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Bacteriophage and Probiotics Used for Infection Prevention in Healthcare Settings:

A Work in Progress

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Hosted by Paul Webber
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Topics for Today

- Novel uses for bacteriophage viruses in health care
- Focus on use of bacteriophage for infection prevention purposes
- Recent research on this topic and the use of probiotics in cleaning solutions
- Benefits and limitations
- General comments about this approach to management of surface bioburden
- Don't forget the Chain of Infection

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Bacterial Viruses

Bacteriophages – an extremely diverse group of viruses, particle structure has different forms

- All bacteria can be infected, but phage generally target specific bacteria
- Can be lytic or lysogenic re: release of newly made virus
- Can pick up genetic material from its host, serves to transfer genetic information from one cell to another
- Lacks the capability to infect eukaryotic cells

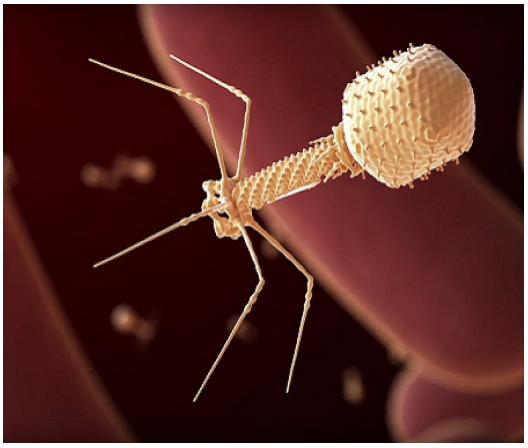

Photo source: NIAID, NIH

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Novel Uses for Bacteriophage

- Phage therapy
- Vaccine carriers
- Gene delivery
- Food preservation and safety
- Biofilm control
- Surface disinfection

An extensive review on these topics can be found in:

Harada LK, etal. Biotechnological applications of bacteriophages: State of the art. *Microbiol Res* 2018; 212-213:38-58

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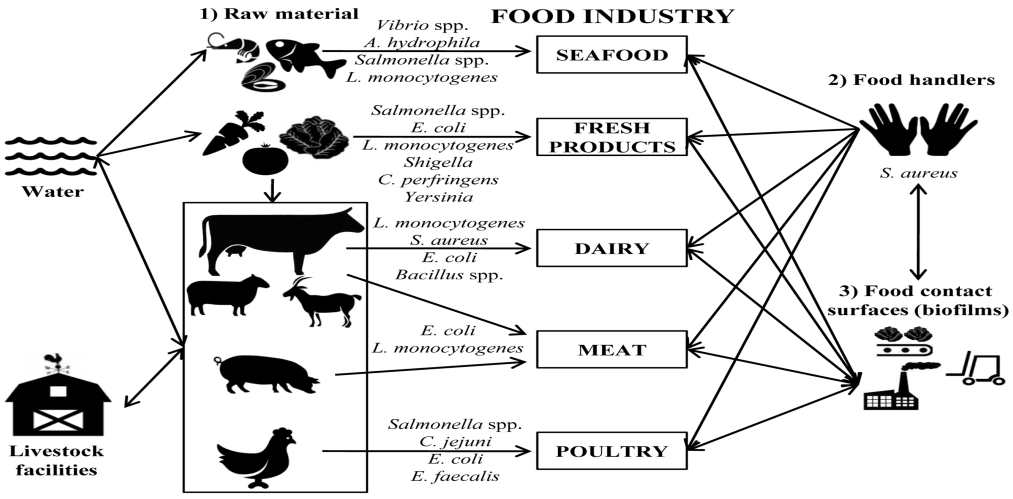
Main Sources of Contamination in the Food Industry



Source: Gutiérrez D, et al. Bacteriophages as weapons against bacterial biofilms in the food industry. *Front Microbiol* 2016; 7: 825 *doi: 10.3389/fmicb.2016.00825*

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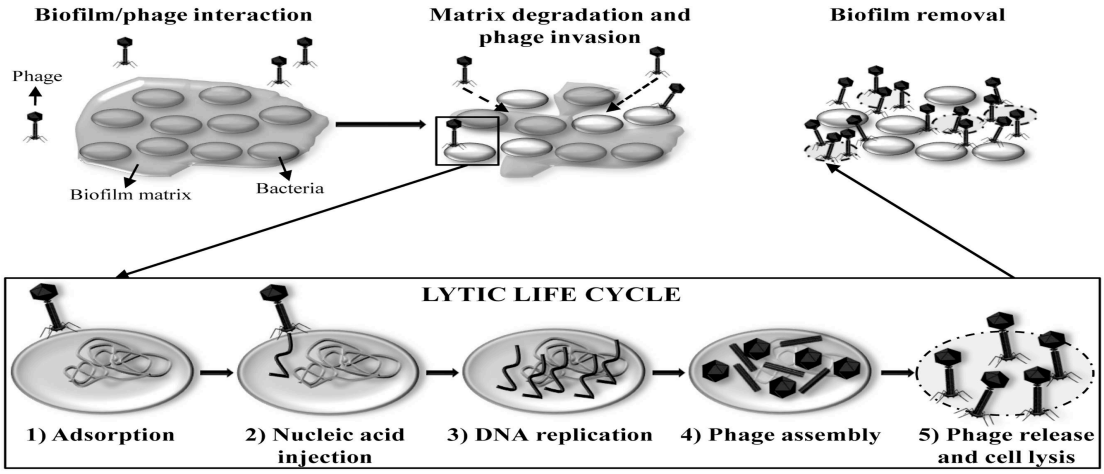
Lytic Life Cycle of Bacteriophages Inside a Biofilm



Source: Gutiérrez D, et al. Bacteriophages as weapons against bacterial biofilms in the food industry. *Front Microbiol* 2016; 7: 825 *doi: 10.3389/fmicb.2016.00825*

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The Attack on the Biofilm!

- Some bacteriophage have polysaccharide depolymerases located in the tail structure
  - Hydrolytic enzymes disperse into the biofilm matrix
- Some phage have lytic enzymes VAPGHs, and double-stranded phages have endolysins
  - Create a hole in the bacterial cell wall
  - Phage DNA enters the bacterial cell, initiates infection
- Enzymatic degradation of the biofilm matrix
- Bacterial cell lysis and release of progeny bacteriophage
- Residual biofilm material now easier to remove

Source: Gutiérrez D, et al. Bacteriophages as weapons against bacterial biofilms in the food industry. *Front Microbiol* 2016; 7: 825 *doi: 10.3389/fmicb.2016.00825*

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Is the successful use of bacteriophage for control/removal of contamination in food industries transferrable for use in healthcare venues?

It's a work in progress…

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Focus on Use of Bacteriophage for Hard Surface Cleaning and Sanitization

- Goal is to reduce and/or eliminate bacterial pathogens from a surface
- Bacteriophages have specific bacterial targets (i.e., a "one size fits all" approach requires a cocktail of multiple different bacteriophages…)
- Is the end result "sanitization" or "disinfection?"
- Current interests focus on reduction of antibiotic resistant bacteria in hopes of reducing healthcare-acquired infections (HAIs)

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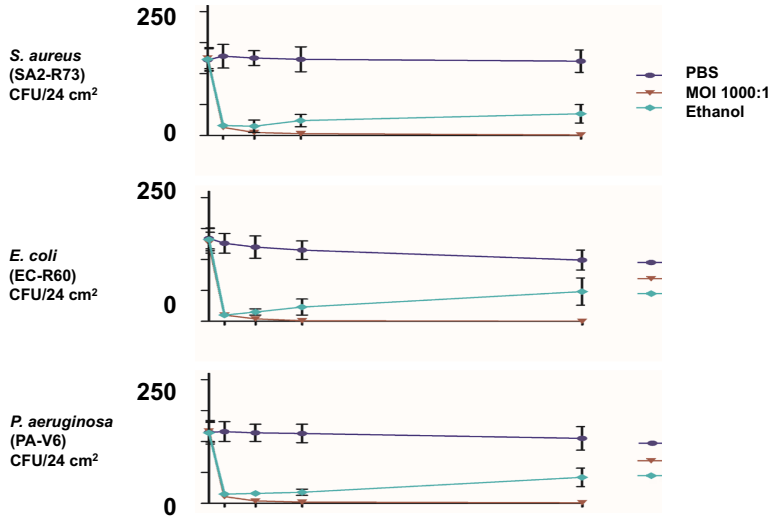
First Phase: Proof of Concept

- Hospital isolates of antibiotic-resistant bacteria
- Surface materials tested: ceramic, glass, plastic
- 100 CFU applied to 24 cm^2
- Specific phages in PBS, multiplicity of infection (MOI) 1000:1
- Sample times: 0, 1, 3, 6, 24 hours
- RODAC plates



D'Accolti, et al. *Infection Drug Resistance* 2018; 11: 1015-1026

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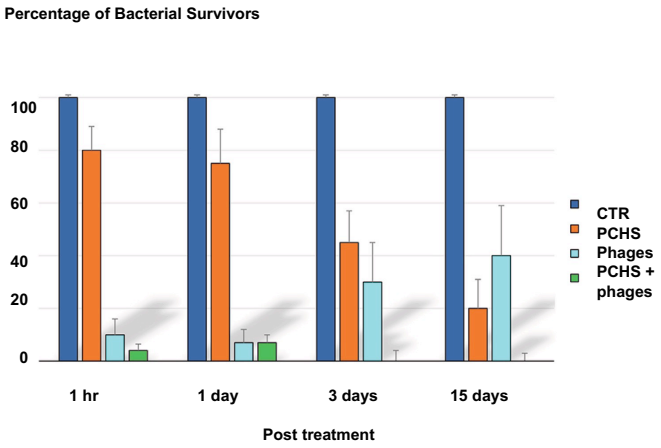
Reduction Effect: Bacteriophages Added to Probiotic Cleaning Solution

- *S. aureus*: 100 CFU added to 24 cm^2 of ceramic sink surface, let dry
- Used a combination of a phage/probiotic cleaning solution
- Duplicate samples from three independent experiments
- RODAC plates



D'Accolti, et al. *Infection Drug Resistance* 2018; 11: 1015-1026

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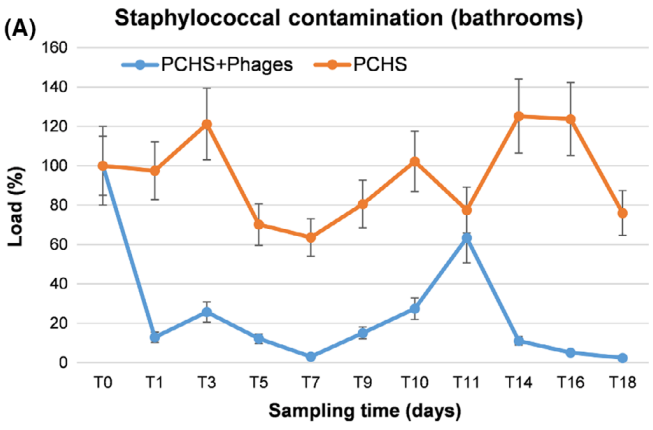
Second Phase: Small Area *in situ* Evaluation of *S. aureus* Reduction Using Phage/Probiotic Cleaning Solution

- *S. aureus* load in bathrooms determined prior to test
- Mean starting level of contamination: 3.7 X 10^4 CFU/m^2
- Floor, sink, shower plate
- Daily phage application by nebulization
- RODAC plates



D'Accolti, et al. *Microbial Biotechnol* 2019; 12: 742-751

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Benefits and Limitations

- Bacteriophages specific for a bacterium (e.g., *S. aureus*) were able to inactivate/destroy their targets regardless of antibiotic-resistant status
- The lytic effect was rapid (previous studies by the research group indicated that 10 minutes was an effective contact time)
- Probiotic cleaning solutions need a long time to achieve a stable decrease in pathogens

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Probiotic Cleaning Solution Without Bacteriophage: What Impact on HAI Incidence Rates?

- Multicenter study of internal medicine units' patients in 6 hospitals in Italy over 18 months
  - One of the 6 hospitals served as a control hospital for > 6 months
- 2 study periods: pre-intervention (normal cleaning processes for terminal cleaning) and intervention (use PCHS cleaning solution with *Bacillus* sp. spores instead of conventional cleaners)
- HAI incidence was measured in both periods
- Surface sampling for bioburden determination
- HAIs observed: UTI, BSI, systemic clinical sepsis, gastrointestinal infection, skin/soft tissue infection, respiratory infection

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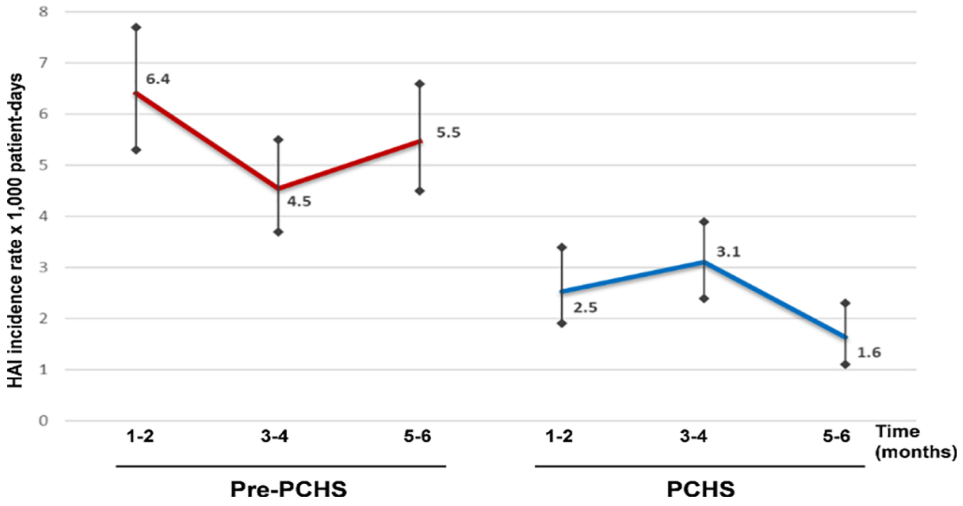
Impact of Using PCHS Cleaning Solution: HAI Incidence Reduction

Total patients surveyed: 11,842.

Total number of surfaces sampled: 24,875.

Use of PCHS: significant decrease in HAI incidence from 4.8% to 2.3% ($p<0.0001$).

Use of PCHS: stable decrease in the numbers of pathogenic bacteria.



Source: Caselli, et al. *PLOS One 2018 https://doi.org/10.1371/journal.pone.0199616*

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Pathogens load on hospital surfaces

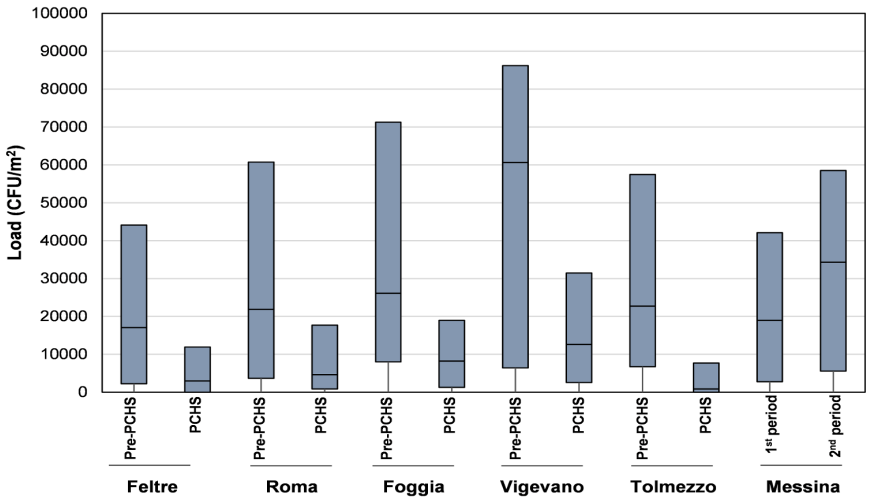
A)

Pathogen load on surfaces expressed as CFU/m^2.

RODAC plates.

Staphyloccus, Enterobacteriacea, Acinetobacter, Candida, Pseudomonas, & Clostridioides.

Results are sum of median values for each pathogen; upper box is 75% percentile value.



Source: Caselli, et al. *PLOS One 2018 https://doi.org/10.1371/journal.pone.0199616*

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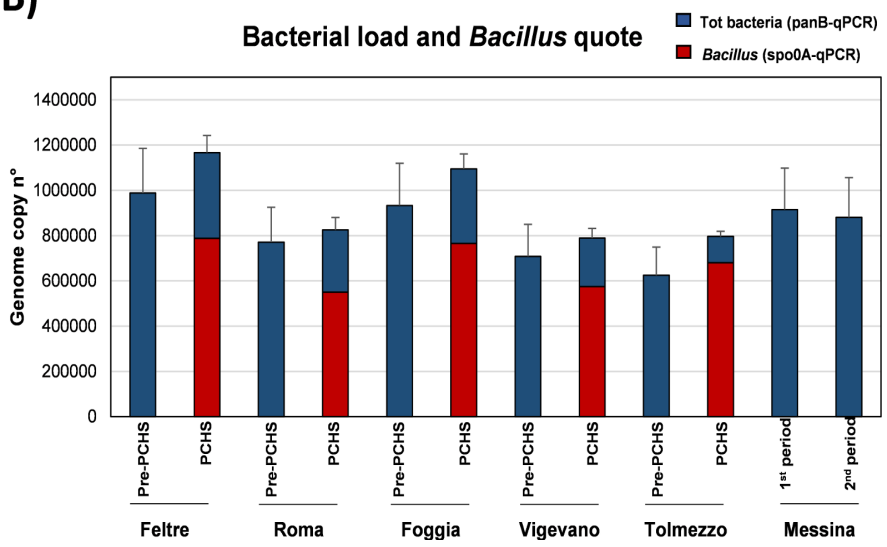
Total bacterial load and PCHS-bacilli count.

Measured by a pan-bacterial qPCR (*panB)* and a specific qPCR for *Bacillus* genus (*spo0A*).

Results expressed as genome copy number per 100 ng of tested DNA.

Median values shown.

B)



Source: Caselli, et al. *PLOS One 2018 https://doi.org/10.1371/journal.pone.0199616*

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Would an Aerosolization Method for Dispersing Bacteriophage be Suitable for this Purpose?

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Taiwan Study of Aerosolization of Bacteriophage Targeting CR-AB

- Large Taiwan hospital, 945 beds
- 14 month Baseline period, and 8 month Intervention period
- 264 cases of carbapenem-resistant *Acinetobacter baumannii* (CR-AB) recorded (191 in baseline period, 73 in intervention period
- Mean percentage of CR-AB isolation dropped to 46.07% from 87.76%
- Rates of CR-AB acquisition dropped to 5.11/1000 patient-days from 8.57/1000 patient-days (p=0.0029)

Ho Yu-Huai, et al. PLOS One 2016; *doi:10.1371/journal.pone.0168380*

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Cleaning Solution Details

- Baseline period:
  - The conventional cleaning solutions used were a 1:100 dilution of chlorine bleach (0.06%) applied to larger surfaces, and 75% alcohol applied to small surfaces
- Intervention period:
  - After routine terminal cleaning, selected bacteriophage (10^7 PFU/mL) in 500 mL was aerosolized by an ultrasonic humidifier, producing a cool fog suitable for a 27 cubic meter space
  - Hospital had a panel of 24 bacteriophage, matched the incoming patient's CR-AB bacterial isolate to a specific phage
  - CR-AB was transferred into the room after the aerosolization was completed and the phage had settled
  - Phage titer on surfaces after settling: 5.5 x 10^4 PFU/cm^2

Ho Yu-Huai, et al. PLOS One 2016; *doi:10.1371/journal.pone.0168380*

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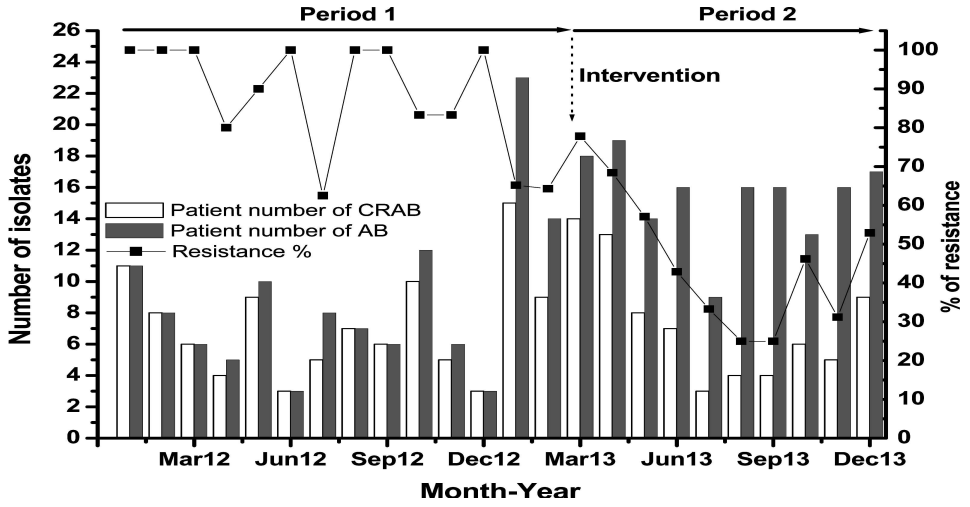
Bacterial Isolates Testing

Percentage of CR-AB isolates dropped from 87.76% to 46.07% in the ICUs.

Percentage of CS-AB isolates increased from 17.21% to 52.60%.



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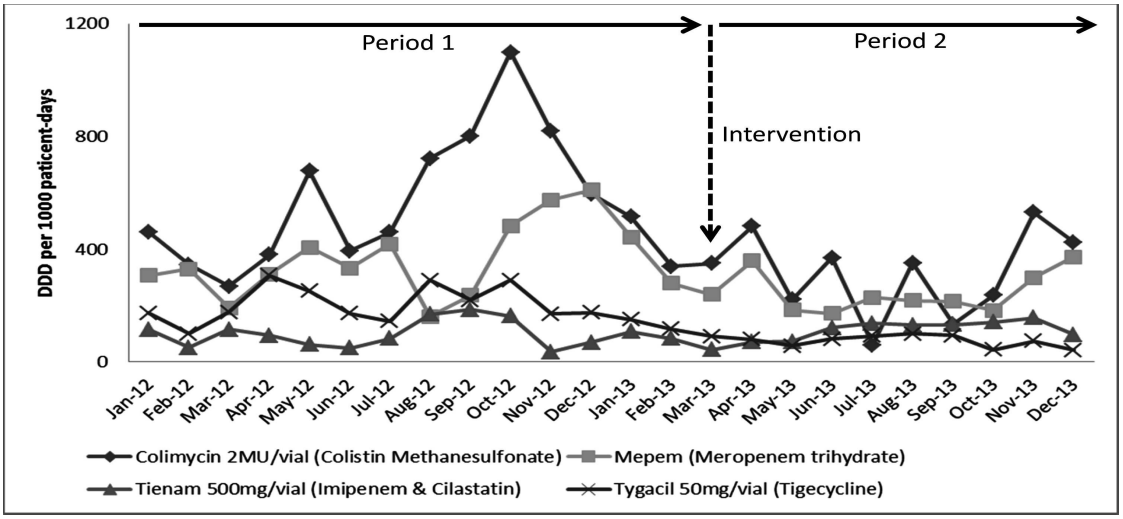
Reduction in Antibiotic Usage



Ho Yu-Huai, et al. PLOS One 2016; *doi:10.1371/journal.pone.0168380*

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General Comments

- The focus on destroying targeted antibiotic-resistant bacteria is possible with this approach
- However, the bioburden on hard surfaces in healthcare facilities can be a complex mixture of bacteria, viruses, fungi
- Has potential build-up of organic matter on surfaces treated with phage/probiotic cleaning solutions been addressed?
- Current standard for surfaces in healthcare facilities is cleaned and disinfected as appropriate
- Is this approach to cleaning surfaces in healthcare facilities envisioned for widespread use in the facility or will it be limited to contact isolation areas?

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Chain of Infection (COI)

- Virulent pathogen:
  - Bacteria, fungi, viruses, parasites
- Sufficient number of pathogen:
  - Infectious dose
- Mode of transmission:
  - Contact, droplet, airborne, fecal/oral, indirect
- Portal of entry:
  - Broken skin, mucous membrane, respiratory tract, ingestion
- Susceptible host:
  - Age, immunity, medical conditions

Other possible links include reservoir, portal of exit

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Thank You!

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www.webbertraining.com/schedulep1.php

September 16, 2021	*(FREE Teleclass)* **ANTIMICROBIAL RESISTANCE AND INFECTION PREVENTION - WE HAVE MET THE ENEMY AND HE IS US** Speaker: **Prof. Allison McGeer**, Sinai Health System, University of Toronto
September 16, 2021	*(FREE South Pacific Teleclass - Broadcast live from the New Zeland Infection Prevention and Control Nurses College conference)* **UNDERSTANDING THE SCIENCE BEHIND AOTEAROA NEW ZEALAND'S COVID-19 RESPONSE** Speaker: **Prof. Michael Baker**, University of Otago, New Zealand **Live broadcast sponsored by Schulke** schülke
September 23, 2021	**TRIDEMICS OF INFLUENZA, COVID, AND NOW RSV** Speaker: **Prof. Robert T. Ball**, Medical University of South Carolina

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