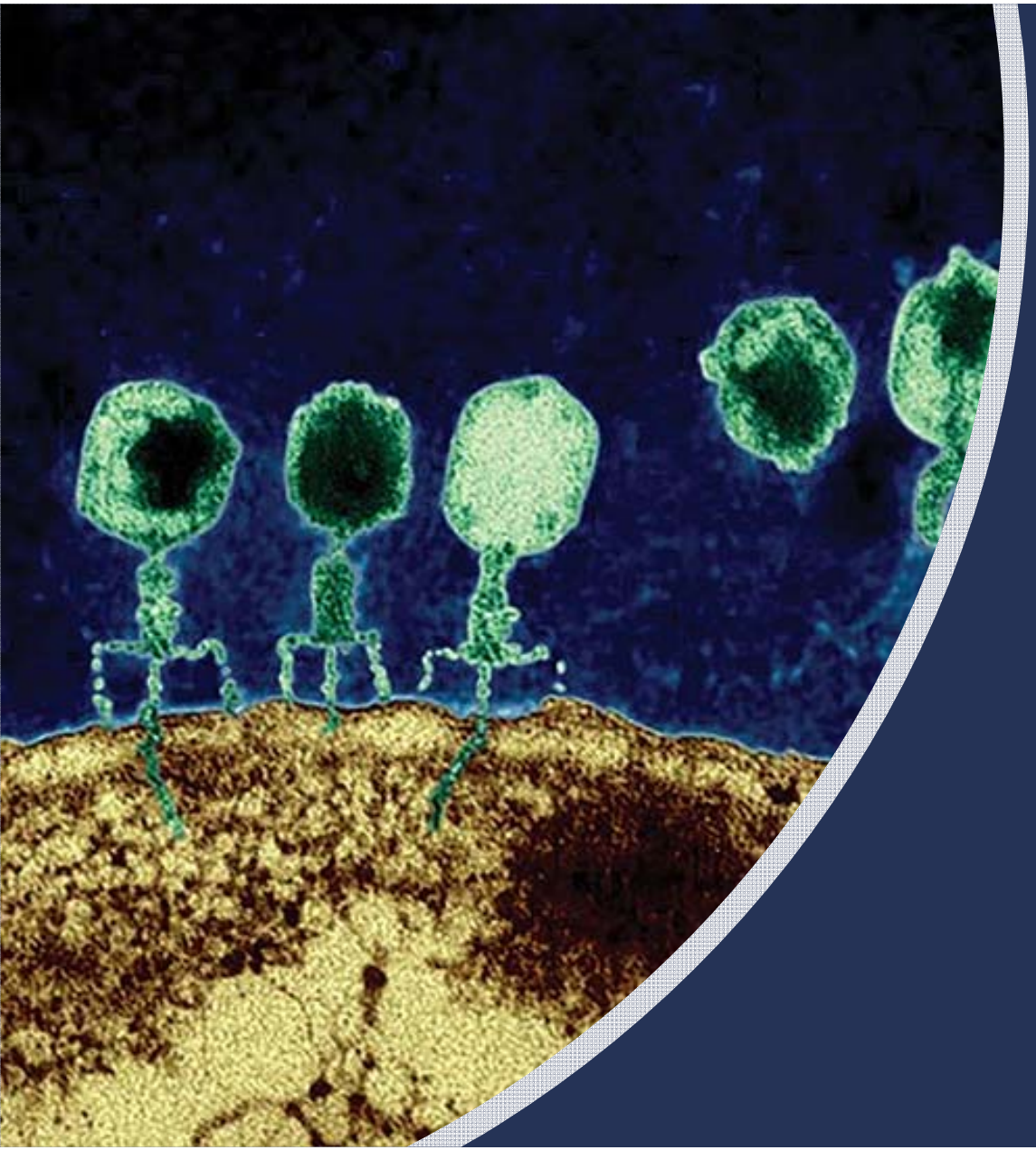

Bacteriophage therapy as a novel tool for foodborne pathogens

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Year 2 PhD Student

PI: Professor Paul KS Chan

18.12.2019

Overview

- Foodborne pathogens and current treatments
- Bacteriophage as an alternative antimicrobial food treatment
- Application of bacteriophage therapy
  - *Salmonella* spp.
- Problems with bacteriophage therapy and how to overcome them
- Conclusion

Foodborne pathogens

- Foodborne Disease Burden Epidemiology Reference Group (FERG) established by World Health Organisation (WHO)
- December 2015 report
  - 600 million foodborne infections in 2010
  - 400,000 deaths
  - USD$1500 cost per person
  - USD$75 billion per year

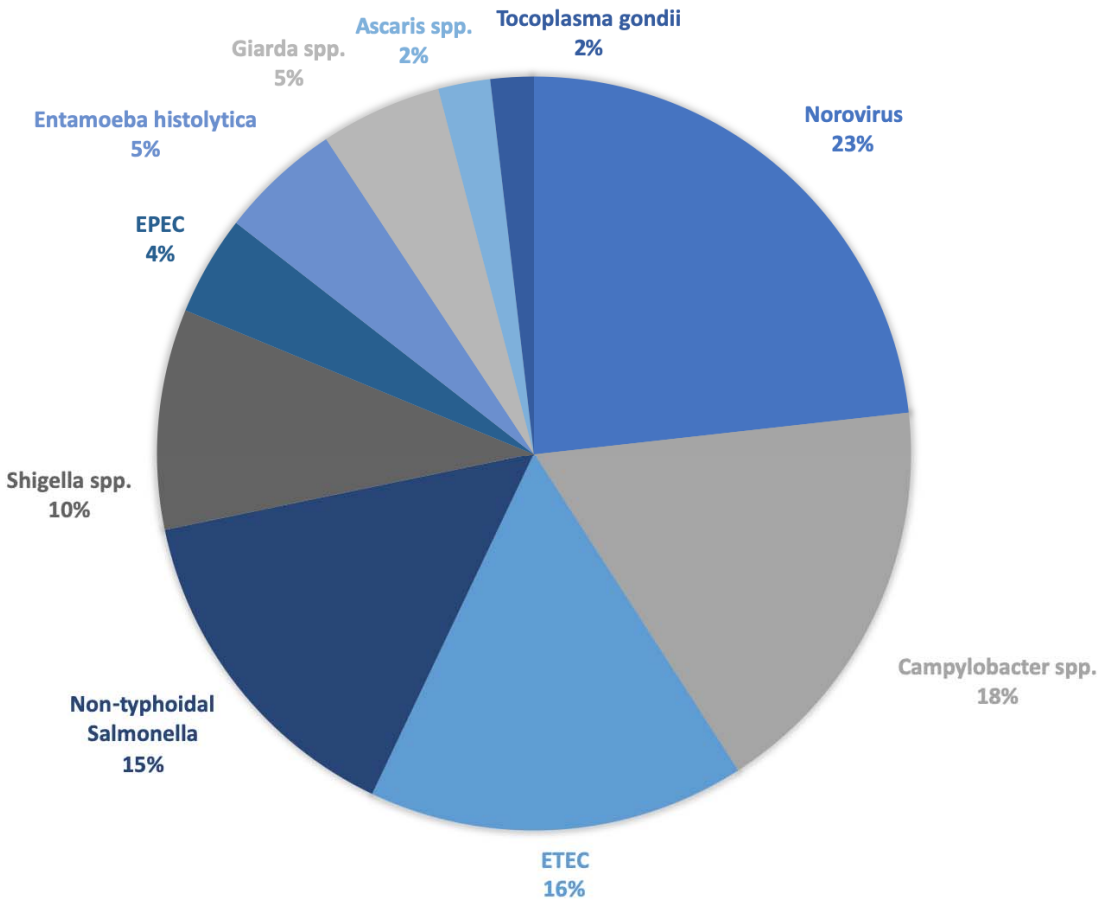


Figure 1. Top 10 global burden of foodborne diseases (December 2015)

(WHO, 2015)

Risk factors

- Immunocompromised
  - E.g. HIV, organ transplant
- Stress
- Pregnancy
- Age
  - <5 or >60

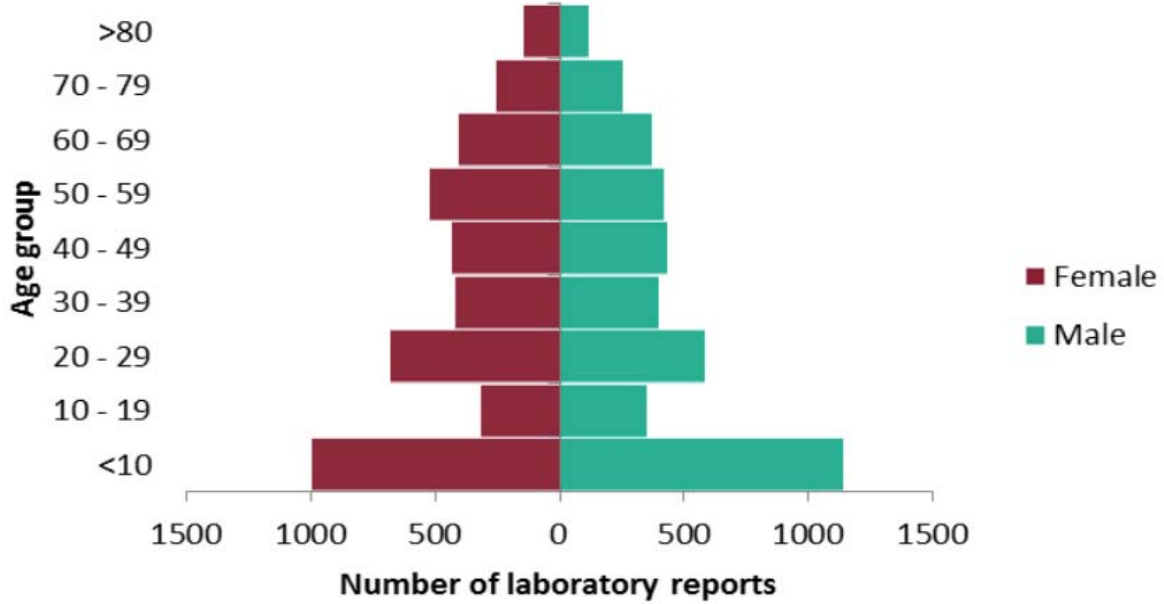


Figure 2. Age distribution of laboratory reports of all non-typhoidal *Salmonella* in England (2016)

Preventative, rather than reactive

(NHS, 2018)

Current food treatment approaches

- Heat pasteurization
  - Liquids, dairy
- High Pressure Processing (HPP)
  - Liquids, precooked meals, frozen food
- Freezing/Refrigeration
- Irradiation/UV light
- Chemical sanitizers: chlorine and peracetic acid
  - Fresh fruit, vegetables, ready-to-eat products

Problems with current food treatment processes

- Kills bacteria indiscriminately
- Not applicable to every food
- Decrease nutritional value
- Consumer belief

Ideal treatment

- Targeted antimicrobial approach
- Can be used alone/in combination
- Kills bacteria rapidly
- Safe
- Retains nutritional value of food

Bacteriophage therapy as an alternate food treatment

What are bacteriophages?

- Viruses
- Infect and replicate in bacterial cells
- RNA/DNA
- Highly specific to their host
- Most ubiquitous entities on the planet – 10^{31}

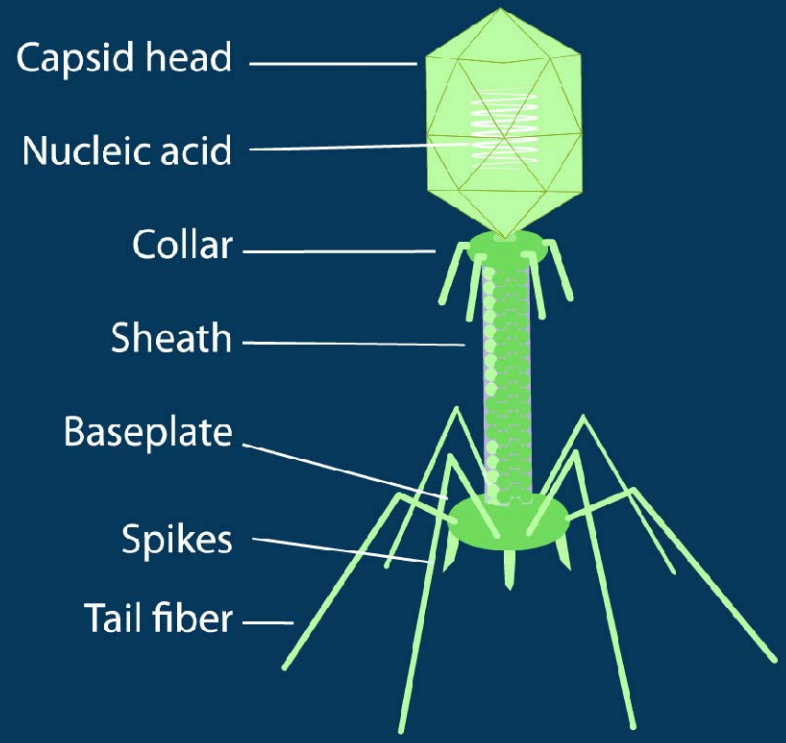
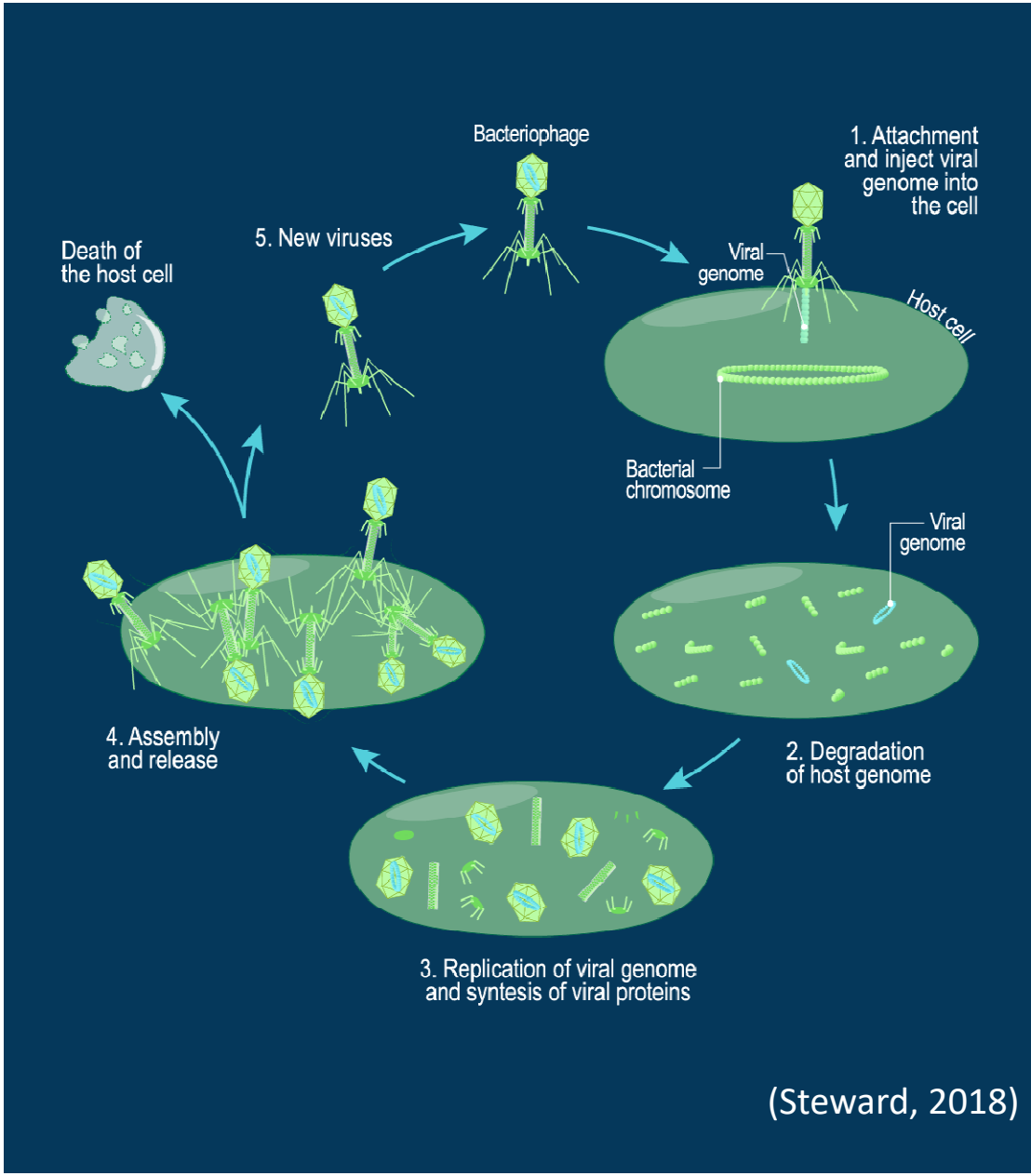


Figure 3. Generic structure of a tailed bacteriophage

(Steward, 2018)

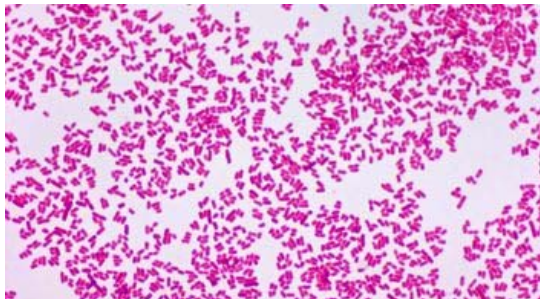
Bacteriophage lifecycles

- Lytic
  - Infect and rapidly kill host
- Lysogenic
  - Integrates into host genome
- Psuedolysogenic
  - Phage enters bacterial cells but does not integrate stably
- Chronic
  - Slowly shed from host via long time period without cell death



(Steward, 2018)

Salmonella enterica



- Gram-negative, rod-shaped
- stomach cramps, fever, nausea and diarrhea
- Death via dehydration or infection beyond gut
- 78 million cases
- 60 000 deaths

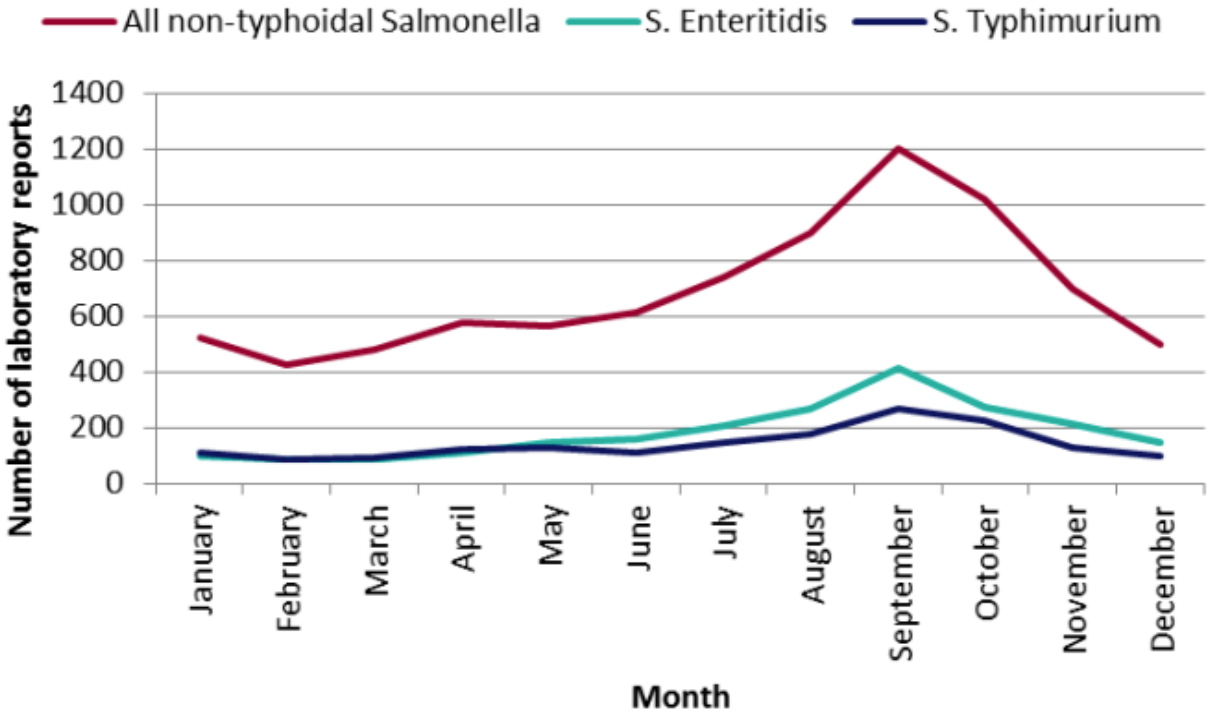
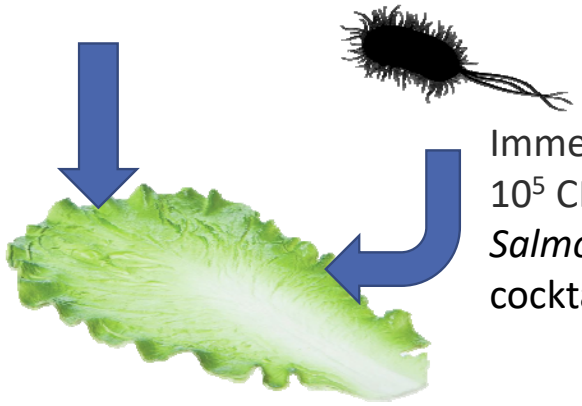


Figure 4. Seasonal incidence of all *Salmonella* in England in 2016

(NHS, 2018)

Effectiveness of SalmoFresh phage cocktail and chlorine wash treatment on *Salmonella*

600g of Lettuce
Washed with tap water (10 °C)
Excess water removed

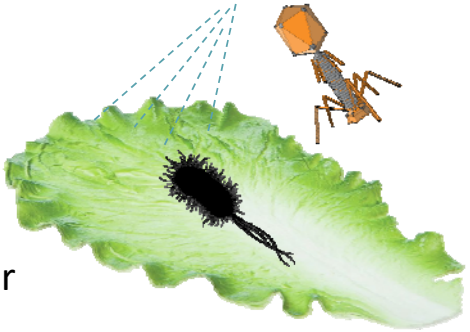


Immersed in 10^5 CFU/ml *Salmonella* cocktail

Air dry for 30mins

Washed with:

1) SalmoFresh™ Phage cocktail
2) Chlorine wash
3) SalmoFresh™ + Chlorine



1)
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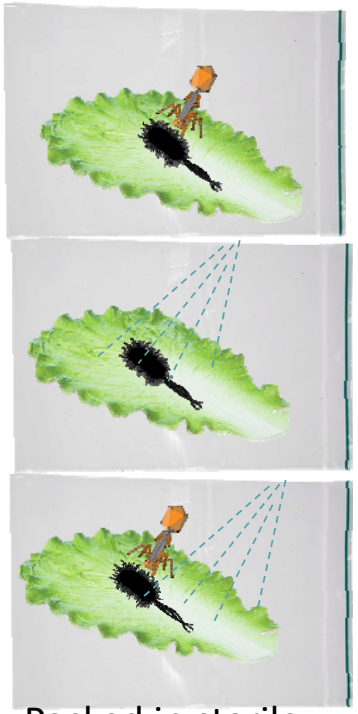


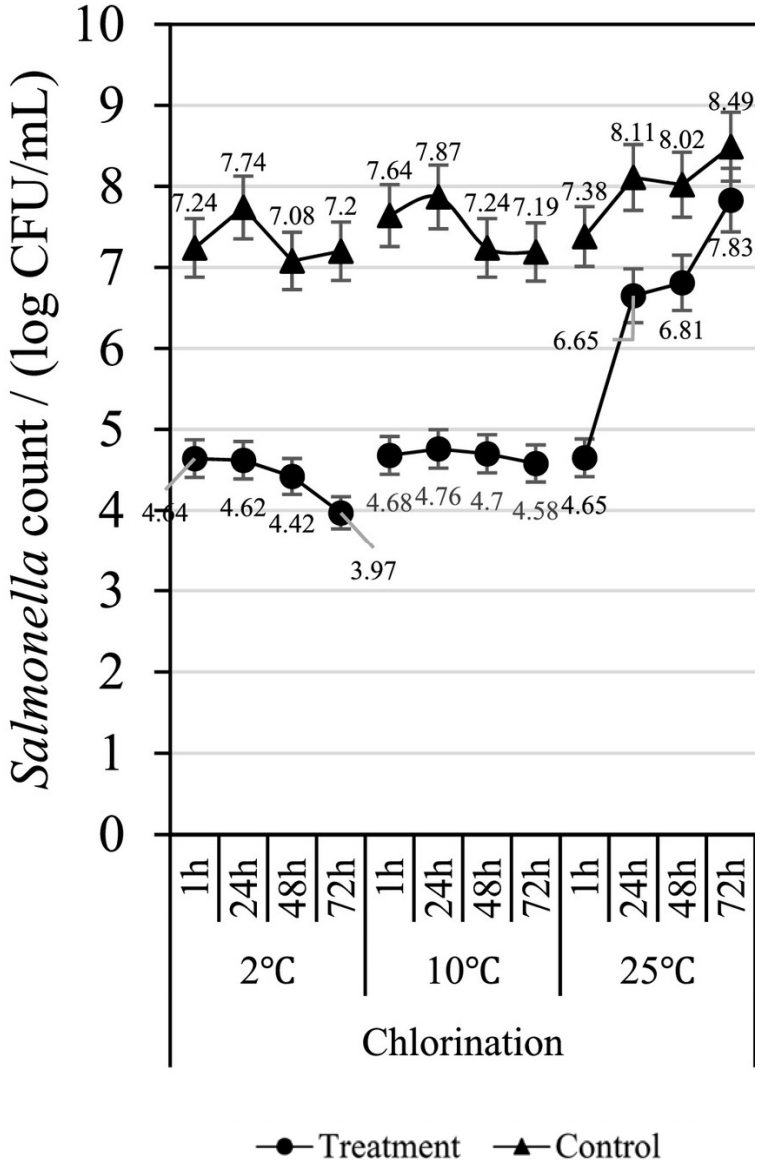
Packed in sterile bags

Temperature:
2, 10, 25 °C
Storage times:
1, 24, 48, 72 h.

Chlorinated water treatment of *Salmonella enterica*

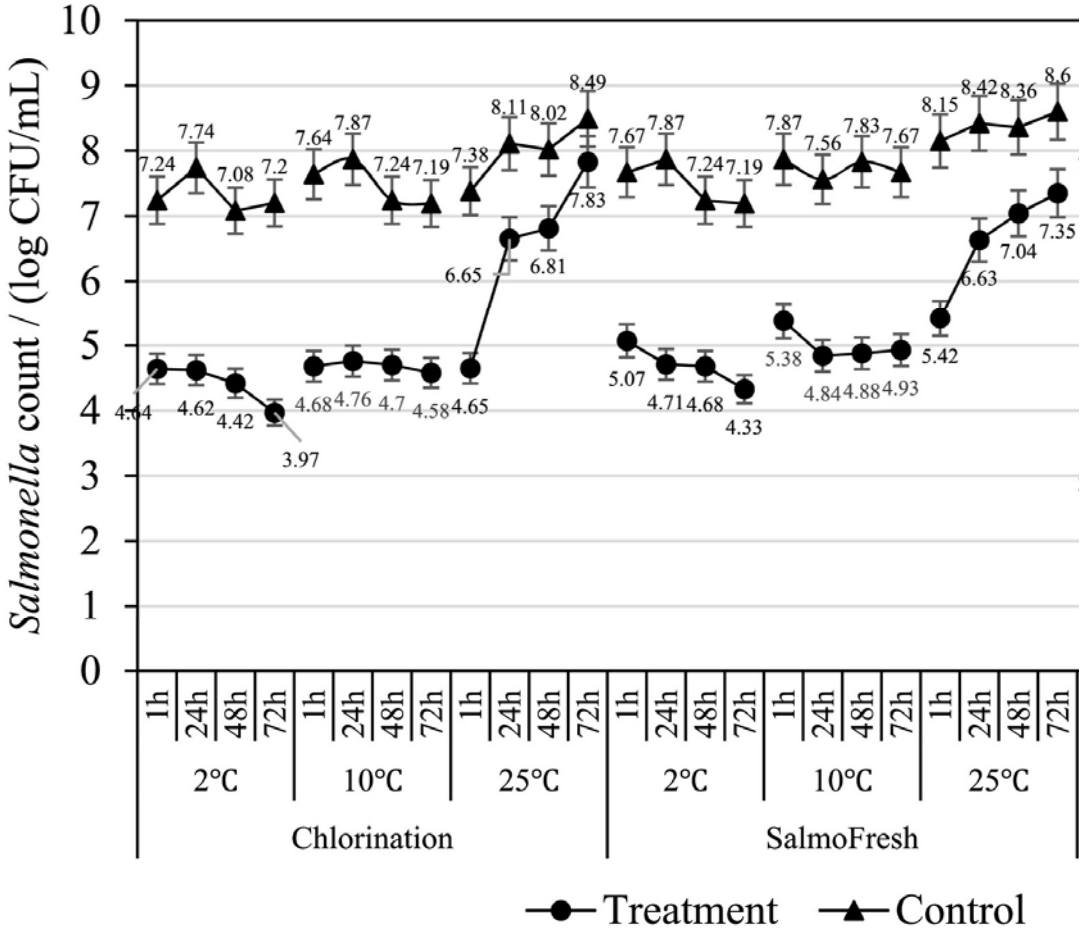
- Effectively lowers Salmonella count at 2°C and 10°C for 72h
- Chlorine is unable to maintain lowered *Salmonella* count at 25°C

- Lowers *Salmonella* load but unable to eradicate
- Unable to treat post-chlorination *Salmonella* proliferation



(Zhang et al., 2019)

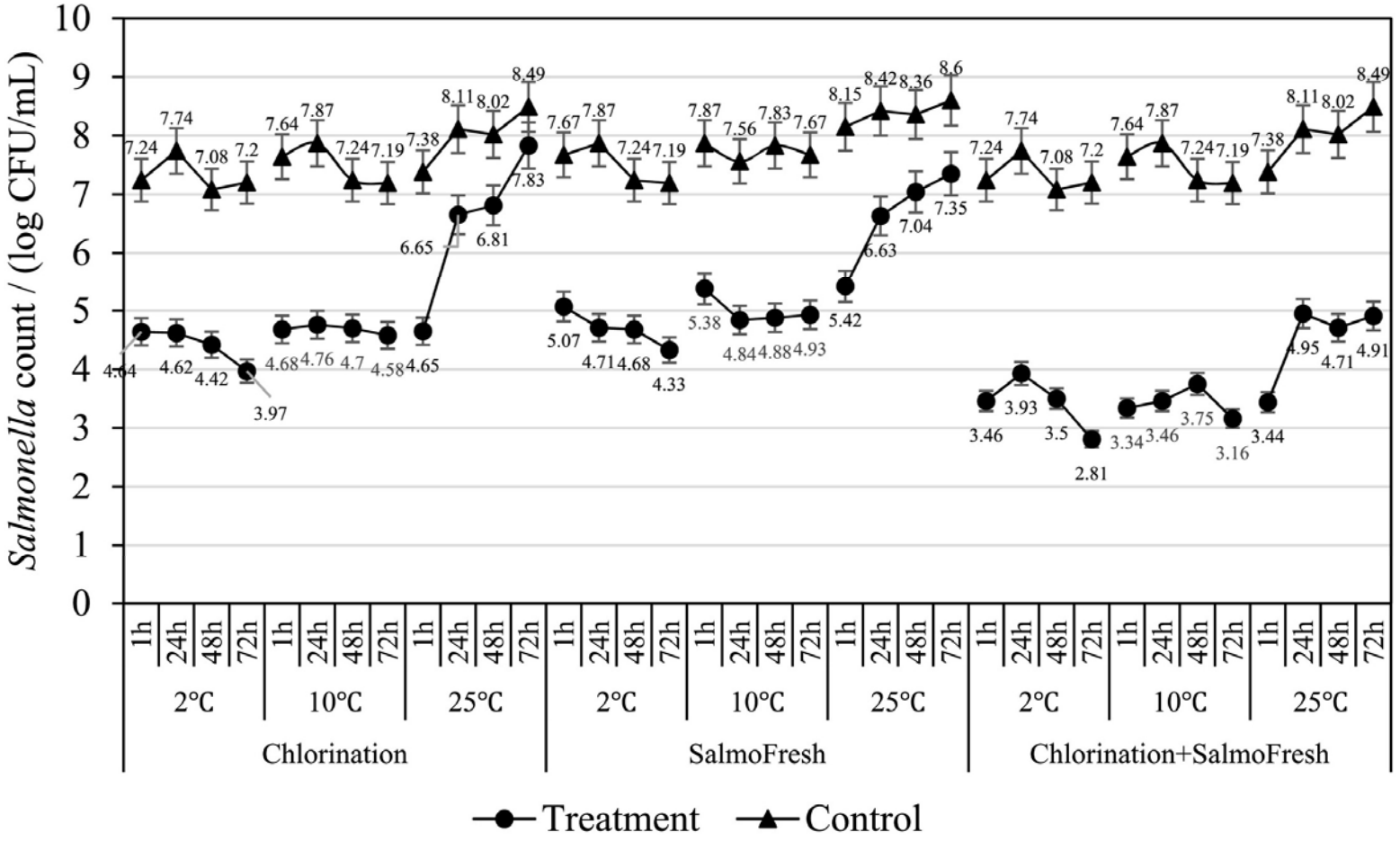
Chlorinated water and SalmoFresh phage Treatment of *Salmonella enterica* on fresh-cut lettuce



- SalmoFresh lowers *Salmonella* count at 2°C and 10°C for 72h
- Increased *Salmonella* count at 25°C
- Comparable to chlorine treatment

(Zhang et al., 2019)

Chlorinated water and SalmoFresh phage Treatment of *Salmonella enterica* on fresh-cut lettuce



(Zhang et al., 2019)

Bacteriophage therapy: an alternate food treatment

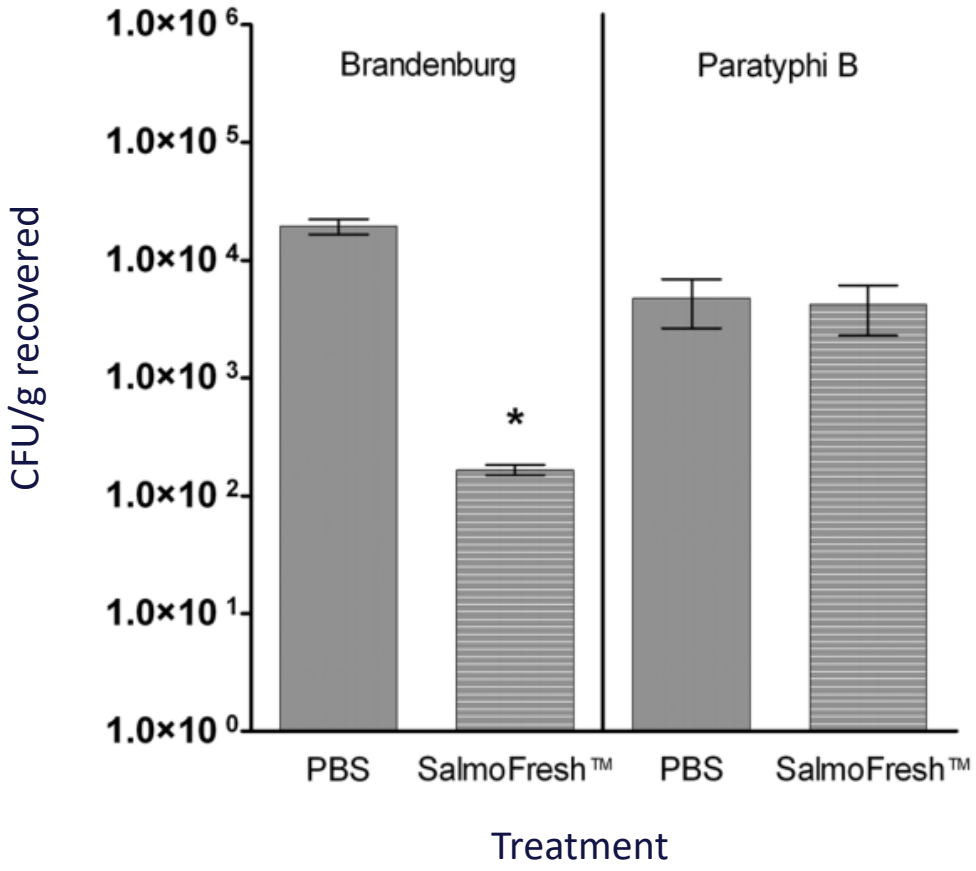
- Targeted antimicrobial approach
- Can be used alone/in combination
- Kills bacteria rapidly
- Safe
- Retains nutritional value of food

Problems with phage therapy

- Resistance
- High specificity
- Refrigeration/storage
- Higher cost

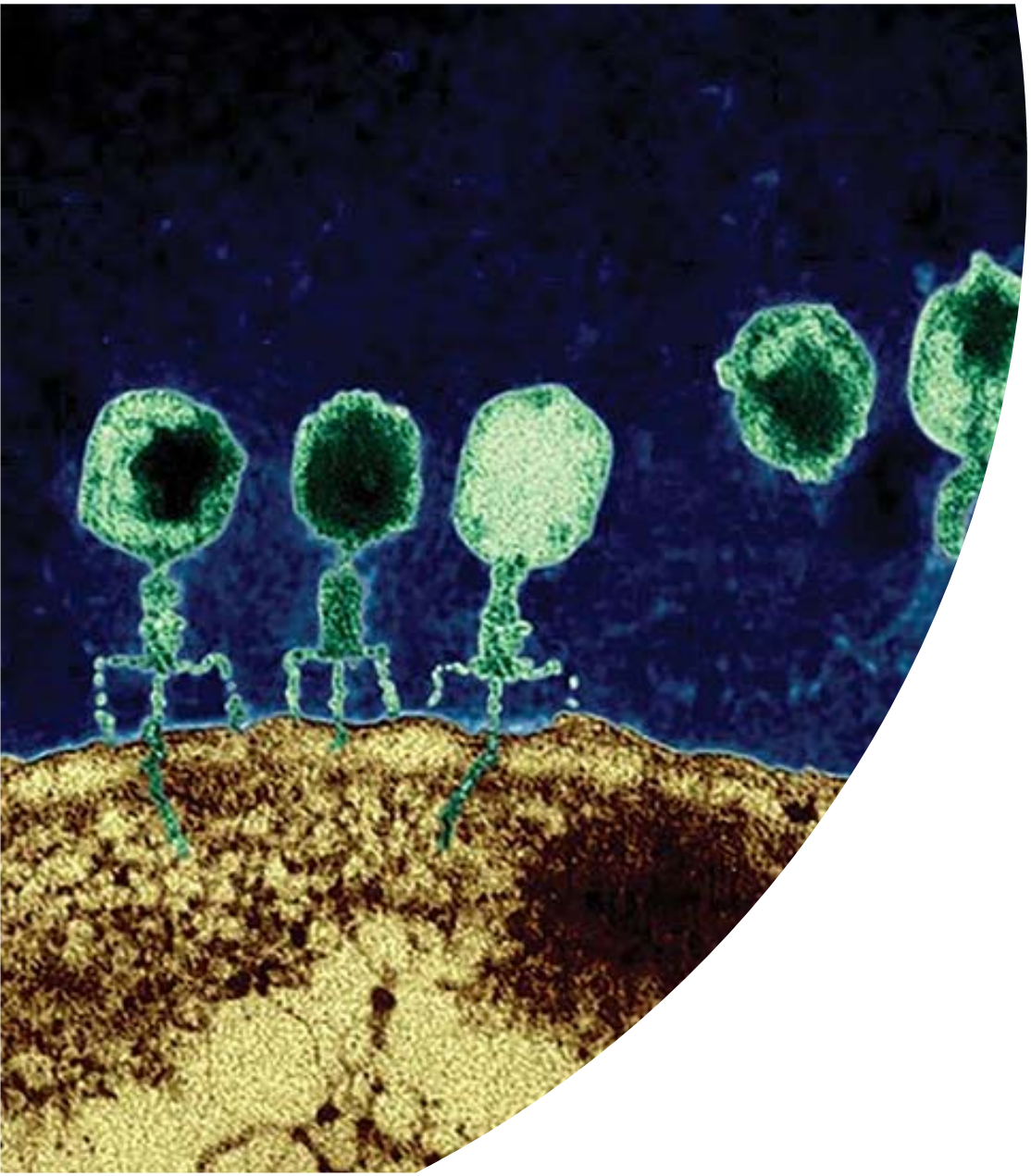
Updating phage strains to combat bacterial resistance

- Phage cocktails contained SPT-1, STML-198, SSE-121, SBA-1781
- SalmoFresh:
  SKML-39 and STML-13-1
- SalmoLyse:
  SEML-239-1 and SNN-387



Conclusion

- Bacteriophage as a safe alternative
- Commercial phage products
  - FDA approval, GRAS status
- Various applications in food production
- Prevent pathogens from reaching high risk consumers



Thank you.
Questions?

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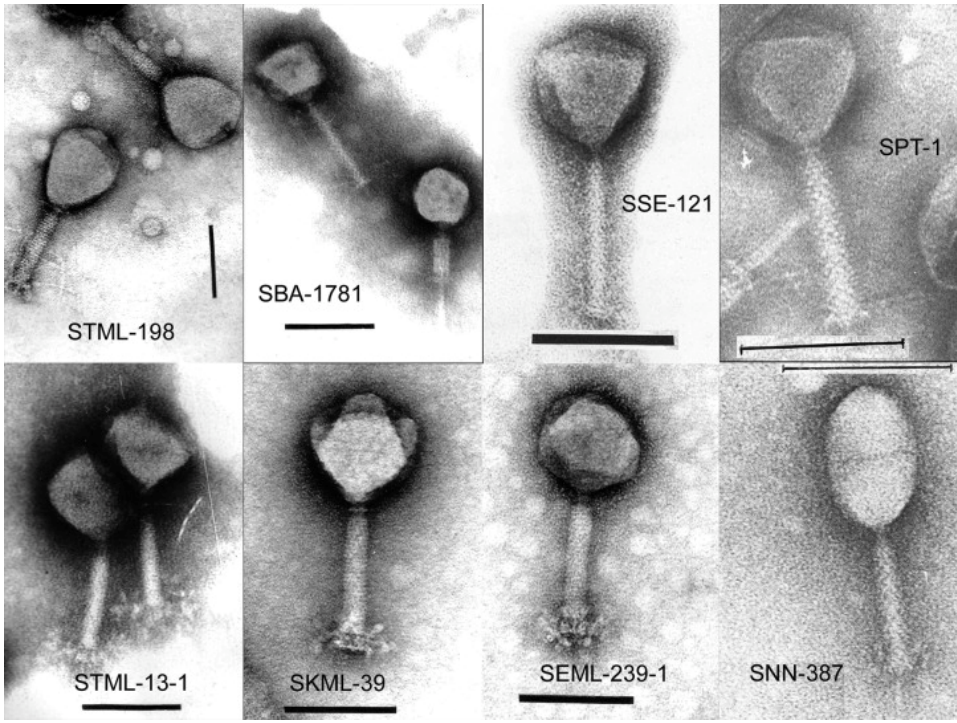
Example of Phage products approved for food applications

Phage Product	Target Organism	Regulatory
Secure Shield E1	*E. coli*	FDA, GRN 724 *pending as of 19 March 2018*
EcoShield™	*E. coli* O157:H7	FDA, FCN 1018; Israel Ministry of Health; Health Canada
SalmoFresh™	*Salmonella* spp.	FDA, GRN 435; USDA, FSIS Directive 7120.1; Israel Ministry of Health; Health Canada
PhageGuard Listex™	*L. monocytogenes*	FDA, GRN 198/218; FSANZ; EFSA; Swiss BAG; Israel Ministry of Health; Health Canada
ShigaShield™ (ShigActive™)	*Shigella* spp.	FDA, GRN 672

(Moye et al., 2018)

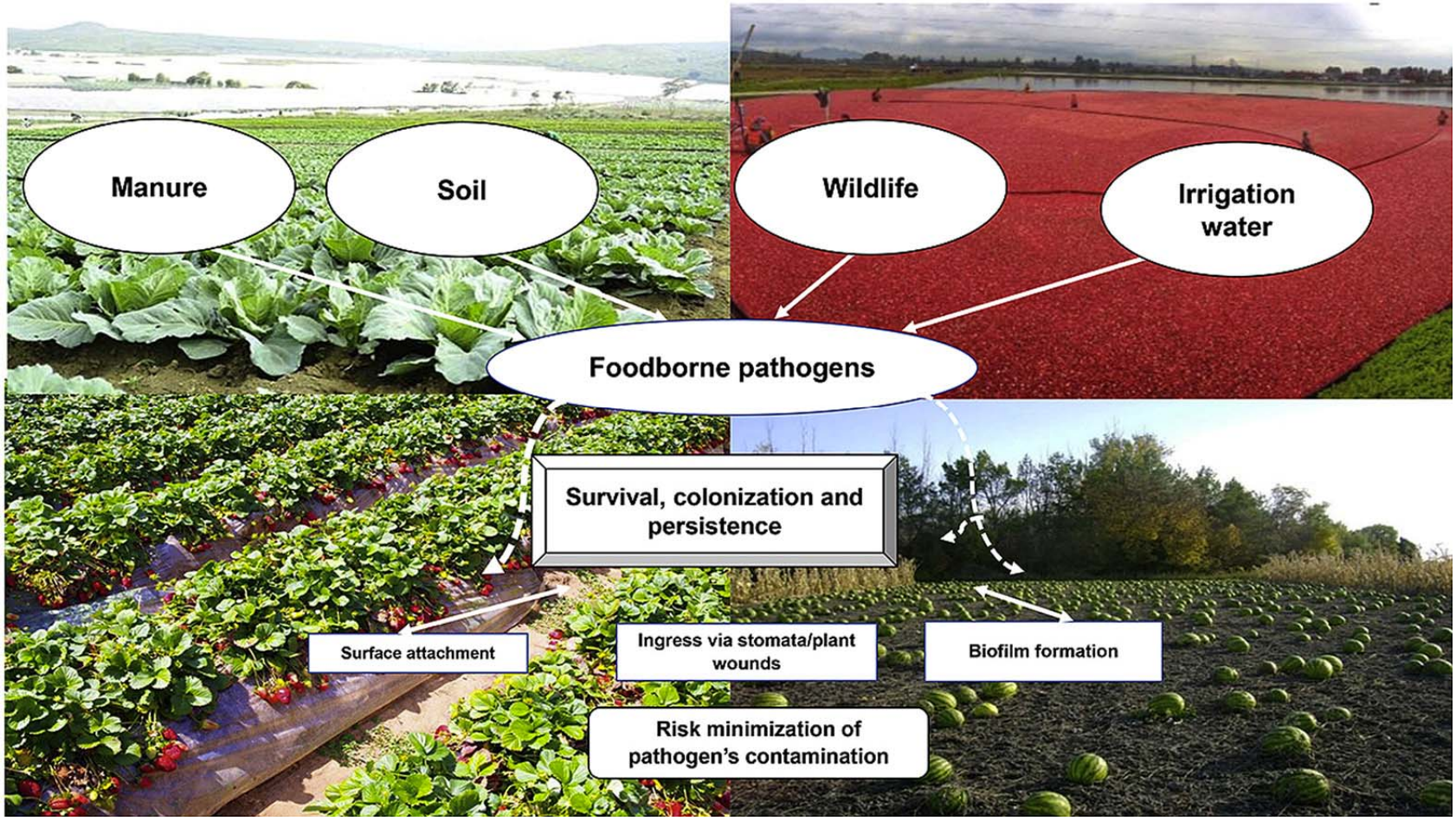
SalmoFresh info

- Patent no. 7674467
- GRAS No. 435
- Kosher, halal and vegan
- RTE meats, fish, shellfish, fruits, vegetables, dairy



- 6 phage cocktail
- SPT-1, SBA-178, SBA-1781, SIT-128, SSE-121, SDT-15
  - ATCC: PTA-5281, PTA-5284, PTA-5282, PTA-5285, PTA-5283 and PTA-5280.
- 3M NaCl, 0.3M Na_3citrate. 2H2O, pH 7.0 at ambient temperature

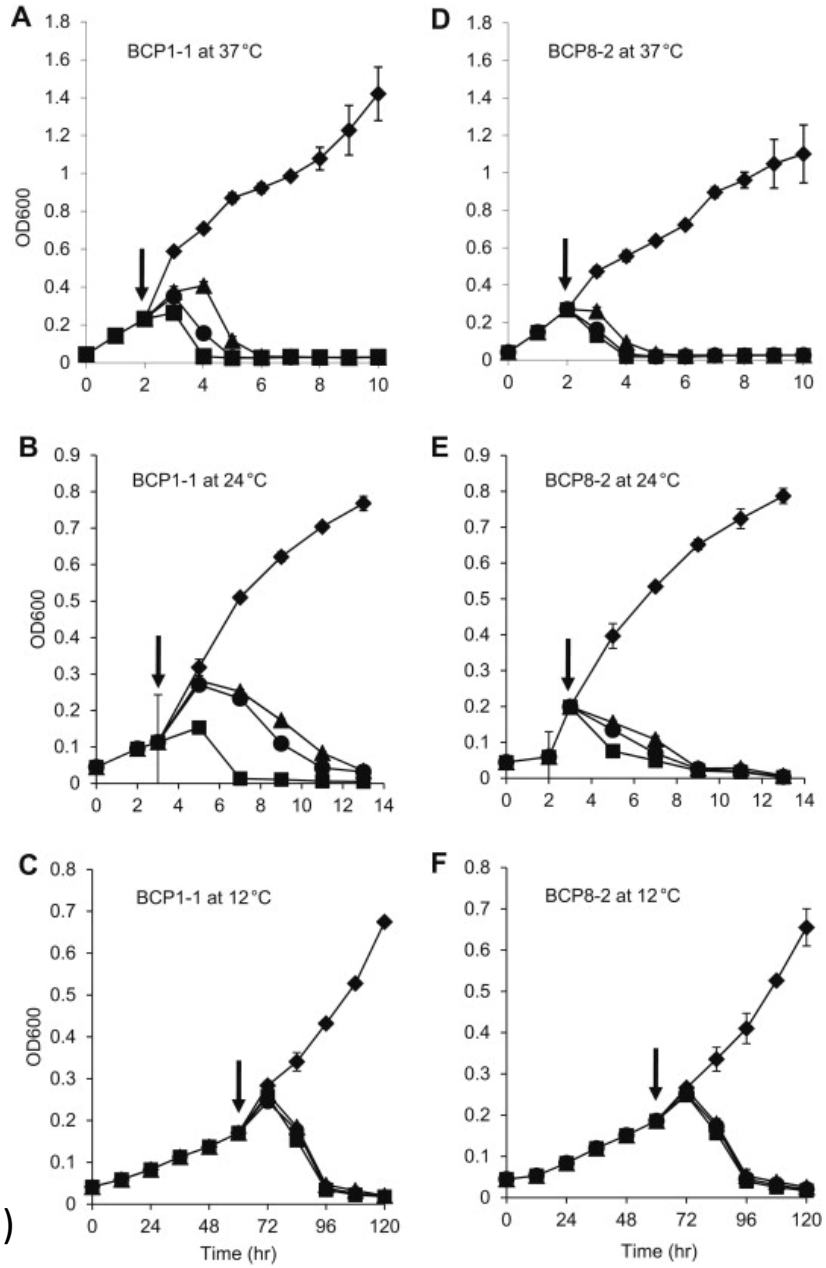
Where does contamination of fresh produce come from?



(Alegbeleye et al., 2018)

Example of fermented product that could benefit from phage therapy

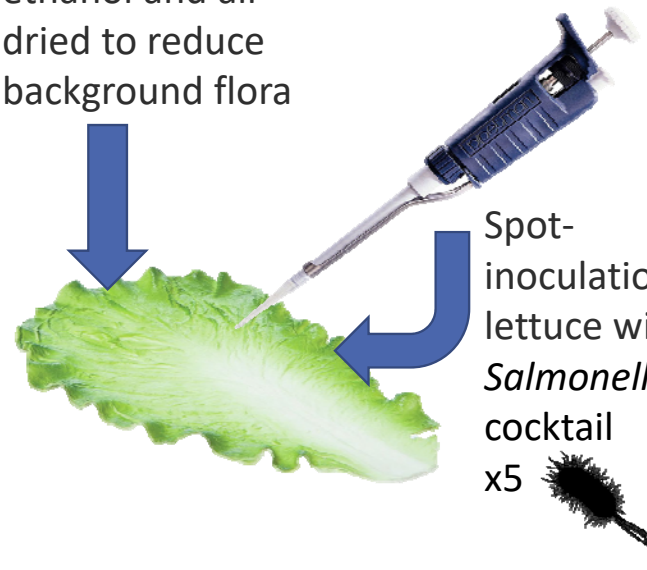
- *Bacillus cereus*
  - causes diarrhoea and vomiting
- Phages isolated from Korean fermented soybean paste
- Phages BCP1-1 and BCP8-2 were able to inhibit *Bacillus cereus* growth at 12, 24, and 37 °C in TA medium



(Bandara et al., 2012)

Effectiveness of SalmoFresh™ phage cocktail on *Salmonella*

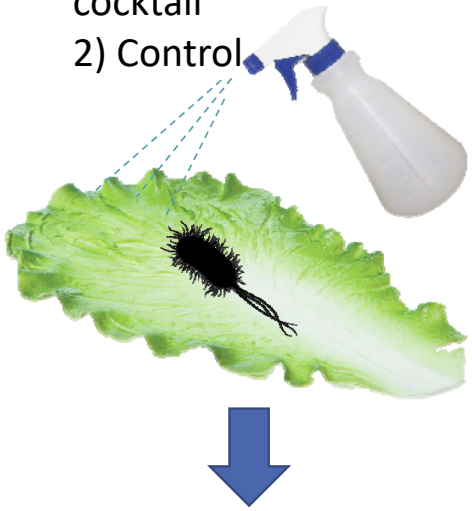
Washed with tap water 10 °C, 70% ethanol and air-dried to reduce background flora



Spot-inoculation lettuce with *Salmonella* cocktail x5

air-dried at 25 °C for 3 h

Sprayed with:
1) SalmoFresh™ Phage cocktail
2) Control



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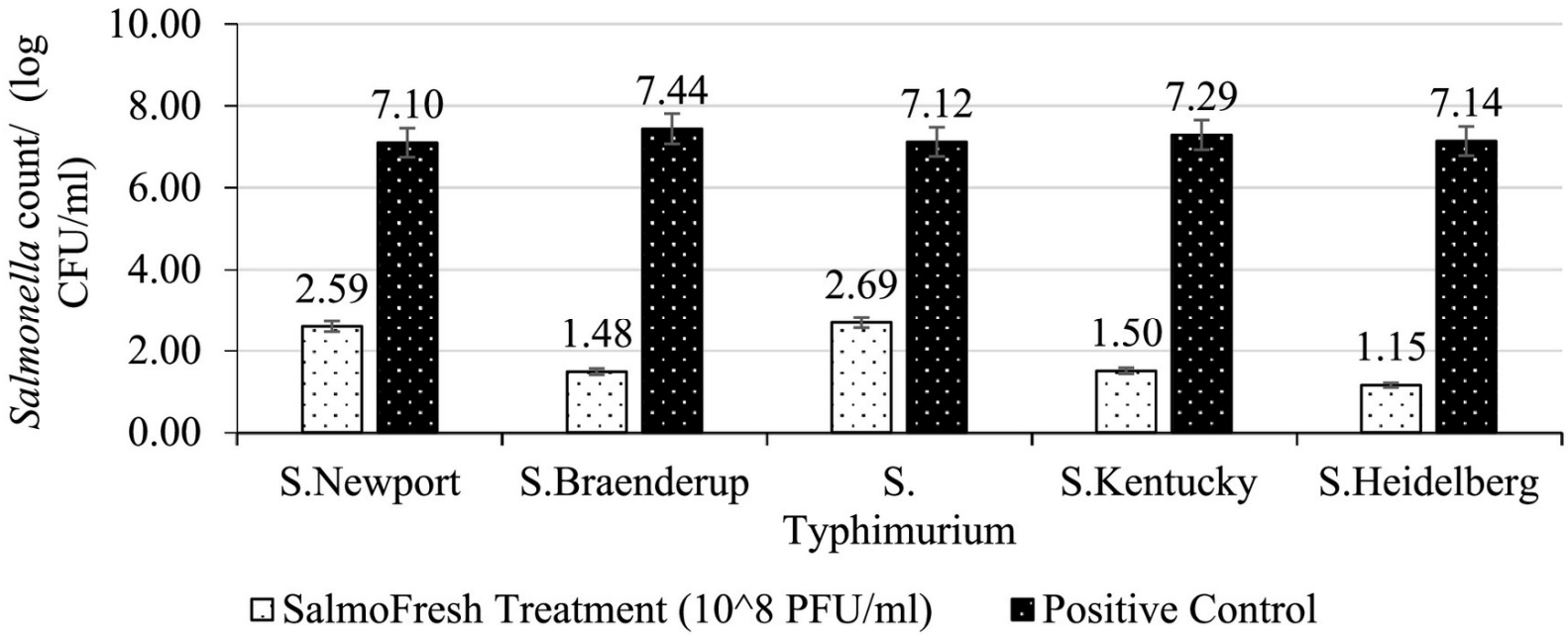
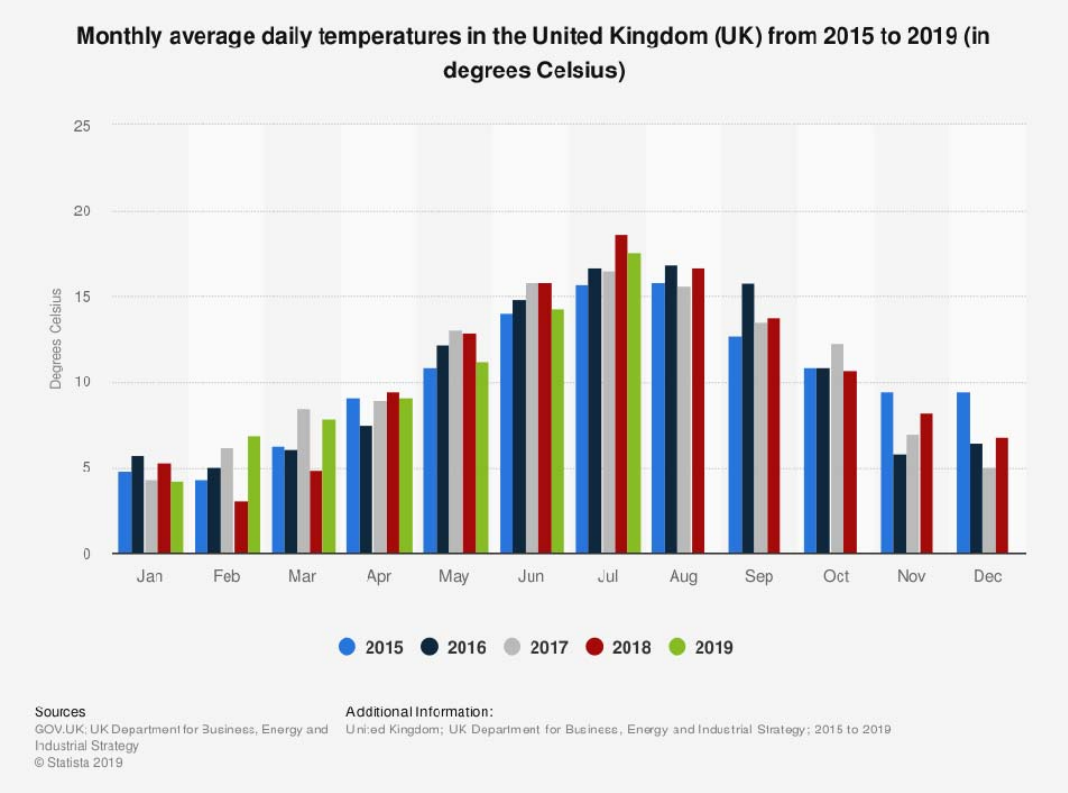
Effectiveness of SalmoFresh™ phage cocktail on *Salmonella*

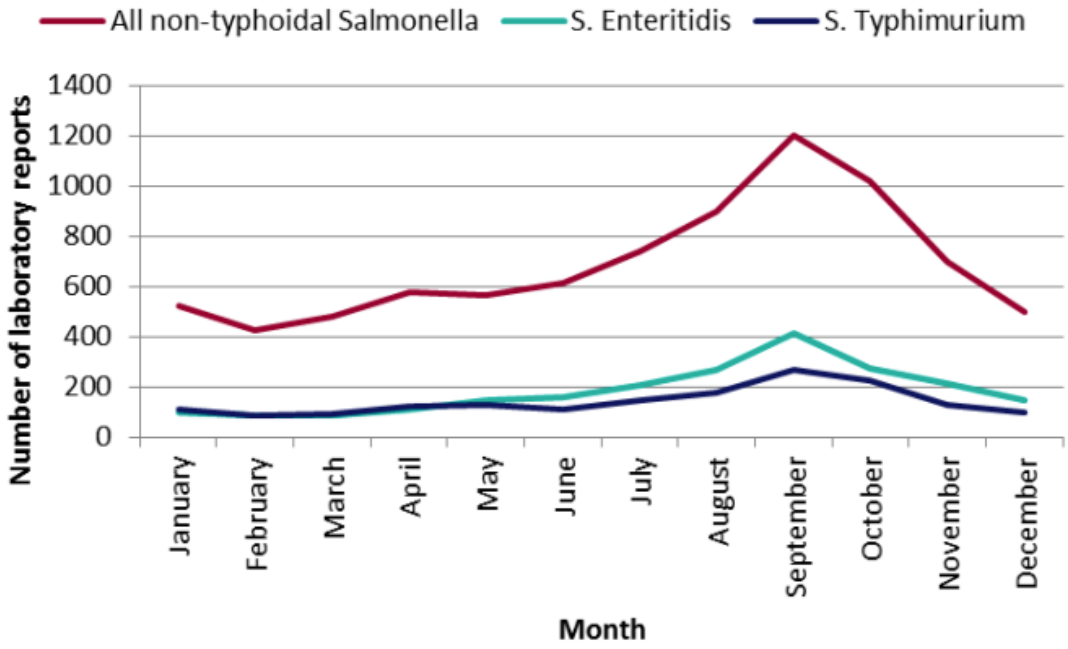


- Target wide range of *Salmonella enterica* serovars
- Applicable to fresh produce production processes *in vitro*

(Zhang et al., 2019)

UK temperature vs Samonella incidence





CDC report: 4th December 2019

- *Escherichia coli* O157:H7
- Lettuce from Salinas, California on recall

- Reported Cases: 102
- Hospitalizations: 58
- Deaths: 0