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Application of Bacteriophages for Biocontrol of Extensively Drug Resistant *Salmonella* Serovars Isolated from Poultry Farms

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Abstract

Salmonella is one of the most common foodborne pathogens causing diseases in humans and animals. Increased resistance to antibiotics necessitates the need for an alternative control strategy. This study aimed to screen, isolate and evaluate the bacteriophage characteristics for biocontrol of pathogenic *Salmonella* serovars. Twelve *Salmonella* isolates, including different *Salmonella enterica* serovars, were obtained from different sources of poultry farms. All isolates were screened for antibiotic sensitivity and showed multiple antibiotic resistance. Two lytic bacteriophages, vB_SalSph_WW1, and vB_SalM_WW2, were isolated from the sewage and characterized against *Salmonella enterica* serovar Enteritidis. Morphological analysis by transmission electron microscopy revealed that the vB_SalSph_WW1 phage belonged to the family Siphoviridae while the vB_SalM_WW2 phage belonged to the family Myoviridae. Both phages showed a broad host range within the *Salmonella* genus. Phages vB_SalSph_WW1 and vB_SalM_WW2 had a lytic effect on 3 (25%) and 4 (33.3%) of the 12 *Salmonella* isolates, respectively. The lytic cycle of each phage was determined by a one-step growth curve and both phages had the same short latent period (15 min). WW1 phage gave a burst size of 90 PFU/infected cell, while the vB_SalM_WW2 phage gave a higher burst size of 150 PFU/infected cell. The stability test revealed that vB_SalSph_WW1 and vB_SalM_WW2 phages were stable at pH 4–9 and pH 4–10, respectively. Both Phages exhibited high degrees of thermal tolerance with active titer as high as 42°C. However, they lost their stability and the titers declined when heated at 50°C for 30 min. This study revealed that vB_SalSph_WW1 and vB_SalM_WW2 phages have the potency to be used as an alternative strategy to control the infection of *Salmonella* in poultry farms and to prevent transmission of *Salmonella* infection to humans and spread of the pathogen into environment.

KEYWORDS
Salmonella, Antimicrobial susceptibility, Bacteriophage, Poultry.

INTRODUCTION

Salmonella is a Gram-negative bacillus and is a genus belonging to the Enterobacteriaceae family. It is the most common food borne pathogen recognized globally and as the second largest zoonotic pathogen reported in the European Union (EFSA, 2018). Over 2,600 serotypes of *Salmonella* have been identified up to date (Sun *et al*., 2022). This pathogen can be found ubiquitously in almost all human food chains. World poultry products are source of 20% of *Salmonella* contamination that can persist in the human and animal environments for a long time through formation of biofilm (Vestby *et al*., 2009). Serovars of *Salmonella* Enteritidis and Typhimurium have been isolated in most of outbreaks of salmonellosis resulted from consumption of poultry products (Vose *et al*., 2011). *S. enterica*, serovar Enteritidis is considered as the most common cause of salmonellosis worldwide (Bao *et al*., 2015). The prevention of salmonellosis is a complicated process due to its wide mode of transmission and complex epidemiology. Recently, the control of salmonellosis with antibiotics is quite non-significant due to emergence and uprising of multi-drug resistance strains of *Salmonella* as a result of over and misuse of antibiotics in animal and human settings (CDC, 2017). Moreover, chemical preservatives are often used in advanced stages of the food industry. However, these preservatives may be harmful to humans and cause deterioration of food quality (Sobiecki, 2017). This demands alternative intervention strategy to control microbial infection. Bacteriophages have been identified as the most prospective alternative method for biocontrol of infections and contaminations caused by drug resistant pathogens (Sonalika *et al*., 2020). Bacteriophages are ubiquitous in the environment, specific to the host and safe not harmful to beneficial bacteria (Magnone *et al*., 2013). Bacteriophages have been identified as effective biocontrol agent in many foods, such as meat (Yeh *et al*., 2017). Some preparations of bacteriophages to control *Salmonella* on chicken farms have been approved for use and put on the markets in many countries such as SalmoFREE (Clavijo *et al*., 2019). In this study, two novel lytic phages using *Salmonella* Enteritidis as a host were isolated from sewage sample in Sharkia, Egypt. The host ranges and biological properties of vB_SalM_WW1 and vB_SalM_WW2 phages such as lytic ability, one-step growth curve, thermal and pH stability were in vitro investigated. The major aim of this research was to enhance phage diversity

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and to evaluate the potential of newly isolated phages to control *Salmonella* in poultry farms and poultry food products.

MATERIALS AND METHODS

Salmonella isolates

Twelve *Salmonella* isolates were kindly provided from Microbiology and Veterinary Public Health Departments, Zagazig University, Zagazig, Egypt that were used for phage isolation, propagation and host range characterization in this study. Six isolates were obtained from Poultry dropping. Moreover, four from farm litter and two isolates from farm water at Sharkia Governorate, Egypt. All isolates were biochemically and molecularly characterized using PCR targeting the 16sRNA and inv A gene (Li *et al*., 2012; Mthembu *et al*., 2019) and kept in Brain Heart Infusion broth (Oxoid, USA) containing 20% glycerol at −20 °C for subsequent use.

Antimicrobial Susceptibility Testing

The susceptibility of *Salmonella* isolates to 24 antimicrobial agents was determined using disk diffusion method according to the standards procedures for disc diffusion method recommended by the Clinical Laboratory Standards Institute (CLSI, 2019). A panel of 24 standard antimicrobial discs (Oxoid, Cambridge, UK) within different 14 antimicrobial categories including penicillins [ampicillin (AM), and amoxicillin (AX)], penicillin combinations [Sublactam –cefoperazone(CES)], cephalosporines [Cefuroxime (CXM), Cephalexin (CL), Ceftazidime (CAZ), and cephradine (CE)], carbapenemes [Imipenem (IPM)], monobactams [azetronam (ATM)], aminoglycosides [tobramycin (TOB), and amikacin (AK)], macrolides [erythromycin (E),and spiramycin (SP)], quinolones [Difloxacin (Dif), levofloxacin (LE), and ciprofloxacin (CIP)], sulfonamides [sulfamethoxazole-trimethoprim (SXT)], amphenicols, and thiamphenicol derivative [chloramphenicol (C), and Floricol (FFA)], polypeptides [colistin (CT)], lincosamides [clindamycin (DA)] and tetracyclines [doxycycline (DO),and tetracycline(TE)], and Aminocyclitol[(Spectinomycin (SH)] The multiple antimicrobial resistance indices were calculated as previously reported (Tambekar *et al*., 2006). Pan drug-resistance (resistance to all antimicrobial agents), extensive drug-resistance (resistance to all classes of antimicrobial agents except 2 or fewer), and multidrug-resistance (resistance to three or more classes of antimicrobial agents) were determined as reported elsewhere (Magiorakos *et al*., 2012).

Isolation of Salmonella phages

The phages were isolated from wastewater samples aseptically collected from three different wastewater sources in Sharkia Governorate, Egypt using enrichment method (Cerveny *et al*., 2002) with some modifications. The filtrate from each sample was incubated with log phase *Salmonella* cultures followed by centrifugation and filtration. The supernatant was then checked for the presence of lytic phages by spot assay (Chang *et al*., 2005) using double layer agar plate method (Sambrock and Russell, 2001).

Propagation and purification of Salmonella phages

The phage filtrate was further purified by repeated plaque assays at least three times to obtain single uniform plaques, then purified by dextran sulfate-polyethylene glycol system (Watanabe *et al*.,1970) and kept at 4°C for subsequent use.

Determination of titer and multiplicity of infection of Salmonella phages

The purified phages were serially diluted and plaque assay was done in triplicate for each dilution by double layer method. The plates had 30-300 plaques from each dilution was counted and the titer was determined as plaque forming unit per milliliter (PFU/ml) (Anderson *et al*., 2011). The multiplicity of infection (MOI) was calculated as the proportion of infectious phage particles (PFU/ml) to number of host cells (CFU/ml) in a sample (Lu *et al*., 2003).

Host range characterization of Salmonella phages

The lytic spectrum of each phage lysate on the log phase culture (108 CFU/ml) of each *Salmonella* isolate was determined by spot assay (Chang *et al*., 2005) using double layer method as previously described. The zone of lysis was categorized as clear (+++), turbid (+) or no lysis (-) The results were confirmed by plaque assay using double layer method in triplicates.

Morphological characterization of Salmonella phages

On 200 mesh copper grids with carbon-coat films, a drop of each purified phage was applied. In the following step, the grids were negatively stained with 2% Na-phosphotungstate (pH 7.6) and seen under a transmission electron microscope (TEM, Hitachi H600A) at 100 kV. Following recommendations made by Kropinski *et al*. (2009) as well as Adriaenssens and Brister (2017) the phages were given names.

Effect of Tempreature and pH on survival of isolated phages

The effect of temperature and PH on the viability of phages was studied by the method described by Shang *et al*. (2021). In sterile test tube, 2 ml of the filtered phage suspension was incubated in the following temperatures: 4, 25, 37, 42, 50°C, for 30 min then cooled by tap water and phages survival was determined by plaque assay technique. The effect of pH degree on the survival and stability of phages was determined using nutrient broth of various pH degrees (4-12). Phages were diluted in test tubes containing 9 ml of liquid medium adjusted to various pH degrees using 0.1 N HCl and/or 0.1 N NaOH. After incubation of the mixtures at 4°C overnight, the residual phage activity was determined by plaque assay technique as previously described.

RESULTS

Antimicrobial Susceptibility Testing

All the *Salmonella* isolates under investigation demonstrated substantial levels of resistance to the various antibiotic classes under investigation, according to the antiprogram (Table. 1). All isolates from dropping were MDR with a high MAR index (0.45-0.79). Litter isolates were XDR with MAR indices ranging from (0.79-0.87). On the other hand, there were two water isolates: one MDR with a MAR value of 0.458 and the other an XDR with a MAR index of 0.875.

Isolation and characterization of Salmonella lytic phages

Two lytic phages (vB_SalSph_WW1 & vB_SalM_WW2) were isolated based on spot and plaque assays vB_SalSph_WW1 produced small single clear plaques (< 1 mm), while vB_SalM_WW2

produced medium size single clear plaques (1–3 mm). Based on TEM micrograph, the isolated vB_SalSph_WW1 phage belonged to family Siphoviridae and vB_SalM_WW2 phage belonged to Myoviridae, vB_SalM_WW2 phage was morphologically like T4 phages with icosahedral head (51.61x66.56nm) and long contractile tail (109.03 nm) with clear tail fibers (Fig. 1A). Meanwhile, vB_SalSph_WW1 phage also had icosahedral head (65.07x66.56 nm) and long non contractile tail 199.11 nm (Fig. 1B).

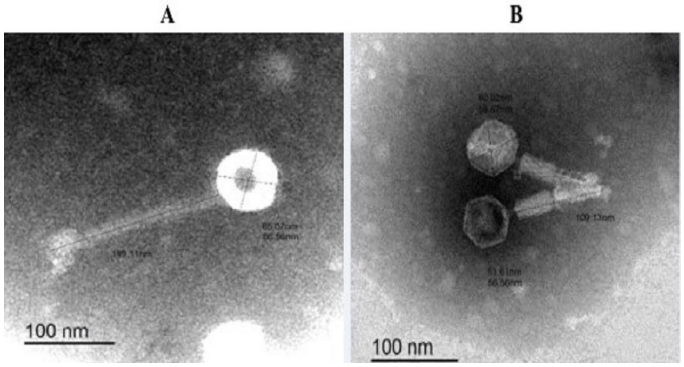

Fig.1. The morphology of *Salmonella* phages under transmission electron microscope negatively stained with 2% Na-phosphotungstate. A: vB_SalSph_WW1, B: vB_SalM_WW2. The scale bar represents 200 nm.

Phage titer and host range characterization

Table. 2 shows the host range for each phage that was determined against 12 *Salmonella* isolates of different serotypes by spot assay and confirmed by plaque assay yielding only three susceptible isolates to phages lysis, and single clear plaques were produced. These isolates were resistant to most of tested antimicrobials.

Single-step growth curve

The growth curve showed that (vB_SalSph_WW1 & vB_SalM_WW2) phages had a one round cycle of infection that took ~60 min (Fig. 2). vB_SalSph_WW1 phage had a latent period of ~15 min, rise period~45 min and burst size of 90 PFU/infected cell. Meanwhile, vB_SalM_WW2 had a latent period of ~15 min, rise period ~45 min and burst size of 150 PFU/infected cell.

pH and Thermal tolerance of the isolated phages

The isolated vB_SalSph_WW1 & vB_SalM_WW2 bacteriophages were very stable showing pH resistance ranging from

Table 1. Antibiogram patterns of twelve *Salmonella* isolates included in the current study:

Salmonella serovar	Source	Resistance profile*	MAR Index[c]	Resistance to antimicrobial (n=14)	
S. Enteritidis	Poultry dropping	AX, CL, CAZ, CE, E, SP, CIP, LE, C, DA, SH	0.46	7	MDR[a]
S. Vejle	Poultry dropping	AX, AM, CXM, CL, CE, ATM, E, SP, DIF, SXT, C, FFA, SH	0.54	8	MDR[a]
S. Kauka	Poultry dropping	AX, CES, CL, CE, TOB, E, SP, DIF, C, FFA, CT, DA	0.5	9	MDR[a]
S. Typhimurium	Poultry dropping	AX, AM, CES, CXM, CL, CAZ, CE, ATM, TOB, E, SP, DIF, FFA, CT	0.58	9	MDR[a]
*S.*Typhimurium	Poultry dropping	AX, CES, CXM, CL, CAZ, CE, ATM, TOB, AK, E, SP, DIF, SXT, C, FFA, DA, SH	0.71	11	MDR[a]
S. Kauka	Poultry dropping	AX, AM, CES, CXM, CL, CAZ, CE, ATM, E, SP, CIP, DIF, SXT, C, FFA, CT, DO, TE, SH	0.79	11	MDR[a]
S. Enteritidis	Litter	AX, AM, CES, CXM, CL, CAZ, CE, ATM, TOB, E, SP, CIP, DIF, SXT, C, FFA, CT, TE, SH	0.79	12	XDR[b]
S. Kauka	Litter	AX, AM, CES, CXM, CL, CAZ, CE, ATM, TOB, AK, E, SP, DIF, SXT, C, FFA, CT, DO, TE, SH	0.83	12	XDR[b]
S. Typhimurium	Litter	AX, AM, CES, CXM, CL, CAZ, CE, IPM, ATM, TOB, AK, E, SP, SXT, C, FFA, CT, DO, TE, SH	0.83	12	XDR[b]
S. Sernftenberg	Litter	AX, AM, CES, CXM, CL, CAZ, CE, IPM, ATM, TOB, E, SP, DIF, SXT, FFA, CT, DO, TE, SH	0.79	13	XDR[b]
*S.*Typhimurium	Water	AX, AM, CES, CXM, CL, CAZ, CE, IPM, ATM, TOB, AK, E, SP, CIP, DIF, SXT, C, FFA, CT, TE, SH	0.88	13	XDR[b]
S. Stratford	Water	AX, CL, CAZ, CE, E, SP, CIP, LE, C, DA, SH	0.46	7	MDR[a]

*Antibiotics panel; ampicillin (AM), amoxicillin (AX), Sublactam –cefoperazone (CES), Cefuroxime (CXM), Cephalexin (CL), Ceftazidime (CAZ), cephradine (CE), Imipenem (IPM), azetronam (ATM), tobramycin (TOB), amikacin (AK), erythromycin (E) spiramycin (SP), Difloxacin (Dif), levofloxacin (LE), ciprofloxacin (CIP), sulfamethoxazole-trimethoprim (SXT), chloramphenicol (C), Floricol (FFA), colistin (CT), clindamycin (DA) and doxycycline (DO), tetracycline(TE), and Spectinomycin (SH).

[a]The isolates were resistant to ≥1 agent in ≥3 antimicrobial categories.

[b]The isolates were resistant to ≥1 agent in all except ≤2 antimicrobial categories.

[c]Multiple antibiotic resistance index (average MAR index = 0.55)

Table 2. Spot test for isolated phages.

Salmonella serovar	Phage 1 (vB_SalSph_WW1)	Phage 2(vB_SalM_WW2)
S. Enteritidis	+++	+++
S. Vejle	-	-
S. Kauka	-	+
S. Typhimurium	+	-
S. Typhimurium	-	-
S. Kauka	+	-
S. Enteritidis	+++	+++
S. Kauka	-	-
S. Typhimurium	+	-
S. Sernftenberg	-	+++
S. Typhimurium	+++	+++
S. Stratford	-	-

The isolates were susceptible to phage and produce clear plaques +++)); The isolates were susceptible but produced non clear plaques (+); No plaques were produced. (−).

4–10 after 2 h (Fig. 3). Recovered vB_SalSph_WW1 phage titers remained active throughout pH 4–9, while vB_SalM_WW2 phage titers remained active throughout pH 4–10. Both phages lost their virulence when applied to pH extremes greater than 10. Both Phages also exhibited a high degree of thermal tolerance with active titer as high as 40°C (Fig. 4) but when heated at 50°C for 30 min the titers declined.

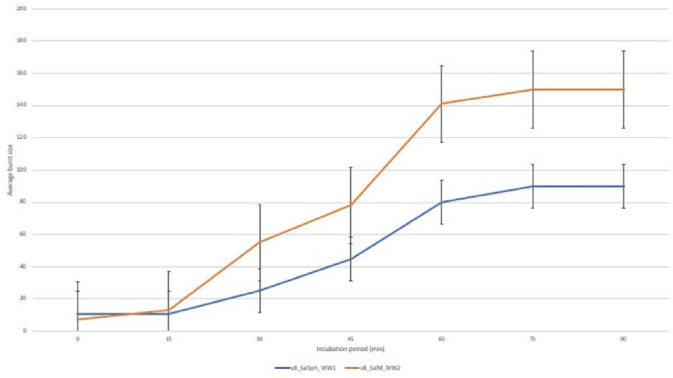

Fig. 2. Single-step growth curve of *Salmonella* phages at 37°C. The plaque forming units (PFUs) per infected cell at different times post infection were shown. The sample was taken every 15 min up to 90 min.

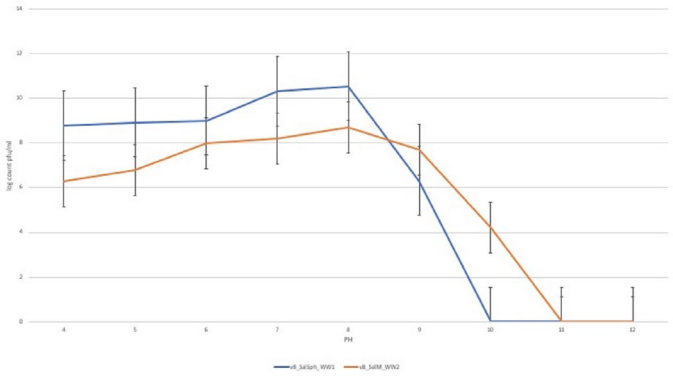

Fig. 3. The effect different pH on isolated *Salmonella* phages.

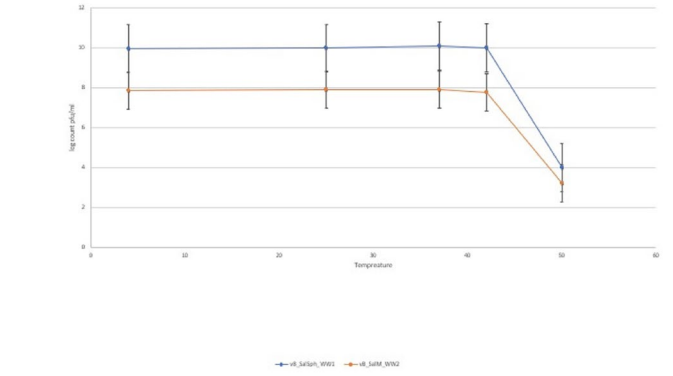

Fig. 4. The effect of different temperatures on isolated phages.

DISCUSSION

Salmonella is the most common enteric pathogen causing diseases in livestock and contamination of animal meat and derived products. Poultry and associated products are among the food sources recognized as the most common vehicles for human salmonellosis (Campos *et al*., 2019). Over and misuse of antimicrobials in the veterinary patterns are considered as a major factor in the emergence of *Salmonella* strains resistant to multiple drugs. That's why multidrug resistant isolates were commonly identified in animal sources rather than human cases (Briers *et al*., 2014). There is an urgent need for new applicable methods for control pathogenic bacteria resistant to antibiotics. Bacteriophages are the most potential alternative to antibiotics and can be utilized in all aspects of food processing and production chains to overcome antibiotic resistant pathogens (Moye *et al*., 2018, Caflisch *et al*., 2019, Gorski *et al*., 2020). The major limitation in utilization of phages as biocontrol agents for *Salmonella* is the narrow host range as most bacteriophages are usually specific. However, the two phages isolated in this study were with high lytic capacity, successfully infecting S. enteritidis and *S.* Typhimurium in addition to *S.* Sernftenberg in case of vB_SalM_WW2. This is similar to vB_SalP_TR2 phage and EcS1 phage affecting wide range of *Salmonella* serovars (Shang *et al*., 2021, Saad *et al*., 2018). Both isolated bacteriophages vB_SalSph_WW1 and vB_SalM_WW2 showing efficient lytic activity and wide host range were successfully isolated and characterized. Regarding to their lytic activity, the latent period for both phages is 15 min, which is equivalent to *Salmonella* phage vB_SenTO17 (10–20 min) (Kosznik-Kwasnicka *et al*., 2020) and is shorter than *Salmonella* phages vB_SPuM_SP116 (20min), vB_SenS_SE1 (40 min) (Bao *et al*., 2019) and SS3e phage (20 min) (Kim *et al*., 2012). The burst size of vB_SalSph_WW1 phage is 90 PFU/cell which is similar to SS3e (98 pfu/cell), while for vB_SalM_WW2 it is 150 PFU/ cell that is quietly equivalent to PSDA-2 phage 120 pfu/cell. The short latent period for the two phages and their high burst size can ensure their efficiency in eradication of host bacteria in a relatively short time (Sun *et al*., 2022). In addition, resistance to pH and heat is essential for phage application in biocontrol of pathogenic bacteria. Both phages were relatively stable between pH 4 - 10, which is compatible with a wide range of applications (Bao *et al*., 2019, Sun *et al*., 2022). For thermal stability, the two phages were relatively stable as the temperature is high as 42 ◦C and they were rapidly inactivated if the temperature increased more than 50 ◦C. The thermal stability was matching with previous studies on *Salmonella* phages as vB_SenS_SE1that yielded low phage titers at temperatures above 50°C (Lu *et al*., 2020).

CONCLUSION

It is concluded that the newly isolated *Salmonella* phages vB_SalSph_WW1 and vB_SalM_WW2 exhibit wide host ranges and superior anti-*Salmonella* activities with relative high acid and thermal tolerance. Both phages show great possibility as efficient alternative for biocontrol of *Salmonella* in the poultry industry and a food-processing environment. Moreover, as both phages are relatively similar in their lytic activity and stability which refer to the possibility for preparation of phage cocktail.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Adriaenssens, E., Brister, J.R., 2017. How to name and classify your phage: An informal guide. Viruses 9, 70.

Anderson, B., Rashid, M.H., Carter, C., Pasternack, G., Rajanna, C., Revazishvili, T., 2011. Enumeration of bacteriophage particles: Comparative analysis of the traditional plaque assay and real-time QPCR-and nanosight-based assays. Bacteriophage 1, 86–93.

Bao, H., Shahin, K., Zhang, Q., Zhang, H., Wang, Z., Zhou, Y., 2019. Morphologic and genomic characterization of a broad host range *Salmonella enterica* serovar Pullorum lytic phage vB_SPuM_SP116. Microb Pathog. 136, 103659.

Bao, H., Zhang, P., Zhang, H., Zhou, Y., Zhang, L., Wang, R., 2015. Biocontrol of *Salmonella* Enteritidis in Foods Using Bacteriophages. Viruses 7, 4836-4853.

Briers, Y., Walmagh, M., Van Puyenbroeck, V., Cornelissen, A., Cenens, W., Aertsen, A., Oliveira, H., Azeredo, J., Verween, G., Pirnay, J., Miller, S., Volckaert, G., Lavigne, R., 2014. Engineered endolysin-based "Artilysins" to combat multidrug-resistant Gram-negative pathogens. mBio. 5, e01379–14.

Caflisch, K.M., Suh., G.A., Patel R., 2019. Biological challenges of phage therapy and proposed solutions: a literature review. Expert Rev

Anti Infect Ther. 17, 1011–1041.
Campos, J., Mourão, J., Peixe, L., Antunes, P., 2019. Non-typhoidal *Salmonella* in the pig production chain: A comprehensive analysis of its impact on human health. Pathogens 8, 19.
CDC (Centers for Disease Control and Prevention), 2017. *Salmonella* Urbana Infections Linked to Imported Maradol Papayas. https://www.cdc.gov/*Salmonella*/urbana-09-17/index.html.
Cerveny, K.E., DePaola, A., Duckworth, D.H., Gulig, P.A., 2002. Phage therapy of local and systemic disease caused by Vibrio vulnificus in iron-dextran-treated mice. Infec. Imm. 70, 6251–6262.
Chang, H.C., Chen, C.R., Lin, J.W., Shen, G.H., Chang, K.M., Tseng, Y.H., 2005. Isolation and characterization of novel giant Stenotrophomonas maltophilia phage ϕSMA5. Appl. Envir. Microbiol. 71, 1387–1393.
Clavijo, V., Baquero, D., Hernandez, S., Farfan, J. C., Arias, J., Arevalo, A., 2019. Phage cocktail SalmoFREE(R) reduces *Salmonella* on a commercial broiler farm. Poult. Sci. 98, 5054–5063.
CLSI (Clinical and Laboratory Standards Institute), 2019. Performance standards for antimicrobial susceptibility testing (29th ed.). Wayne, PA: CLSI CLSI supplement M100.
EFSA (European Food Safety Authority), 2018. European Centre for Disease P, Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 16 (12).
Gorski, A., Miedzybrodzki, R., Wegrzyn, G., Jonczyk-Matysiak, E., Borysowski, J., Weber-Dabrowska, B., 2020. Phage therapy: Current status and perspectives. Med Res Rev. 40, 459–63.
Kim, S.H., Park, J.H., Lee, B. K., Kwon, H.J., Shin, J.H., Kim, J., 2012. Complete genome sequence of *Salmonella* bacteriophage SS3e. J Virol. 86, 10253–10254.
Kosznik-Kwasnicka, K., Grabowski, L., Grabski, M., Kaszubski, M., Gorniak, M., Jurczak-Kurek A., 2020. Bacteriophages vB_Sen-TO17 and vB_Sen-E22, Newly Isolated Viruses from Chicken Feces, Specific for Several *Salmonella enterica* Strains. Int. J. Mol. Sci. 21, 8821.
Kropinski, A. M., Prangishvili, D., Lavigne, R., 2009. Position paper: The creation of a rational scheme for the nomenclature of viruses of bacteria and archaea. Environmental Microbiology 11, 2775–2777.
Li, Q., Cheng, W., Zhang, D., Yu, T., Yin, Y., Ju, H., Ding, S., 2012. Rapid and sensitive strategy for *Salmonella* detection using an InvA gene-based electrochemical DNA sensor. Int. J. Electrochem. Sci. 7, 844–856.
Lu, M., Liu, H., Lu, H., Liu, R., Liu, X., 2020. Characterization and Genome Analysis of a Novel *Salmonella* Phage vB_SenS_SE1. Curr Microbiol. 77, 1308–13015.
Lu, Z., Breidt, F., Jr., Fleming, H., Altermann, E., Klaenhammer, T., 2003. Isolation and characterization of a Lactobacillus plantarum bacteriophage, ΦJL-1, from a cucumber fermentation. Int. J. F. Microbiol. 84, 225–235.
Magiorakos, A-P. ; Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Micro. Infect. 18, 268-281.
Magnone, J.P., Marek, P.J., Sulakvelidze, A., Senecal, A.G., 2013. Additive approach for inactivation of Escherichia coli O157:H7, *Salmonella*, and Shigella spp. on contaminated fresh fruits and vegetables using bacteriophage cocktail and produce wash. J. Food Prot. 76, 1336–41.
Moye, Z.D., Woolston, J., Sulakvelidze, A., 2018. Bacteriophage Applications for Food Production and Processing. Viruses 10, 205.
Mthembu, T.P., Zishiri, O.T., El Zowalaty, M.E., 2019. Detection and Molecular Identification of *Salmonella* Virulence Genes in Livestock Production Systems in South Africa. Path. 8, 124.
Saad, A.M., Askora, A., Soliman, A.M., Nariya, H., Kawasaki, T., Fujie, M., Shimamoto, T., Yamada, T., 2018. Full genome sequence of a polyvalent bacteriophage infecting strains of Shigella, *Salmonella*, and *Escherichia*. Arch Virol. 163, 3207-3210.
Sambrock, J.W., Russell, D., 2001. Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
Shang, Y., Sun Q., Chen, H., Wu, Q., Chen, M., Yang, S., Du, M., Zha F., Ye, Q., Zhang, J., 2021. Isolation and Characterization of a Novel *Salmonella* Phage vB_SalP_TR2.Front. Microbiol. 12, 664810.
Sobiecki, J. G., 2017. Vegetarianism and colorectal cancer risk in a low-selenium environment: effect modification by selenium status? A possible factor contributing to the null results in British vegetarians. Eur. J. Nutr. 56, 1819–32.
Sonalika, J., Srujana, A.S., Akhila, D.S., Juliet, M.R., Santhosh, K.S., 2020. Application of bacteriophages to control *Salmonella* Enteritidis in raw eggs. Iran. J. Vet. Res. 21, 221-225.
Sun, Z., Mandlaa, Wen, H., Ma, L., Chen, Z., 2022. Isolation, characterization and application of bacteriophage PSDA-2 against *Salmonella* Typhimurium in chilled mutton. PLoS. ONE. 17 (1).
Tambekar, D.H., Dhanorkar, D.V., Gulhane, S.R., Khandelwal, V.K., Dudhane, M. N., 2006. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. Afri. J. Biotech.,5, 1562–1565.
Vestby, L. K., Moretro, T., Langsrud, S., Heir, E., Nesse, L.L., 2009. Biofilm forming abilities of *Salmonella* are correlated with persistence in fish meal-and feed factories. BMC. Vet. Res. 5, 20.
Vose, D., Koupeev, T., Mintiens, K., 2011. A Quantitative microbiological risk assessment of *Salmonella* spp. in broiler (*Gallus gallus*) meat production. EFSA Supporting Publication. 8, 183E.
Watanabe, K., Takesue, S., Jin-Nai, K., Yoshikawa, T., 1970. Bacteriophage active against the lactic acid beverage-producing bacterium Lactobacillus casei. Appl. Envir. Micro. 20, 409–415.
Yeh, Y., Purushothaman, P., Gupta, N., Ragnone, M., Verma, S.C., de Mello A.S., 2017. Bacteriophage application on red meats and poultry: Effects on *Salmonella* population in final ground products. Meat Sci.127, 30–34.