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Bacteriophage is considered as a natural and promising tool for safety and preservation of food (Anany et al., 2014; Moye et al., 2018). Bacteriophages are viruses that have ability to infect and replicate within bacterial pathogen in a strain-specific manner (Beke et al., 2016). The ubiquitous and specificity of bacteriophages makes them ideal for the bio-control of pathogenic bacteria in food and environmental samples (Zhan et al., 2015; Anany et al., 2018; El-Dougdoug et al., 2019).

*S.* *Typhi* caused outbreaks that may be related to contaminate tap water with sewage, or milk and other foods that is a threat of the human health (Kaur et al., 2018; Liu et al., 2018). Bacteriophages have a bio-control application advantages over a chemical and conventional preservation of food (Huang et al., 2018).

Therefore, the present investigation was conducted to isolate *S.* *Typhi* from clinical cases and use lytic bacteriophages to control antibiotic-resistant *S.* *Typhi* in water and milk.

**Materials and Methods**

**Sampling**

Laboratory work was conducted at Benha Fever Hospital, Benha city and Microbiological lab, Faculty of science, Benha university, Egypt. A total of 233 sera specimens were screened for typhoid fever infection. Clinical isolates of *S.* enterica were detected from aseptically collected blood specimens with suspected enteric fever. The specimens investigated in this study were available from microbiology analysis lab in the Benha fever hospital.

**Isolation of Salmonella spp.**

Isolation and identification of *Salmonella* isolates was performed by standard microbiological techniques. Clinical specimens were enriched in blood culture bottles (5ml). All bottles were incubated at 37°C then a loopful of the blood culture was sub-cultured each two days on blood agar, macconkey agar and selective medium; *Salmonella Shigella* agar (SS agar). The inoculated plates were incubated at 37°C for 18-24hrs. Once a positive culture bottle was detected, Gram staining was prepared from colonies grown on SS agar plate. Negative bottles were incubated for further 2 days and then incubated again up to 7 days before they were reported as negative samples.

Identification of *Salmonella* spp.

The positive colonies were sub-cultured three successive times for purification on SS agar medium. *Salmonella* isolates were identified by colonial morphology, gram’s staining and other conventional biochemical tests (Bergey’s manual 2009). In addition, clinical isolates were identified using the VITEK® 2 GN system (Version 08.01) (Andrews et al., 2014) and the serotypes of *S.* enterica isolates was identified serologically using Widal test (Mukesh et al., 2018). Widal test was performed as described by (Widal, 1896) that based on revealing the presence of antibody (agglutinin) in the infected serum, against the flagellar (H) and somatic (O) antigens of *S.* Typhi.

**Antibiotic sensitivity test**

The antimicrobial susceptibility of *Salmonella* isolates were tested by modified Kirby-Bauer disk diffusion method on Müller-Hinton Agar as per CLSI recommendations. (CLSI, 2019). The antibiotics tested in this study include Ampicillin (10µg), Amoxicillin (25µg), Tetracycline (30µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Cefadroxil (30µg), Co-trimoxazole (25µg), Ciprofloxacin (5µg), Levofloxacin (5µg) and Nalidixic acid (30µg). Interpretation the results of antibiotic susceptibility tests was made according to standard interpretative zone diameters suggested in CLSI guidelines (CLSI, 2019). The bacterial response to antibiotic was interpreted as: R: Resistant, I: Intermediate and S: Sensitive.

**Isolation of bacteriophages.**

Different samples of sewage water collected from Virology Lab, Department of Microbiology, Faculty of Agriculture, Ain Shams University were examined for isolation of bacteriophages. Each sample (about 10ml) was enriched in an equal volume of blood culture broth medium and 100µl of an overnight culture of *S.* Typhi isolates were added. The mixture was incubated for 24-48hrs. with shaking at 37°C. After that, the mixture was centrifuged at 4,000×g for 20min at 4°C. The supernatant was filtered through a 0.45µm sterile filter. The spot test technique was performed to detect the presence of lytic phages (Sambrook et al., 1989).

**Purification of phages**

The soft agar overlay method (plaque assay) was used for purification the isolated phages (Sambrook et al., 1989). The plaque assay was performed by addition 100µl of overnight
culture of S. Typhi to 100µl of phage lysate. The mixture was incubated for 10-15min at 37°C for attachment. Then, 4ml of semisolid media were added, mixed and poured on agar plate. The plates were incubated at 37°C for 24hrs. The individual plaques were selected based on their morphologies and sizes. They were picked from the plates and placed separately in 300µl CM phage buffer. The plaque assay for individual plaques were repeated three times for highly purification. Then the phage lysate was stored at 4°C before propagation.

**Propagation of bacteriophage.**

The propagation of isolated phages was processed using the overlay (soft agar) method (Sambrook et al., 1989). Then, 4ml of CM buffer was added onto each plates and phage lysate was scraped off from the upper layer of soft agar. The suspensions containing phage lysate were put on ice for 15min. The phage lysate was centrifuged at 4,000×g for 20min at 4°C and filtered using a 0.45µm membrane filter. The propagated phage lysate was stored at 4°C.

**Titration of bacteriophages**

The phage lysates were diluted in CM buffer. The titers of the propagated phages were determined by spotting 10µl from decimal dilutions of phage suspension onto soft overlay prepared as described previously. The plates were incubated upright for 16-20hrs at 37°C. The titers of the phages were determined using the following equation:

\[
\text{Phage titer (PFU/ml)} = \frac{\text{Number of plaques}}{\text{Volume spotted} \times \text{Dilution factor}}
\]

**Characterization of S. Typhi phages**

Morphology of the isolated phages using TEM. The isolated S. Typhi phages were examined using transmission electron microscopy (TEM). The phage sample was prepared as described in (Ackermann, 2012) and examined by TEM (JEOL-JSM-5500LV, the Regional Center of Mycology and Biotechnology, Cairo, Egypt) using high vacuum mode at an acceleration voltage of 80 kV.

**Stability of phages.**

pH stability: the stability of isolated S. Typhi phages was tested at different pH values (5.0, 7.0 and 9.0) for 1, 3, 5 and 24hrs by adding 100µl of the phage lysate to 900µl of CM buffer and then incubated at 37°C. Plaque assay technique was performed in triplicate and phage counts determined. The logarithmic reduction in phage titer was determined.

Temperature stability: A wide range temperature degrees (-20°C, 4°C, room temperature, 37°C) were chosen to test phage stability for 1 day, 1 week, or 2 weeks. Temperature treatments were repeated and the average of phage counts was determined.

About salinity, Phage was tested against several salinity conditions (5%, 10%, and 20%) for 1hrs and one day. The lytic activity of phages was tested using spot test.

**Determination of the Bacteriophage Insensitive Mutant frequency**

The frequency of bacteriophage insensitive mutant (BIM) was tested through overlay method as mentioned previously (O'Flynn et al., 2006) at a multiplicity of infection MOI of 10. The BIM of phage cocktail, containing same volume and titers of four phages, was tested. The emerged colonies were counted. The BIM frequency was determined as:

\[
\text{Number of surviving colonies divided/ The original bacterial titer.}
\]

**Bio-control application using the cocktail of Salmonella phages**

*In vitro:* The bio-control efficacy of a phage cocktail composed of 4 lytic S. Typhi phages was assessed in broth medium. MOI of 5 was used by mixing 10^5 CFU/ml of overnight S. Typhi with phage cocktail suspension. Samples were incubated for 1, 3, 5, 7 and 24hrs at 37°C. After incubation, the surviving bacterial cells were counted in sterile saline to determine the bacterial log_{10} reduction.

*In vivo:* Treatment of tap water, full fat and skim milk.

Tap water, full and skim milk were initially screened for contamination with Salmonella spp. on specific SS agar medium. An equal volume of the 4 lytic S. Typhi phages were applied to tap water artificially contaminated by 10^3 CFU/ml S. Typhi culture suspended in saline. The artificially contaminated tap water was treated with phage cocktail with MOI 5. Positive controls (untreated) were made with 10^3 CFU/ml bacterial culture and CM buffer. Treated, untreated and negative
controls of tap water were stored for 0, 1, 3, 5 and 24 hrs at room temperature (25°C). On the same approach, full fat and skim milk were artificially treated with S. Typhi and phages. Treated, untreated and negative control of full fat milk samples were stored 0, 1, 3, 5, and 24 hrs at 25°C and 4°C. For skim milk sample, treated, untreated, negative control were stored at 25°C and 4°C for 1 hrs, 1 day, 2 days and 3 days. The developed Colonies were counted and calculated in (CFU/ml). The viability of phages was determined using plaque assay.

**Statistical analysis**

All experiments were performed in triplicates; the averages and standard errors were then calculated. The data represent the mean± standard deviation (SD). A t-test was performed and considered significant at P< 0.05, for three independent trials.

**Results**

**Sero-typing of Salmonella spp.**

The Widal test in diagnosing typhoid fever was assessed in Specimens with suspected enteric fever in Benha, Egypt. The test was done on sera specimens from 233 samples, 133 specimens with non-typhoidal fever and 100 bacteriologically proven specimens of typhoid fever. This study suggests that a positive Widel test is of considerable importance in diagnosing typhoid fever and identified the clinical isolates of Salmonella as Salmonella Typhi.

**Identification of Salmonella isolates**

Twenty out of 100 of S. Typhi isolates which gave positive blood culture and found sensitive to isolated phages were selected and identified. The colonies were detected as white, smooth and non-hemolytic on blood agar media; and as smooth colonies non-lactose fermenting, on Macconkey agar media; while colonies with circular shape, moderate to large in size with black centers were detected on SS agar media.

A variety of biochemical tests conventionally used to identify S. isolates was performed on S. Typhi isolates and showed that all the bacterial isolates were identified as gram negative bacilli and catalase positive. Whereas they gave negative result with indole, citrate and urease test.

Identification of the S. Typhi isolates using VITEK® 2 GN analyzer, modified version 2018, was carried out in Benha university hospital and reported that the tested isolates were identified as Salmonella spp. with 94% similarity.

**Antibiotic sensitivity test**

Using antibiotic susceptibility testing by disk diffusion, the twenty S. Typhi isolates, sensitive to phages, were tested against different antibiotic groups. They showed resistance to conventional antibiotics such as Ampicillin (100%), Amoxicillin (100%), Tetracycline (80%), Chloramphenicol (75%), Ceftriaxone (55%), Cefadroxil (65%) and Co-trimoxazole (75%). On the other hand, most of S. Typhi isolates showed reduced resistance to Fluoroquinolone including Ciprofloxacin (40%), Levofloxacin (50%) and Nalidixic acid (45%). The sensitivity of the four S. Typhi isolates selected for phage isolation was reported in Table 1.

**Characterization of S. Typhi phages.**

Four phages specific to S. Typhi were isolated from sewage water and selected based on their lytic activity by spot test. They were showed different plaque morphologies and named as vb_StyM EN 1, vb_StyM EN 2, vb_StyS EN 3 and vb_StyP EN 4. The titration of phages was determined using overlay method and counted approximately 10^9 PFU/ml. The size, morphology and turbidity of plaques were determined as showed in Table 2.

**Morphology of the phage isolates.**

The selected S. Typhi phages were examined using TEM and represent four different morphotypes (Fig. 1). Salmonella phage EN-1 and EN-2 have contractile tails and related to the family Myoviridae (Fig. 1 A, B). EN-3 is member of the Siphoviridae family as determined by the presence of a flexible, long tail and the absence of a contractile sheath (Fig. 1 C), whereas Salmonella phage EN-4 is characterized by icosahedral head without tail that related to Podoviridae (Fig. 1 D). The diameter of head and tail lengths of phages were calculated (Table 3).

**Phage stability**

Wide range of stability criteria of S. Typhi phages were characterized due to their possible application in bio-control of food borne pathogens in food matrix usually exposed to different processing conditions such as pH, temperature and salinity.
### TABLE 1. The antibiotic susceptibility test on pure cultures of four S. Typhi isolates.

<table>
<thead>
<tr>
<th>t group</th>
<th>Antimicrobial agents</th>
<th>Dose</th>
<th>S. Typhi 1</th>
<th>S. Typhi 2</th>
<th>S. Typhi 3</th>
<th>S. Typhi 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactam</td>
<td>Ampicillin-AM</td>
<td>10µɡ</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin-AX</td>
<td>25µɡ</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tertracycline-TE</td>
<td>30µɡ</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Chloramphenicol-CMP</td>
<td>15µɡ</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Ceftriaxone-CRO</td>
<td>30µɡ</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Cefadroxil-CFR</td>
<td>30µɡ</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>Trimthoprim-TMP/</td>
<td>1.25/23.75</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole-SMZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin- CIP</td>
<td>5µɡ</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>Levofloxacin- LEV</td>
<td>5µɡ</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Nalidixic acid-NA</td>
<td>30µɡ</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>I</td>
</tr>
</tbody>
</table>

### TABLE 2. Plaques morphology of S. Typhi phages.

<table>
<thead>
<tr>
<th>Phage isolates</th>
<th>Plaque morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>vb_StyM EN-1</td>
<td>Turbid, regular, mediate, circular plaques with 1.5 to 2mm</td>
</tr>
<tr>
<td>vb_StyM EN-2</td>
<td>Turbid, irregular, Large, circular plaques with 2.5 to 3.5mm</td>
</tr>
<tr>
<td>vb_StyS EN-3</td>
<td>Clear, regular, mediate, circular plaques with 2 to 3mm</td>
</tr>
<tr>
<td>vb_StyP EN-4</td>
<td>Clear, regular, small, circular plaques with 1 to 2mm</td>
</tr>
</tbody>
</table>
The stability of phage cocktail was tested in buffers of various pH values (5.0, 7.0 and 9.0) and temperatures (-20°C, 4°C, 25°C and 37°C). Then the count and log reductions (PFU/ml) of phage cocktail were determined through overlay technique. The result showed that the storage of phage cocktail under tested pH values for up to 24hrs did not affect- (P> 0.05) their stability (Fig. 2 A). In addition, there was no reduction of phage titre under different temperatures (P> 0.05) up to 2 weeks (Fig. 2 B.).

**Table 3. Head diameters and tail lengths of phages.**

<table>
<thead>
<tr>
<th>Phage</th>
<th>Head diameter (nm)</th>
<th>Tail length (nm)</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>vb_StyM EN-1</td>
<td>76.03±1</td>
<td>103.21±3</td>
<td>Myovirida</td>
</tr>
<tr>
<td>vb_StyM EN-2</td>
<td>79.47±1</td>
<td>98.44±2</td>
<td>Myovirida</td>
</tr>
<tr>
<td>vb_StyS EN-3</td>
<td>69.98±0.5</td>
<td>211.54±2</td>
<td>Siphovirida</td>
</tr>
<tr>
<td>vb_StyP EN-4</td>
<td>88.02±2</td>
<td>-</td>
<td>Podovirida</td>
</tr>
</tbody>
</table>

The stability of phage cocktail was tested in buffers of various pH values (5.0, 7.0 and 9.0) and temperatures (-20°C, 4°C, 25°C and 37°C). Then the count and log reductions (PFU/ml) of phage cocktail were determined through overlay technique. The result showed that the storage of phage cocktail under tested pH values for up to 24hrs did not affect- (P> 0.05) their stability (Fig. 2 A). In addition, there was no reduction of phage titre under different temperatures (P> 0.05) up to 2 weeks (Fig. 2 B.).

The *Salmonella* phages cocktail were subjected to different salinity conditions (5%, 10% and 13%). Phages did not encounter any significant reduction in their titer (P> 0.05) when subjected to different salinity conditions (5%, 10% and 13%) after 1hrs and 24hrs.

**Bacteriophage insensitive mutants (BIM)**

BIM against the isolated phages was tested using phage cocktail. Resistant mutants of *S. Typhi* were developed against phage cocktail. Using phage cocktail resulted in a frequency of BIM about 1.62x10⁻⁵. Phage cocktail caused significantly low BIM frequency when compared to the effect of the other *Salmonella* phages against *S. Typhi*.

**S. Typhi challenge using Salmonella phage cocktail.**

**In broth media**

The efficacy of phages cocktail to control *S. Typhi* was tested in blood culture broth medium. Phage cocktail was used to achieve significant reduction in *S. Typhi* growth and to reduce the chance of resistance development. A significantly high number of phages are required to ensure sufficiently rapid contact and infection of the few targeted bacterial cells present. In blood culture broth, the addition of phage cocktail with MOI 5 resulting in 2 log reduction of bacterial growth after 1hrs (P< 0.05). While significant reduction and complete inhibition (below detection limit 1 log CFU/ml) of bacterial growth was achieved after 3, 5, 7 and 24hrs at 4°C and 25°C compared to control counts (Fig. 3 A, B).
CONTROL OF ANTIBIOTIC-RESISTANT *SALMONELLA ENTERICA* SEROVAR *TYPHI* IN ... Egypt. J. Bot. 60, No. 1 (2020)

**Fig. 2.** Stability of *Salmonella* phage cocktail under different pH values (A) and temperatures (B).

**In tap water**

The efficacy of the phage cocktails to control *S. Typhi* in a drinking water was examined. The tap water was artificially contaminated with *S. Typhi*, and then the corresponding phage cocktails were added and incubated at room temperature. The count of the surviving *S. Typhi* was determined at regular intervals and compared to the control. Figure 4 showed the effect of phage cocktail with MOI 5 to reduce *S. Typhi* count in tap water incubated at room temperature. Treatment using with MOI 5 resulted in inhibition of *S. Typhi* after 1hrs (P< 0.05) and complete reduction after 3 h compared to the control (below 1 log CFU/ml) (Fig. 4).

**Fig. 4.** bio-control of *S. Typhi* in tap after addition of phage cocktail with MOI 5 [Statistically significant treatments compared to the control were indicated by "*" where P< 0.05. The population count below detection limit (less than 1 log CFU/ml) is indicated by "**"].

**In full fat and skim milk**

Bio-control of *S. Typhi* using phage cocktail resulted in decrease of viable *Salmonella* counts of at least 1 log CFU/ml after 1h. (Fig. 5 A, B) in treated full fat milk stored at 4 °C and 25 °C, followed by regrowth during the remaining incubation period at 25 °C (Fig. 5 A). On the other hand, a complete elimination of viable bacteria in pasteurized full fat milk was observed at 4°C after 3hrs (Fig. 5 B) compared to the control (less than 1 log CFU/ml).

Further, the efficacy of the phage cocktail in skim milk was better than for full fat milk. At 25°C, the growth of *S. Typhi* was reduced by about 2 log CFU/ml in treated skim milk after 1hrs. followed by a small amount of regrowth during the remaining incubation period with about 5 log reduction after 3 days (Fig. 5 C) compared to the control (P< 0.05). The efficacious phages showed complete eradication in treated skim milk at 4 °C over the incubation period (below detection limit) (Fig. 5 D).

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Typhi in full fat milk using phage cocktail at 4°C, (C) Treatment of Typhi in skim milk using phage cocktail at 25°C and (D) Treatment of Typhi in skim milk using phage cocktail at 4°C. [Statistically significant treatments compared to the control were indicated by “*” where P< 0.05. The population count below detection limit (less than 1 log CFU/ml) is indicated by “**”].

**Stability of phage cocktail on samples**

The concentration of phage cocktail added to the samples was monitored over different incubation periods. In tap water, we observed a slight rising (approximately 2 log) in the phage titer at 25°C. In milk samples, no significant loss in titers of phage cocktail inoculated into full fat milk at 4°C (P> 0.05), in contrast the titer of phage cocktail was reduced up to 6 log after 24h incubation at 25°C (P<0.05) (Fig. 6 A). On the other hand, phage titers remained stable at 4°C and decreased by 1 log at 25°C in skim milk after 3 days (Fig. 6 B).

**Discussion**

*S. Typhi* is globally a major clinical problem causing typhoid fever. In order to develop effective strategies to control these pathogens, a mixture of lytic bacteriophage specific for *S. Typhi* with antibacterial activity is ready to use for bio-control of bacterial pathogen was used. In our study, twenty isolates of *S. Typhi* were obtained from blood cultures (Tack, 2019) and identified phenotypically by biochemical tests as described previously (Karmakar et al., 2016). Additionally, *S. Typhi* isolates were characterized morphologically by observing colonies on the plate and using light microscope to investigate cell morphology and all the bacterial isolates were identified as being Gram-negative rods (Asmelash et al., 2016).

![Figure 5](image)

**Figure 5.** Histogram showing bio-control of *S. Typhi* in treated full fat and skim milk after addition of phage cocktail with MOI 5 (A) Treatment of *S. Typhi* in full fat milk using phage cocktail at 25°C, (B) Treatment of *S. Typhi* in full fat milk using phage cocktail at 4°C, (C) Treatment of *S. Typhi* in skim milk using phage cocktail at 25°C and (D) Treatment of *S. Typhi* in skim milk using phage cocktail at 4°C. [Statistically significant treatments compared to the control were indicated by “*” where P< 0.05. The population count below detection limit (less than 1 log CFU/ml) is indicated by “**”].

![Figure 6](image)

**Figure 6.** Stability of *S. Typhi* phages in (A) Full fat milk and in (B) Skim milk at 4°C and 25°C. [Statistically significant treatments compared to the control (initial conc.) were indicated by “***” where P< 0.05].
The clinical isolates were identified using Vitek analyzer as Salmonella spp. (Andrews et al., 2014; Pincus, 2014). Furthermore, they were identified serologically as Salmonella enterica serotype Typhi using Widal test (Somily et al., 2011; Amey et al., 2017). Widal test is used to diagnose typhoid fever in the developing countries. The test measures agglutination antibody levels against O and H antigens (Tankeshwar, 2015).

Emergence of resistance in S. Typhi is a serious public health concern (Karkey et al., 2017). The antibiotic sensitivity test for S. Typhi was performed and showed resistance to fluoroquinolones and other families. The resistance to the fluoroquinolones has reduced therapeutic options in this completely treatable disease (Raveendran et al., 2010). Resistance mechanism of S. Typhi against antibiotics was reported (Ugboko & De, 2014) and was attributed to drug inactivation, alteration of active efflux and target site in Salmonella. These resistance mechanisms could either be plasmid or chromosomal mediated.

Bacteriophages have been considered as a promising trend to detect and control foodborne pathogenic bacteria (Anany et al., 2017; Moye et al., 2018). The main step for successful application and to boost the food safety is cautiously selection of lytic phages. In this study, S. Typhi phages, targeting the causal of typhoid fever; S. Typhi, were isolated and identified with various plaque morphologies and sizes. These phages are belonging to Myoviridae, Siphoviridae and Podoviridae. The behavior and susceptibility of isolated phages depends on several phenomena such as restriction endonuclease modification and blocking the adsorption step (Pires et al., 2016). Host range is one of main criteria affecting the selection of lytic phages to be used for bio-control applications (Holmfeldt, 2007). Most of Salmonella phages has a broad host range against different Salmonella serovars (Whichard et al., 2003). Using a cocktail of phages could overcome the limitations of phages with a narrow host range (Goodridge & Bisha, 2011). Consequently, in this work, a cocktail of S. Typhi phages was used.

The applications of bacteriophages for bio-control were based on stability of phages at various environmental conditions. The phages showed high tolerance under alkaline and acidic conditions for up to 24hrs without a significant decrease in their titers which was in agreement with previous work (Joniczyk et al., 2011).

The low frequency of emergence of resistant mutants against S. Typhi phages was investigated, as shown in a previous study (O’Flynn et al., 2006). The BIM result of S. Typhi phages in the current work suggests the phages are able to overcome resistance mechanisms (Labrie et al., 2010). The development of resistant strains of phage is considered as one of the problems of using phages for bio-control against pathogenic bacteria (Maura & Debarbieux, 2011).

Interestingly, bacteriophages with lytic activity specific to S. enterica have been considered as a new and alternative strategy to manage and control pathogenic bacteria in food matrices (Goodridge & Bisha, 2011; Moye et al., 2018). Currently, phages are considered as new antimicrobials to fight multidrug-resistant bacteria. Bacteriophages possess a number of properties helping them to combat pathogenic bacteria such as specificity which is a very valuable property, as it would prevent interference with the microflora or inhibition of starter culture in dairy production in addition to their auto-replication which gives lifetime controlling (Molina et al., 2018). Outbreaks of S. Typhi thought to be related to the consumption of tap water contaminated with sewage, raw and pastorialized milk represents a threat of the basic public health (Kaur et al., 2018; Liu et al., 2018). Therapeutic application of bacteriophage has many pros over chemical conventional preservatives including natural activity, specificity, lack toxicity, and a high degree of safety (Huang et al., 2018). Treating contaminated tap water with a cocktail of S. Typhi phages using MOI 5 resulted in complete eradication in the count of bacterial pathogen comparing with the untreated samples after 24hrs.

EFSA and ECDC, (2017) reported that Salmonella being the most frequently causative agent in outbreaks associated with milk and dairy products (37.7%). Furthermore, pathogenic bacteria can be emerged during food processing and storage, that is mainly linked to cross-contamination, changes in temperature and poor handling practices.

In milk, the phage cocktail showed high efficacy on the bacterial growth as described previously (Huang et al., 2018; Phontang et al., 2018).
Bacteriophages can also be an effective control in pasteurized whole milk contaminated with *Salmonella* spp. The inhibitory effect of this phage cocktail was higher at 4°C than at 25°C (Bao et al., 2015). Thus, phages were able to successfully inhibit the growth of targeted bacteria in milk even at refrigeration temperatures (Lee et al., 2017; Molina et al., 2018. Gutiérrez et al., 2019).

In spite of this, the effectiveness of phages decreases in full fat milk than in skimmed milk and this may attributed to the organic substances such as proteins, fat content, inhibitory substances and the conditions of medium. The adsorption of phages can affected by changes in temperature, pH or ionic strength of the medium which is particularly troublesome for biocontrol application (Hosseinidoust et al., 2014). The composition and pH of milk (Slightly acidic) have a lower impact on the activity of phages. Where the phages are prone to aggregates at low pH (Langlet et al., 2008). In addition to that, the tail fiber of phage, responsible for attachment to bacterial cell, has a positive charges (Serwer, 1987) could attracted to the negative charges on Fatty acids in milk rather than those on bacterial cells thus render the interaction between phage and its host. Consequently, this study showed no significant reduction in bacterial growth in full fat milk at 25°C comparing to skimmed milk.

Nevertheless, other studies showed the lytic activity of bacteriophages for bio-control *Salmonella* spp. and other pathogenic bacteria with relevance in different food matrices and dairy industry (Heyse et al., 2015; Gutiérrez et al., 2019).

**Conclusion**

*S. Typhi* showed resistance against traditional antibiotics such as ampicillin, chloramphenicol, and trimethoprim-sulfonamide combinations. Although fluoroquinolones such as ciprofloxacin are used to treat severe *Salmonella* infections, some of *S. Typhi* showed resistance to fluoroquinolones. Consequently, many trends are looking for new safe, effective and potential antimicrobials alternative to antibiotic. Therefore, this study led to isolation of lytic phages specific to *S. Typhi*. Stability of the isolated phages in different environmental conditions suggests their applicability and use as bio-control tools throughout the food supply chain. This study proves the efficacy of these lytic phages for controlling the growth of *S. Typhi* in milk and drinking water, and so potentially increases the shelf life of milk and enhances the safety of tap water.

**References**


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