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Review article

A systematic review and modeling of the effect of bacteriophages on *Salmonella* spp. Reduction in chicken meat

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ABSTRACT

Prevention and control of foodborne pathogens are of vital public health importance, and poultry meat is recognized as a major source of *Salmonella* infection in humans. Therefore, it is necessary to reduce the presence of *salmonella* in poultry meat. This article provided a systematic review and modeling to assess the effect of various factors on bacteriophages' function on *Salmonella* spp. Reduction in poultry meat. Twenty-two studies were included based on the inclusion and exclusion criteria mentioned in the methodology. The results showed that each unit increase in bacterial dose, phage dose, and temperature increases the *Salmonella* reduction by about 7%, 20%, and 1%, respectively. In addition, wild-type phages were more efficient than commercial-type phages, and this result was statistically significant ($\beta = 1.124$; p-value <0.001). This multivariate analysis is a helpful tool to predict the role of various factors in the role of phage in reducing *Salmonella* in poultry meat.

1. Introduction

Salmonella is one of the most important foodborne pathogens worldwide and the causative agent of the most common foodborne disease known as non-typhoidal salmonellosis. More than 2500 *Salmonella* serotypes have been described in the genus of *Salmonella enterica*. Salmonellosis symptoms are abdominal pain, vomiting, inflammatory diarrhea, headache, and nausea [1–3]. Salmonellosis, caused by non-typhoidal *Salmonella* spp, is currently the leading foodborne disease of bacterial etiology in the United States, causing approximately 1.35 million cases and 420 deaths annually. According to the Center for Disease Control and Prevention (CDC), *Salmonella Enteritidis* (*S. Enteritidis*) is the most commonly reported serotype. Between 2002 and 2017, there were 4265 food poisoning cases in Korea, 332 were related to *Salmonella*, and after enteropathogenic *Escherichia coli* and norovirus, *Salmonella* was the third most common food poisoning [4]. *Salmonella* is estimated to be responsible for around 85% of foodborne diseases worldwide. In 2007, the United States Department of Agricultural Economic Services (USDA) estimated that the United States had suffered 2.5 million $ in

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economic losses from 1.4 million cases of *Salmonella* [2]. In 2015 and 2008, 94,625 and 2551 cases of salmonellosis were reported in Japan and Europe, respectively [5]. In China, 70 salmonellosis outbreaks were reported from 2008 to 2012, resulting in 4151 hospitalizations and four deaths [3,6]. The above cases make clear the importance and impact it has worldwide.

Poultry meat is one of the essential sources of *Salmonella* infection in humans [4,5,7,8]. The animal intestine is an important reservoir for *Salmonella*, which can be transferred to meat during manufacturing and slaughtering procedures [4,9]. The spread of *Salmonella* in poultry meat can cause economic and health damage. With the increasing consumption of these products, there are concerns that *Salmonella* infectioncould pose a crucial public health risk [10,11]. Therefore, reducing *Salmonella* in poultry meat is necessary for human health, and prevention methods must be applied appropriately [5,12].

Conventional, physical, and chemical methods of reducing *Salmonella* can have various side effects [5,13]. Physical processes, such as washing with hot or cold water, freezing, cooling, and ionizing radiation can cause deterioration in the properties of raw meat and consumer satisfaction. Heat treatment changes the color, but radiation oxidizes the fats and changes the organoleptic properties of meat [10,14]. Chemical disinfectants, namely ascorbic acid and calcium carbonate have detrimental effects on texture, taste, and color. Besides, chemical preservatives, including sodium benzoate and benzoic acid can lead to complications, like asthma, hives, and seizures [3,15]. Conventional methods; therefore, do not meet the needs of the consumer, and new techniques have to be developed.

Bacteriophage is a virus with a bacterial host that injects its genetic material into the bacterial cell. After replication and rupture of the host cell, more phages are released [16–18]. The clinical use of phages to treat a wide range of infections began in the early 1920s [19]. Bacteriophages have drawn increased attention as a new approach to combat pathogens because of their advantages, like ubiquitous nature, easy extraction, cost-effectiveness, and safety [20–22]. However, phage cocktails are used to improve phage efficiency and performance, which several studies have shown to be effective [23–25]. Such strategies as combination therapies and genome engineering can further help prevent the spread of phage resistance in the future [19].

Recently, such phages as SalmoFresh® and Salmonelex™ have been identified as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) [26]. Many studies have examined the effects of phage in various foods that have shown phages are used as a biologically safe agent. The first factor in phage successfully is phage stability. Several external factors, namely temperature, pH, a_w, and salt concentration can influence the lytic activity of phage [22].

According to various studies evaluating the effect of phage in reducing *Salmonella* in poultry meat, numerous factors can affect the effectiveness of phage, including temperature, time, phage inoculation method, bacterial dose, and phage dose. Sukumaran et al. (2015), for example, concluded that the dip treatment affect phage performance [8]. Thung et al. (2017) found that at 4 °C, the effect of phages increases slightly over time [27]. Duc et al. (2018) established that with an increase in temperature from 8 °C to 25 °C, phage efficincy increases [5]. Moon et al. stated that with an increase in phage dose, the effect of phages increases, and phages have a greater influence on single bacteria than a bacterial cocktail [11]. Adriana et al. performed a systematic review and meta-analysis on the effects of dietary additives, vaccinations, and processing aids as control measures for *Salmonella* spp. In chicken meat. They found that the interventions were effective in reducing *Salmonella* [28]. However, since there is no centralized data on the effect of different factors on phage function, this study aimed to systematically review previously published articles on bacteriophage-mediated *Salmonella* reduction and to model the effect of various factors on phages function *Salmonella* reduction in poultry meat.

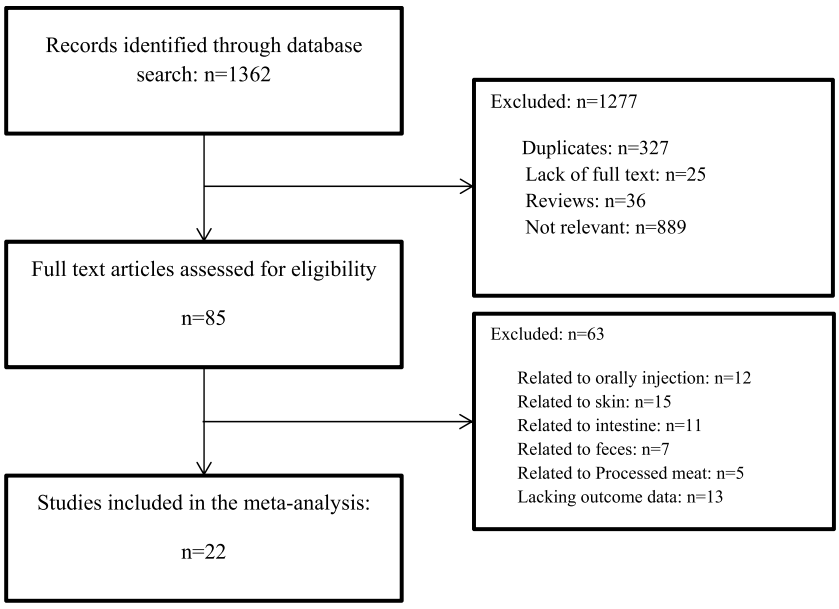

Fig. 1. Flow chart of the systematic literature research.

2. Methods

2.1. Definition and literature search

A comprehensive search for the effect of different phage factors on *Salmonella* reduction in poultry meat carried out using similar keywords in three major global electronic databases, including PubMed, Scopus, and Science Direct. The search was performed by systematic query in title, abstract, and keywords using key terms encompassing the following: *Salmonella*, phage, bacteriophage, poultry, and chicken. Articles based on their titles, abstracts, and full text included, and papers without the English language in the main text, review articles, and book chapters excluded. Studies on the reduction of *Salmonella* in poultry meat, articles with phage cocktails, and experimental studies were included; papers that examined the effects of phages with other substances were excluded. Following, full-text screening of the eligible studies was done from the databases. There were relevant articles in which the abstracts were the only text available and required additional efforts to access full text; so, they were excluded (Fig. 1).

Table 1
Different Salmonella reduction factors investigated in poultry meat.

Reference	Factors investigated	Result
[3]	Phage dose: 106 PFU/g and 107 PFU/g Temperature: 4°C and 25 C Time: 1, 3, 6, 12, 24, and 48 h	These findings demonstrated that the phage cocktail described in this study can be potentially used as a biological control agent against Salmonella in food products.
[33]	Temperature: 4°C and 25 C Time: 1, 2, 3, 4 and 5 h	A significant reduction in bacterial numbers (1.5–4 log CFU/sample,$p < 0.05$) was observed in all tested foods.
[4]	Phage dose: 108, 109, and 1010 PFU/g Time: 24, 48, 72, 92, 120, 144, and 168 h	The potential efficacy of the bacteriophage cocktail as a biological agent against S. Enteritidis in raw chicken breast meat.
[25]	Time: 24, 48, 72, and 96 h	A developed phage cocktail suggests a potential biocontrol against Salmonella in fresh foods.
[1]	Temperature: 4°C and −20 C Time: 3, 6, 24, 48, and 72 h	Significant reduction of SSL1-010 (0.4–1.0 log CFU/cm2,$P < 0.05$) throughout 72 h of storage at 4°C. At −20°C, phage treatment significantly decreased. The number of SSL1-010 by 0.4 log – 0.7 log CFU/cm2 ($P < 0.05$) during 0–24 h of storage. These findings demonstrated that phage cocktail can be used for controlling growth of SSL1-010 on chicken meat during storage at 4 C. Besides, phage cocktail can be employed for reduction of Salmonella load during the first hs of storage at −20 C.
[50]	Time: 48, 120, and 168 h	The potential effectiveness of this bacteriophage cocktail as a biocontrol agent of Salmonella in several food matrices under conditions similar to those used in their production.
[27]	Time: 12, 24, and 48 h	Bacterial population was reduced by 2.0 log cycles on the bacteriophage treated chicken meat samples.
[8]	Phage dose: 108 PFU/g and 109 PFU/g Time: 24 and 168 h	The surface applications of phage significantly reduced Salmonella counts on chicken breast fillets.
[51]	Time: 4, 8, 24, and 168 h Temperature: 4°C and 25°C	Lytic phage preparation was effective in reducing Salmonella on chicken breast fillets.
[18]	Time: 0.5 and 8 h	Bacteriophage reduction was dependent on Salmonella's susceptibility to the bacteriophage, and treatment time.
[11]	Phage dose: 1.1 × 108 PFU/g, 1.1 × 109 PFU/g, and 108 × 2.2 PFU/g	The combined treatments resulted in significantly greater reduction of Salmonella than individual bacteriophage or essential oil treatments.
[10]	Bacterial dose: 3 and 6 log 10 CFU/g Time: 3, 24, 48, 96, 72, 120, and 192 h Temperature: 4°C and 25 C	SE-P3, P16, P37, and P47 phages have the potency to be used as a biocontrol strategy to control Salmonella in the poultry industry.
[5]	Temperature: 8°C and 25 C Time: 2, 4, 6, and 24 h	Phages isolated from raw chicken meats are potential agents for controlling Salmonella in raw meats.
[2]	Temperature: 4°C and 25 C Time: 2, 6, 12, 16, 24, 36, and 48 h	A phage cocktail of SPHG1 and SPHG3 is considered as a promising candidate as a biocontrol agent against foodborne salmonellosis.
[7]	Phage dose: 108 and 106 PFU/g Time: 1, 2, 3, 4, 5, 6, and 7 h	Two phages (vB_SalS_1–23 and vB_SalS_3–29) with lytic effects show a high potential to inhibit the growth of Salmonella contaminants and can be used as candidate biocontrol agents.
[22]	Temperature: 4°C and 25 C Time: 3, 6, 9, 12, 24, and 48 h	Thermostable phages could be applied as complementary tools to control post-contamination after thermal processing of food products.
[9]	Phage dose: 108 PFU/g and 107 PFU/g Time: 0.5 and 6 h	Bacteriophage application during tumbling of red meat trim and poultry can provide additional Salmonella control in ground products.
[52]	Time: 2, 4, 6, 8, 10, 12, and 24 h	These findings highlighted phage vB_SalP_TR2 as a potential antibacterial agent for the control of Salmonella in food samples.
[53]	Time: 0.08, 0.25, 0.5, 1, 24, and 168 h	ST-W77 and SE-W109 are ideal phages for further development as Salmonella biocontrol agents for food production.
[54]	Time: 24 and 48 h	Phages may be useful in the control of food-borne pathogens.
[55]	Phage dose: 106 PFU/g and 107 PFU/g Temperature: 4°C and 25°C Time: 1, 3, 6, and 12 h	Significant reductions of viable Salmonella were observed in diverse foods.
[56]	Temperature: 4°C and 25 C Time: 1, 3, 6, 12, 24, and 48 h	Phage LPST94 is a promising candidate for biological control agents against pathogenic Salmonella and has the potential to be applied across different food matrices.

2.2. Data analysis

All data were entered into a file and analyzed with Stata software (version 13.0). The *Salmonella* reduction was presented descriptively. Univariate linear regression models were used to predict the regression coefficient for *Salmonella* reduction. In order to eliminate possible confounding factors, the variables were entered into the multivariate linear regression model with a significance level <0.2. The variance inflation factor (VIF) was used in the multivariate regression model to remove the linearity effect. Therefore, variables with a VIF greater than four were eliminated.

This equation ($y_i = \widehat{\beta}_0 + \widehat{\beta}_1 X_i + e_i$) was used to predict *Salmonella* reduction. R^2 was used as a criterion to select the best model. The significance level was 0.05 for two-sided tests.

3. Results

3.1. Eligible studies and characteristics

The initial search identified 1362 potentially relevant studies, of which 85 studies were further evaluated. Finally, 22 articles were included in this study. A total of 417 independent data were extracted from the 22 articles and entered into the regression analysis. The baseline characteristics of the included studies are shown in Table 1. The results showed that studies on the reduction of *Salmonella* bacteria were carried out between 2013 and 2021; the main topics examined are the effect of phage on the reduction of *Salmonella* in chicken meat, the main dose of phage is between 6 log10 PFU/mL and 9 log10 PFU/mL, the most used temperatures are 4 °C and 25 °C. The effect of the phages was mainly examined within 1–24 h. The results indicated that the phage could be effective at killing *Salmonella* and reducing it by up to 10 log10 CFU/g under some conditions.

3.2. Univariate and multivariate analysis

Table 2 presents the regression coefficient as a measure of association for each exposure variable with the presence of *Salmonella* reduction. The Univariate regression coefficient (i.e., a separate logistic regression model for each exposure) was highly significant for all variates. The univariate analysis showed that with each unit of increase in the bacterial dose (i.e., from 10^6 CFU/mL to 10^7 CFU/mL) and phage dose (i.e., from 10^6 PFU/mL to 10^7 PFU/mL), the rate of *Salmonella* reduction increases by about 20% (p-value <0.001). In addition, commercial phage decreases *Salmonella* reduction by about 90%. The use of surface treatment increases *Salmonella* reduction by around 76% (p-value <0.001). They were significant for *Salmonella* serotypes, *S. Enteritidis* (β = 0.66, p-value <0.001), and other serotypes (β = 0.25, p-value = 0.025) showed a greater reduction in *Salmonella*.

Since all of the variables were highly correlated (colinear variables), the multivariable model was presented separately for each of these exposures. The results of the multivariate regression model are presented in Table 2. All of the variables were found to be highly significant with this outcome, except the method of inoculation (β = −0.151, p-value = 0.391). However, it was also shown that a one-unit increase in bacterial dose, phage dose, and temperature; increases the *Salmonella* reduction by about 7%, 20%, and 1.5%, respectively, and these results were statistically significant. Time, unlike the other factors, has a negative effect on the process, but it does affect the reduction of *Salmonella*; that is, each extra h in phage treatment is associated with a 0.1% decrease in *Salmonella* reduction (p-value = 0.108) … However, the imperative role of other factors and different test conditions should not be underestimated. The extraction environment had a higher effect on phage function, and commercial phage decreases *Salmonella* reduction by 1.12 units, and this result was statistically significant (p-value <0.001). Lastly, *S. Enteritidis* (β = 0.445, p-value <0.001) and other species (β = 0.356, p-value = 0.002) showed more reduction in *Salmonella*.

Table 2
Univariate and multivariate linear model for regression coefficient of Salmonella reduction in poultry meat.

Univariate model					Multivariate model			
Variable	β	95% Cl		P value	β	95% Cl		P value
		Lower	Upper			Lower	Upper	
Bacterial Dose	0.197	0.142	0.252	0.000	0.071	0.010	0.132	0.022
Phage Dose	0.201	0.162	0.240	0.000	0.202	0.150	0.254	<0.00
Temperature	0.011	0.002	0.020	0.014	0.015	0.007	0.022	0.000
Time	0.003	0.001	0.005	0.001	−0.001	−0.003	−0.0003	0.108
Method of inoculation								
Surface treatment	Ref	–	–	–				
Deep treatment	0.764	0.470	1.058	0.000	−0.151	−0.451	0.149	0.391
Phage type								
Wild phage	Ref	–	–	–				
Commercial phage	−0.900	−1.240	−0.554	0.000	−1.124	−1.445	−0.803	0.000
Type of *Salmonella*								
S. Typhimurium	Ref	–	–	–				
S. *Enteritidis*	0.66	0.43	0.88	0.000	0.445	0.230	0.661	0.000
Other	0.25	0.032	0.48	0.025	0.356	0.133	0.579	0.002

4. Discussion

4.1. Temperature

This is the first time that multivariate analysis has been applied to investigatethe effect of various factors on bacteriophage function on *Salmonella* spp. Reduction in poultry meat with such a broad set of data, revealing the external factors that can affect phage activity and their importance. Temperature is a vital factor in bacteriophage survival. It plays an essential role in adhesion, penetration, proliferation, and the length of the latent period. At lower than optimal temperatures, fewer phages' genetic materials penetrate bacterial host cells; therefore, fewer of them can be involved in the proliferation phase. Higher temperatures can extend the duration of the latent period [29]; therefore, temperature is an important factor in phage function. This study indicated that an increase in temperature is positively correlated with *Salmonella* reduction; a 10-unit increase in temperature results in a 15% increase in *Salmonella* reduction. The positive relationship between temperature and *Salmonella* reduction observed in this study was similar to recent findings from China, Thailand, and Chile [30–32]. According to the present study, increasing the temperature from 4 °C to 25 °C can lead to rise in the rate of *Salmonella* reduction by about 32%. Guo et al. (2021) found that with an increase in temperature from 4 °C to 25 °C, bacterial reduction rises by approximately 30% [33]. In this regard, Greer (1988) argued that possibly low temperatures are responsible for the lysing of a very small proportion of the infected cells and blocking the lytic development of phage [34].

4.2. Time

It was shown that the potency of bacteriophage is significantly improved following the early times of inoculation [35]. According to Table 2, the negative effect of time is negligible so that after 24 h, bacterial reduction decreases by around 2.5%. These results are also supported by El-Dougdoug et al., Kim et al. and Li et al. [36–38]. In one study [39], the negative effect of time was considerably higher on the order of 50% per h. Jassim et al. (2012) indicated that longer exposure time do not remarkably increase the number of attacking phages [35]. However, it can result from various external factors anddisparate test conditions.

4.3. Phage dose

Phage dose has a notable effect on the reduction of *Salmonella;* a 5-unit increase in the phage dose entirely reduced the population of bacteria. The efficacy of phage therapy highly depends on phage dosage [40] and the positive relationship between phage dose and *Salmonella* reduction observed in other studies that have been done in Asia, Europe, and North America with similar trends [36,41,42]. Zhang et al. (2021) in their study found that a one-unit increase in phage dose rises *Salmonella* reduction by about 18%, which is similar to current results [43]. This may be the consequence of a positive correlation between the phage dose and the phage recovery [44].

4.4. Bacterial dose

The present study also showed that increasing the bacterial dose can have a positive impact on phage function. After increasing the bacterial dose by two units, there was a 15% reduction in *Salmonella* counts. Some researchers report that a 2-unit increase in the bacterial dose increases *Salmonella* reduction by around 12% [45]. In another study, Greer showed that phage load had no effect until the initial bacterial density reached a certain level, and at higher levels of bacterial contamination, fewer phages induced a significant increase [46].

4.5. Type of salmonella

Concerning the different serotypes, this study determined that phage biocontrol is more effective for *S. Enteritidis* than *S. Typhimurium.* After phage treatment of poultry meat contaminated with *S. Enteritidis* and other types, *Salmonella* was reduced by over 45% and 35%, respectively, compared to *S. Typhimurium.* These reductions are broadly consistent with those previously recorded [37,38,47] that have been performed on poultry skin, egg yolk, egg white, and liquid egg. Moreover, Vaz et al. found that *S. Typhimurium* had the highest phage resistance, and *S. Heldiberg* and *S. Enteritidis* was the most sensitive [40]. According to these studies [48,49], more decline in *S. Enteritidis* compared to other species could be due to the higher prevalence of the poultry products. However, if this study could be conducted under different conditions and more factors affecting the phage potency, the results might be equal for both strains of *Salmonella.*

4.6. Method of inoculation

Furthermore, one of the objectives of this study was to compare the effectiveness of dip treatment and surface bacteriophage application. Dip treatment with lytic bacteriophages is15% more effective against *Salmonella* thansurface treatment. Sukumaran et al. found that the bacterial reduction obtained after immersion treatment was 13% higher than surface treatment [8]. In the study by Jassim et al. at 103 PFU/mL, the sprayed mode showed a lower log reduction and was less effective than the immersion mode in reducing *Salmonella* overall. The immersion method may indeed provide better reductions in *Salmonella*. However, this effect declines rapidly with prolonged use because of contaminating the solution with various other foodborne bacteria. In addition, the optimal phage concentration for the spraying mode was higher than the immersion mode. The lower effectiveness could be due to the inability

of the spray mode to cover samples with phage as efficiently as the immersion method [35]. It could also be due to the different phage doses used in the various studies.

4.7. Phage type

The current study also investigated the impact of phage type on the reduction rate of *Salmonella* in poultry meat, so employing wild type phages results in a two-fold reduction in the Salmonella population. Multiple passages of the commercial phages are responsible for reducing their efficiency compared to the wild-type. Vaz et al. (2020) proposed that long-term phage-therapy can lead to the evolution of host bacterial resistance, which negatively affects the efficacy of the treatment. The development of bacterial resistance is mainly due to the modification of the phage receptors on their surface, which prevents adsorption [40]. More utilization of commercial phages can be responsible for greater bacterial resistance.

Overall, this is the first study to globally examine the effect of the factors on phage performance through a systematic review and modeling. Nevertheless, there are obvious ways to survey the parameters, such as studying the time interval between the phage inoculation and bacterial reduction, which is not mentioned in all studies. A broader approach is currently being developed with the aim expanding the pool of data and enhancing the value of applicability and predictability.

5. Conclusion

It has been shown that the effectiveness of phage applications depends on several factors. The wild-type phages have the greatest potency and doubles phage performance. In addition, increasing the phage dose and temperature increases its effectiveness. Furthermore, *S. Enteritidis* has the lowest phage resistance; an immersive type of inoculation is more efficient than surface inoculation (e.g., spray). Ultimately, immersion treatment of wild-type phage with higher doses of bacteria and phage at elevated temperatures can be more effective, making phage a helpful tool for reducing *Salmonella* in poultry meat. However, further studies on the role of other factors, including MOI (multiplicity of infection), the exact number of phages, packaging systems, and combination use of phage with other antimicrobial agents, are advised.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Authorship contribution

Mohsen Shahdadi, Maryam Safarirad, Enayat Berizi, Seyed Mohammad Mazloomi, Saeid Hosseinzadeh, and Morteza Zare: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Zahra Derakhshan and Saeed Rajabi: conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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