their intensity and structure. Positive results with complete clear zones are known as confluent lysis, not fully cleared bacterial cultures were known as semi-confluent lysis, single bacterial colonies that overgrew a complete clear zone was known as overgrown lysis; however, individual clear or opaque plaques may appear as multiple small clear zones on the bacterial culture, according to Sybesma *et al*., (2016).

Plaques were isolated in normal saline. The process was repeated several times to obtain high titer of stock phages. These stocks were stored with 1% chloroform at 4°C, as described by Sheng *et al*., (2006).

2.5. Determination of the host range

According to Lee *et al*., (2016), the host range of the isolated phages was tested using a spot assay method, by spotting of a high titer phage stock (10^{10} PFU/ ml) against the previously mentioned reference bacterial strains spread on the surface of MHA plates; including *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 10031 and *S. aureus* ATCC 6538. The assays were repeated three times.

2.6. Transmission electron microscopy (TEM)

The purified phages were adsorbed on carbon coated copper grid, and then these grids were left to dry. They were stained using 4% phosphotungestic acid or 2% uranyl acetate for 2 min., and then dried for 15 min., (Ackermann and Heldal, 2010). The grids loaded by phages were examined by the transmission electron microscopy (TEM) (EF-TEM; JEM1010, JEOL, Tokyo) (Electron microscope unit, Assuit University). The phages were classified morphologically according to the guidelines of the International Committee on Taxonomy of Viruses (ICTV) (King *et al*., 2012).

3. Results

Twenty *E. coli* isolates were recovered from the 30 urine samples. Two isolates (10%) of the clinically isolated *E. coli* were found to be sorbitol-non fermenters showing colorless colonies. The agglutination antisera confirmed that these isolates were *E. coli* O157:H7, as it binds to the antibodies thus caused the latex particles to agglutinate.

3.1. Antimicrobial susceptibility assay

It was found that all the tested *E. coli* showed resistance to more than one antibiotic in 3 antimicrobial categories, and identified as multi-drug resistant *E. coli*. About 100 % of the isolates were resistant to Amoxicillin/ clavulanic, while 85 and 70 % were resistant to trimethoprim/ sulfamethoxazole and ciprofloxacin, respectively. The most effective drugs were imipenem followed by amikacin, as demonstrated in Fig. (1). Results are averages of three replicate plates.

3.2. Bacteriophages isolation

The isolated phages from sewage water expressed high lytic activity with wide plaques on the *E. coli* seeded MHA plates (Fig. 2). They showed lytic activities on the *E. coli* O157:H7 ATCC 43894, *E. coli* NRRL B-3008, in addition to 15 isolates (75%) of the *E. coli* isolated from urine (2 isolates of O157:H7 *E. coli* and 13 non-O157 *E. coli* isolates). However they did not exhibit any activity against 5 non-O157 *E. coli* isolates.

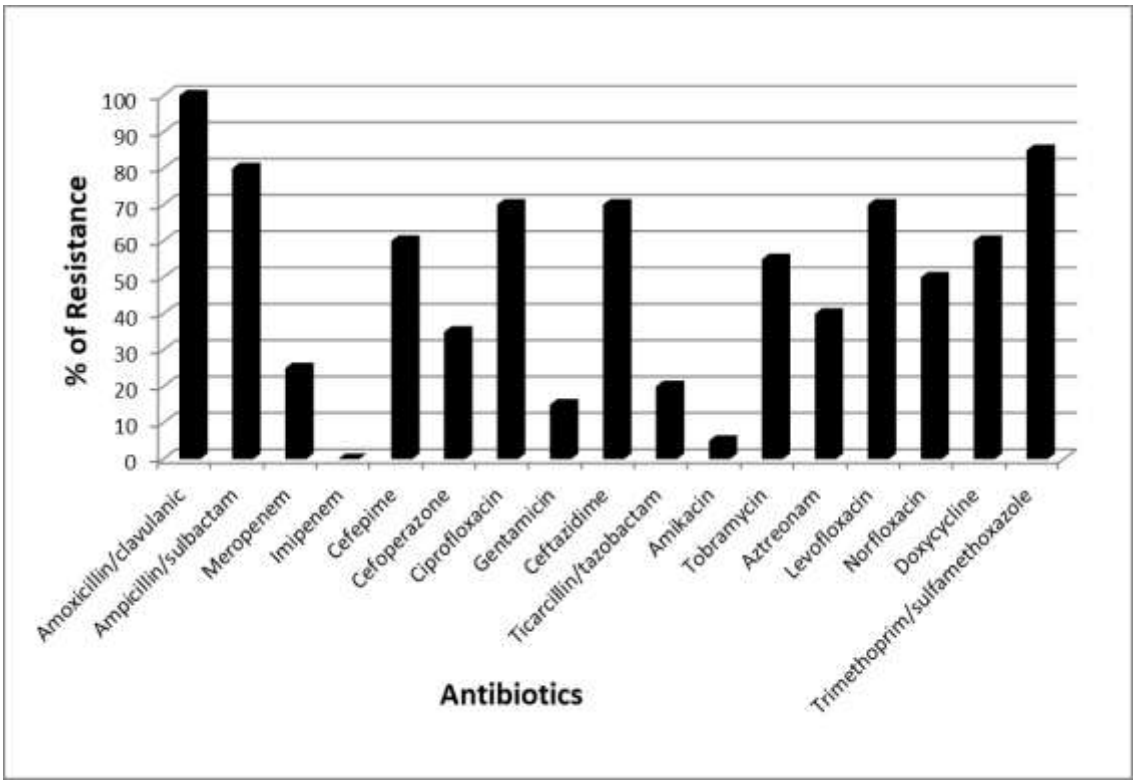


Fig. 1: Antibiotics resistance pattern of the 20 *E. coli* isolates recovered from urinary tract infections.

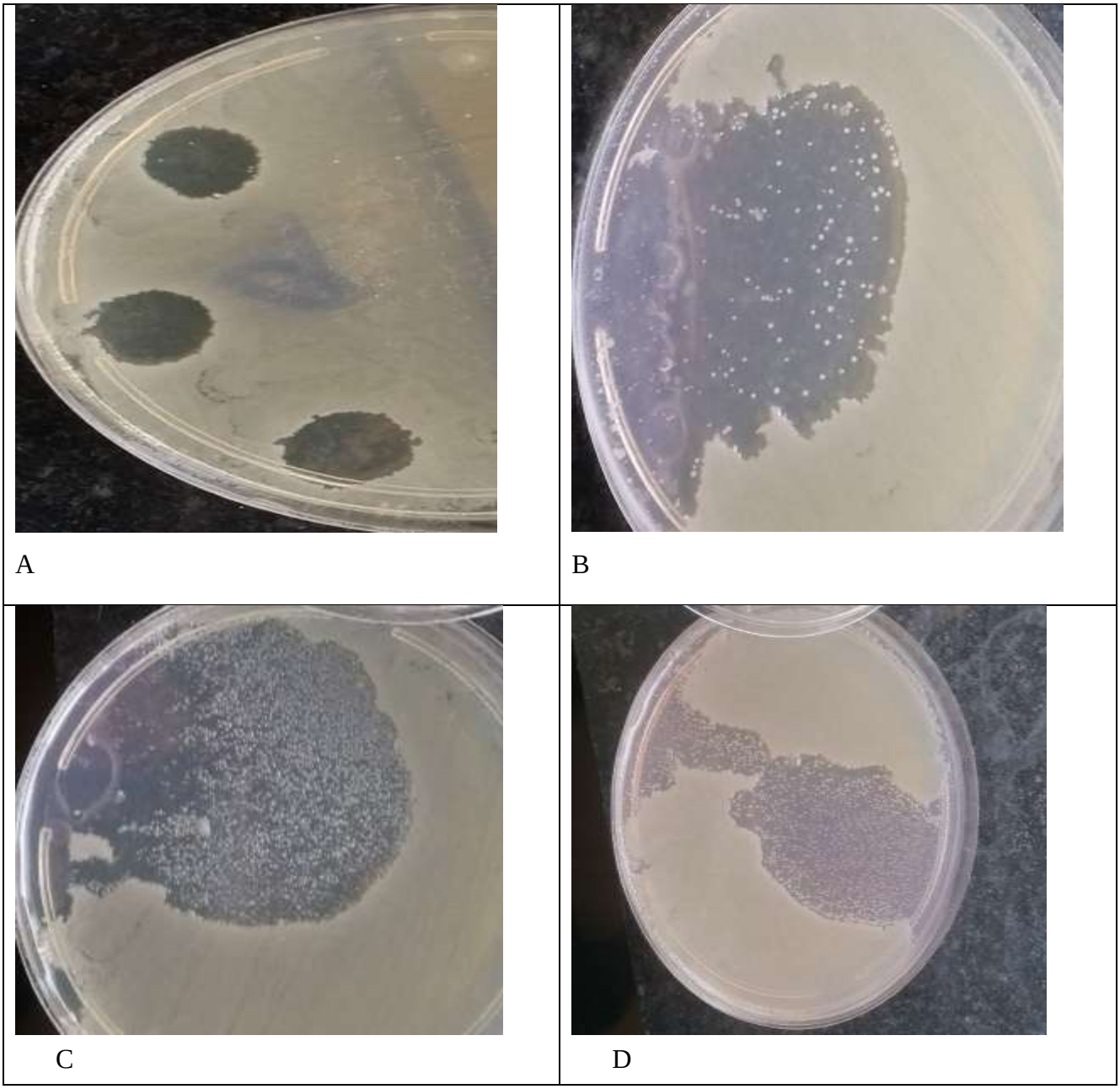


Fig. 2: A: The lytic activity of the propagated phages on *E. coli* O157: H7 ATCC 43894 (confluent lysis), B: shows the lytic activity of the propagated phages on *E. coli* NRRL B-3008 (semi-confluent lysis), C: the lytic activity of the propagated phages on *E. coli* O157: H7 urine isolate (semi-confluent lysis) and D: shows lytic activity of the phages on the *E. coli* non-O157 urine isolate (semi-confluent lysis).

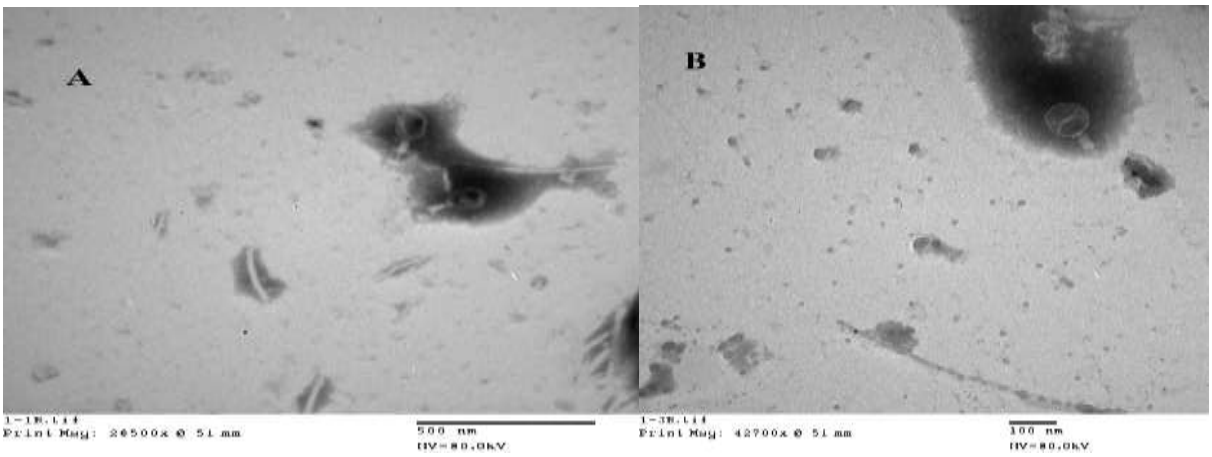
3.3. Host Range

The propagated phages were tested against different bacterial strains to determine their host range. They showed lytic activitiy on *P. aeruginosa* ATCC 27853 strain (Fig. 3). These phages did not exhibit any activities against *K. pneumoniae* ATCC 10031, *S. aureus* ATCC 6538.

Fig. 3: The lytic activity of the phages on the *P. aeruginosa* ATCC 27853 (semi-confluent lysis)

3.4. Transmission electron microscopy (TEM)

The TEM examination of the isolated phages revealed a T4 like phages of the family Myoviridae, subfamily Teequatrovirinae. The T4 like phage capsid is an elongated icosahedron, 120 nm long and 86 nm wide. Fig. 4 (A-D) demonstrate the contracted T4 like phages with contracted tail and icosahedral head, while Fig. 4 (E) shows complete T4 like phage.



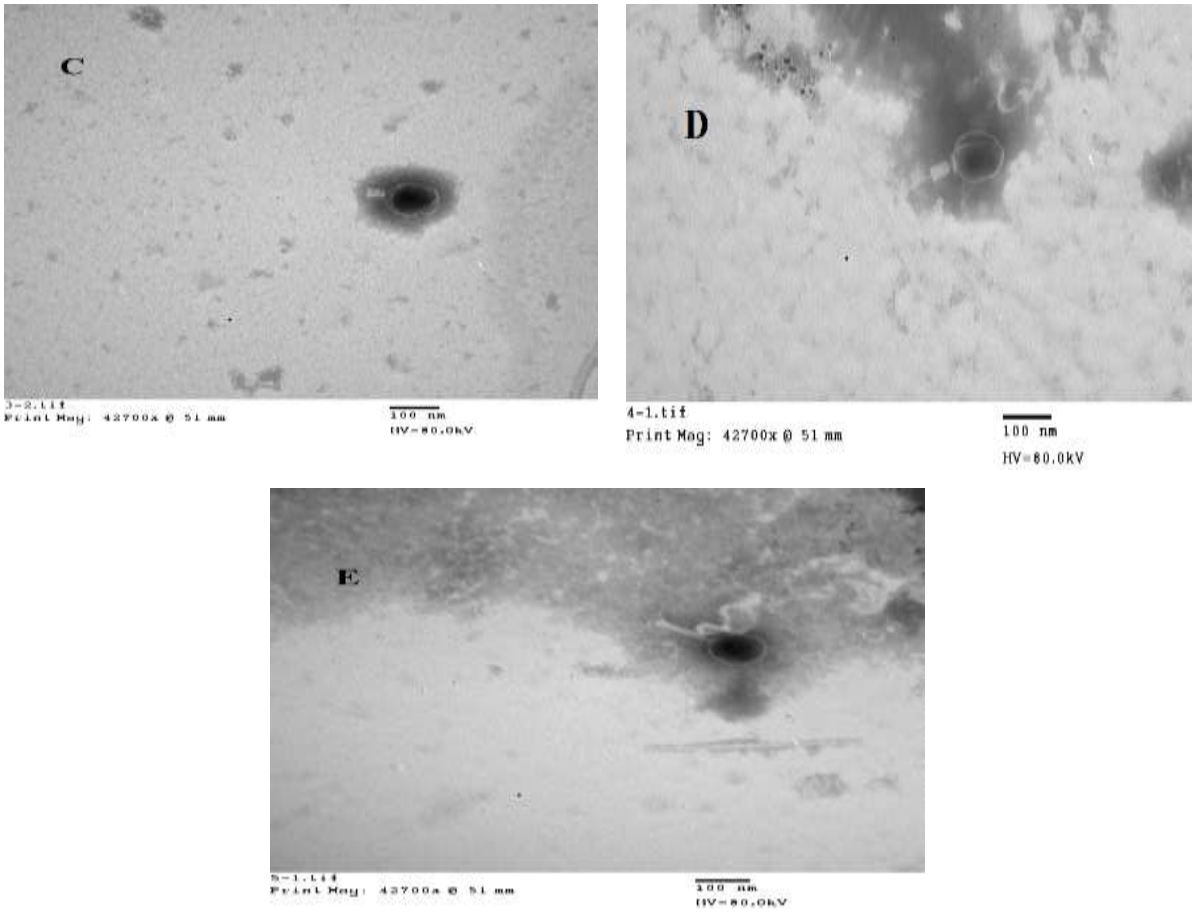


Fig. 4: The isolated phages under the Transmission electron microscope (TEM)

4. Discussion

Urinary tract infections (UTIs) are the most common infections worldwide. Due to the increasing resistance of the uropathogens towards antibiotics, our study was held to investigate the activity of bacteriophages as antibacterial agents. *Escherichia coli* was the most abundant pathogen, about 20 isolates (66.67%) were isolated from the 30 urine samples, in accordance with Tajbakhsh *et al*., (2015). Out of the 20 *E. coli* isolates, 2 isolates (10%) were found to be O157:H7 *E. coli*. Similar to the current results, a previous study conducted by Al-Dawmy and Yousif, (2013) reported the low incidence of O157:H7 *E. coli* among *E. coli* strains isolated from UTI of children. The present isolates were 100% resistant to amoxycillin/ clavulanic, 70% resistant to ciprofloxacin and levofloxacin. The most effective drugs were the imipenem and amikacin, which agree with the previous works of Akram *et al*., (2007); Jafri *et al*., (2014). The bacteriophages isolated in this study were obtained from sewage water, but we did not undergo filtration for sewage samples, as recommended by Anany *et al*., (2011); Lone *et al*., (2016); Hyman, (2019). As they reported that using 0.45 or 0.22 µm pore size filters will remove the bacterial solid

particles and the large phages as well. Moreover, Hyman, (2019) reported that several groups of bacteriophages can infect the host bacteria, even when their heads are attached to solid surfaces. The bacteriophages were tested for their host range. Phages were found to have lytic potential against *E. coli* O157:H7 ATCC 43894, *E. coli* NRRL B-300, and the tested clinical O157:H7 *E. coli* and the non-O157 *E. coli* isolates, in accordance with the previous results obtained by Sheng *et al*., (2006). In addition, it was recorded that the isolated phages exhibited lytic activity against *P. aeruginosa* ATCC 27853 strain, but showed no activity against *K. pneumoniae* ATCC10031, *S. aureus* ATCC6538, that agreed with the findings obtained by Alsaffar and Jarallah, (2016).

The morphology of the isolated bacteriophages was assessed by TEM examination and classified according to King *et al*., (2012). Results revealed the isolated phages were T4 like bacteriophages (lytic phages) of Myoviridae family, which is similar to results of the recent study conducted by Ribeiro *et al*., (2018). In general, O157:H7 *E. coli* is a Shiga toxin producing member of Enterohemorrhagic *E. coli* (STEC), which was first identified as a major human pathogen during outbreak of hemorrhagic colitis in 1982 (Riley *et al*., 1983). It is of particular concern, especially in children and elderly infected persons as it may results in hemolytic uremic syndrome (HUS) that can cause damage kidney (Boyer and Niaudet, 2011). So, attention for how to make safe food and free of foodborne pathogens has emerged. Previous studies of Carter *et al*., (2012) focused on the use of bacteriophages in preserving food from lyse and\or eliminate the food pathogens without affecting the normal microflora such as O157:H7 *E. coli*. Several studies of Letkiewicz *et al*., (2010); Khawaldeh *et al*., (2011); Sybesma *et al*., (2016) highlighted the use of bacteriophages in the treatment of UTIs, and reported the success of bacteriophage therapy against the uropathogens and the multi-drug resistant bacteria such as; *P. aeruginosa* and *E. coli*, which coincide with the results obtained in this study.

Conclusion

The isolated T4 like phages showed lytic potential toward different bacterial strains, including human uropathogenic *E. coli*, and O157:H7 *E. coli*. This presents bacteriophages as good alternatives for the antibacterial therapy, especially against multi-drug resistant pathogens. However, there is an urgent need to carry out *in-vivo* studies to make phage therapy accessible for the human use.

Conflict of interest

No conflict of interests exists between the authors of this study.

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Ethical approval

The protocols used in the study were approved by the Ethics Committee of the Minia University Hospital, El-Minia, Egypt.

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