Phages as Antibiotic Alternatives and their use in Humans, Agriculture, and Aquaculture

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Thank you so much for having me. I am very honored to be part of the discussion today.

At Phagelux, our scientific efforts are driven by the growing need of finding alternative solutions to resistance both in the US and the developing world.

I think that we all recognize that antimicrobial resistance is a present and growing unmet medical need. There needs to be a novel way to combat drug resistance. Ironically, this novel way might just be the prehistorical predator of bacteria: bacteriophages.

Bacteriophages are viruses that specifically infect bacteria, they are present in very large quantities in the human body and in our environment.

And although they were discovered a bit over a century ago before the modern antibiotic era, we are convinced that bacteriophages will turn out to be important therapeutics in combatting antibiotic-resistant infections.
Antimicrobials: Approaching a post-antibiotic era?
Bacteriophages adhere to their host bacterium by specific receptors, inject their DNA into the bacteria and use its replication machinery to replicate its DNA, assemble its components, lyse the bacteria and release its progeny phages into the environment. This is called the lytic cycle. And for the purposes of this presentation we are going to concentrate on phages that are lytic and non-transducing.

What is cool about phages is that they have the potential to clear biofilms or at least help disperse them, and if we don’t want to use the entire phage, there is a possibility to harvest the power of phage enzymes, called endolysins to be able to lyse the bacteria from without. In addition, there's the potential to engineer phage to target bacteria that develop resistance and resensitize bacteria to antibiotics.
Phagelux is a platform company that utilizes phages, lysins, and other biological solutions as adjuncts or alternatives to antibiotics, particularly targeting antibiotic resistant bacteria (AMR).

The **Human Health Division** has 5 products under development:
- Burn care
- Ulcers
- Acne
- Eczema
- Medical devices

The **AgriHealth Division** has 4 franchises:
- Crop Sciences
- Food Safety
- Animal Health
- Feed Additives

- **18** Products under development
- **1** HH product in clinical trial
- **2** Commercial AH products in USA
- **5** Commercial AH products in China
In recent years, Phagelux Inc. has been at the forefront of phage therapy, leading a global endeavor to provide solutions for the bacterial infections of crops, foods, animals and humans.

It is Cayman registered company with headquarters in Shanghai, laboratories in China and North America and manufacturing facilities in the United States and China. Our shareholders WuXi AppTec, the Management Team, Fosun Pharma, are a major part of our success.

Phagelux’s lead products are for the prevention and treatment of ulcer related infections. We are under IND for the treatment of Infected burns, and partnered with industry leaders such as JnJ for other products targeting acne, eczema, and the prevention and treatment of periprosthetic joint infections.

**AgriHealth Division is valued at $35M USD with 8 registered products and 13 under development.** Our Agrihealth partners are Certis/Mitsui, NutriQuest, or Church & Dwight.
Bacteriophages for the treatment of multi-drug resistant infection in wounds

Global Diabetic Ulcers Treatment Market Value (US$ Bn), by 2020

Invasive infection is responsible for 51% of deaths after burn injury

D. Jikia et al Clinical and Experimental Dermatology, 30, 23–26

Phage production Biodegradable polymers Burns/Ulcers
• Despite renewed interest in bacteriophages, there is not yet any product broadly available for human application. There are however a few exceptions where bacteriophages can be applied to human patients under eIND and as a therapeutic option in a few European countries.

• In our hands, we have developed a phage cocktail targeting S. aureus, Ps. Aeruginosa and K. pneumonias. This product is currently under IND and Phase I clinical trials are to resume shortly.

• But in the long run, we must consider that phage therapy can be affected by multiple factors such as rapid clearance by the immune system, inactivation by unfavorable environmental factors such as adverse enzymes, pH, and temperature, and long term storage.

• In view of this, administration of phages in humans requires an appropriate delivery system.

• So our lead products use amino acid biodegradable polymers to encapsulate the phages. These biopolymers are easy to produce, have low cost, maintain phage viability, increase the phage stability, they allow for high phage loading, and have a proper phage release rate.

• Phagelux ProPatch is the third generation of biodegradable patches comprising phages, developed since 1991. The first generation was a patch marketed as Fagobioderm and sold in the Republic of Georgia. Fagobioderm underwent extensive pre-clinical trials to determine its safety and efficacy. The second generation of the patch, PhagoBioDerm (Figure 3), is currently sold in Eastern Europe. Although no formal, controlled clinical trials were undergone, PhagoBioDerm was used to treat hundreds of ulcer and burn patients. Encouraged by years of positive clinical experience, Phagelux acquired the technology in 2014, and improved upon it in an effort to bring it to the North American market.

• Another area of research at Phagelux, is the microencapsulation of bacteriophages used for the prolonged release of phages in wounds and on medical devices. The goal is to create a thin permeable film when topically applied on wounds to prevent and treat multi drug resistant infections caused by S. aureus, Ps. aeruginosa and K. pneumonia. These allow These systems also allow for ease of application and removal, diminished pain and increased comfort for the patient.
**Bacteriophages for the prevention and control of bacteria in crops**

- Natural, Safe, Organic, Eco-Friendly, and Effective EPA registered Pesticides
- AgriPhage™ Has Been Successfully Treating Crops in North America Since 2005

<table>
<thead>
<tr>
<th>Phage Type</th>
<th>Biological Control</th>
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<tbody>
<tr>
<td><strong>AgriPhage</strong></td>
<td><strong>Biological control for bacterial spot and bacterial speck on tomatoes and peppers</strong></td>
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<tr>
<td>- <em>Xanthomonas campestris</em> pv. <em>vesicatoria</em></td>
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<tr>
<td>- <em>Pseudomonas syringae</em> pv. <em>tomato</em></td>
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<tr>
<td><strong>AgriPhage-Cmm:</strong></td>
<td><strong>Biological control for bacterial canker disease on tomatoes</strong></td>
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<tr>
<td>- <em>Clavibacter michiganensis</em> subsp. <em>Michiganensis</em></td>
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<tr>
<td><strong>AgriPhage Fire Blight:</strong></td>
<td><strong>Biological control for fire blight disease on apples and pears</strong></td>
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<tr>
<td>- <em>Erwinia amylofera</em></td>
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<tr>
<td><strong>AgriPhage Citrus Canker:</strong></td>
<td><strong>Biological control for citrus canker</strong></td>
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<tr>
<td>- <em>Xanthomonas axonopodis</em> pv. <em>citri</em></td>
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Now switching on to our AgriHealth Division. University plant pathologists estimate that over 90% of bacterial strains are resistant to current copper-based alternatives in the US.

Many growers confirm this in their experience with traditional treatments.

Our sister company in Salt Lake City, Omnilytics has been treating tomatoe and pepper plants since 2005 in North America. Our product, Agriphage is EPA registered, safe, eco-friendly and effective for the prevention and control of harmful bacteria on tomato and pepper plants, apple and pear trees, and citrus trees.

These products are currently produced and sold in the US. We are spraying 1 quart per acre and are producing 75 000 L per year of Agriphage.

Our patented technology along with thousands of plant samples analyzed over a decade from all over the country. We know what strains of bacteria are most prevalent in the fields and greenhouses form season to season and this allows us to constantly update our formulation to ensure its effectivity throughout the US.
Bacteriophages for the prevention of bacterial contaminations for food safety

1.2M

CDC estimates Salmonella causes about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths in the United States every year.

265K

CDC estimates that 265,000 STEC infections occur each year in the United States. E. coli O157:H7 causes more than 36% of these infections.

SalmoPro

FDA & USDA evaluated GRAS Notification #752 for Salmonella control for use as an antimicrobial agent to control Salmonella in food (including meat, poultry, and egg products).

E.clipse-STECEclipse-STECEclipse-STECEclipse-STEC (currently under review)

GRAS Notification #827 for E. coli control (STEC O157:H7, O26, O45, O103, O111, O121, and O145) for use as an antimicrobial agent to control E. coli in food (including meat, poultry, and egg products).
We also produce 2 products that target E. coli in food. The first, Finalyse is a product that is on the market since 2007. It’s a higher concentrated product. We produce 2000 L/year that are sprayed on 3500 head of cattle immediately before slaughter. Our second product target Shiga toxin producing e.coli and is currently under review for GRAS applications. Our in vitro data shows great promise and in vivo trials will resume after obtaining a GRAS status.

We also produce SalmoPro to control salmonella on meat, poultry and egg products. This product was launched last year and is used inside the meat processing plants. We are currently trialing with 4 customers owning large plants. Our results demonstrate that all controls are positive for PCR testing for the presence of Salmonella, and negative for all sample treated with SalmoPro. This product shows great promise.
Bacteriophages for the treatment of bacterial infections in livestock

Million pigs slaughtered every year in China

Million tons of antibiotics are administered yearly in China

Exudative epidermidis S. aureus, S. hyicus, S. sciuri

Group A
- Intramuscular injection of Penicillin G (500,000 IU/Piglet) once a day for 3 days
- Flushing the skin with 0.1% purple salt, twice a day for 5 days

Group B
- Spray 5 mL of a phage cocktail (~ $10^7$ pfu/ml $\Phi J21P1, \Phi J27P1, \Phi J49P1 = 1:1:1$) once a day for 5 days
As I mentioned previously, our headquarters are located in Shanghai and we are present throughout mainland China, notably in Nanjing, Wuhan and Suzhou.

688 million pigs are slaughtered each year in China. China is responsible for half of the world’s pork production and administers over 170 million tons of antibiotics to pigs annually.

As with the US, the Chinese government is pressing for the drastic reduction in the use of antibiotics in livestock.

Pigs in China are currently plagued with a condition called exudative epidermidis which is a staph infection that creates localized lesions on the flanks and behind the ears of the piglets. The skin changes to a brown colour gradually, becomes wrinkled and it has a greasy feel. Eventually, the skin turns black due to necrosis and the piglets dies within 5 to 60 days. Depending on the region, morbidity is 10%–90% and mortality of 5%–90%.

In this field trial we have given the standard of care to pigs affected by exudative epidermidis, which is intramuscular injections of penicillin once a day for 3 days and flushing the skin with 0.1% purple salt, twice a day for 5 days. The second group was sprayed with 5 mL of a staph phage cocktail once a day for 5 days.

You can see from the images that group A piglets were still affected by the condition while group B pigs made a full recovery.
Bacteriophages for the treatment of bacterial infections in aquaculture

Hepatopancreatic necrosis syndrome (AHPNS) in *P. vannamei* shrimps caused by *Vibrio parahaemolyticus*

$1B$ In economic losses/year

80% Output losses
Since 2009, a new emerging disease in *P. vannamei* shrimp called early mortality syndrome (EMS), or acute hepatopancreatic necrosis syndrome (AHPNS) has caused serious economic losses in shrimp farms in China, Malaysia, Vietnam, Thailand and most recently Mexico. In China, the output loss was close to 70-80% in the past few years. This disease causes more than one billion USD economic losses globally every year. By using a laboratory challenge model, a unique strain of *Vibrio parahaemolyticus* had been identified as the causative agents for AHPNS.

We have isolated 800 strains of *V. parahaemolyticus* from the shrimp pools in southern China, of which 150 pathogenic strains of *V. parahaemolyticus* (carrying toxin gene PirA and PirB) had been identified.

We isolated Lytic bacteriophages from Fujian and Mexico and retained 3 lytic podoviridae bacteriophages which were effective in vitro against 91.5% of AHPND-associated V. parahaemolyticus strains and non-VPAHPND 92.3%

Respectively
Bacteriophages for the treatment of bacterial infections in aquaculture

Guang Xi

Guangdong Province

Fisheries Research Institute (Zhuhai city)

In shrimp length for ponds treated with phage cocktail

14%

Increase in yields for larvae ponds treated with phage cocktail. Inhibition of *Vibrio parahemolyticus* in ponds treated with 1E7 PFU/mL

24h

Inhibition of *Vibrio parahemolyticus* within 24 hours of application

Vibrio within phage
We ran some safety trials in shrimp farms in Guang Xi, and found that the phages were safe and the application of phages resulted in a positive increase in shrimp length when compared to the untreated ponds.

Efficacy was tested in Guangdong province and fisheries research institute in Zhuhai city, where we saw a 14% increase in yields for larvae ponds treated with phage cocktails when compared to control ponds and inhibition of vibrio parahemolyticus in ponds treated with a cocktail concentration of log 7 PFU/mL. At the fisheries institute where we were treating larger ponds, we saw complete inhibition of vibrio within 24 hours of phage application.
Conclusion

• Phages are naturally-occurring, safe, and effective in preventing/treating bacterial infections
• Phages can re-sensitize resistant bacteria to antibiotics
• Therapeutic synergy exists between phages and antibiotics
• Phages are <<non-traditional>> therapies that can be of benefit to human health, agriculture, aquaculture, and food safety.
• Using phages prophylactically could reduce infection and the costs associated with them.
the prophylaxis or treatment of postoperative and postraumatic infections.

*Shigella* phages were successfully used for prophylaxis of bacterial dysentery.

One of the most, if not the most, extensive studies evaluating the utility of therapeutic phages for prophylaxis of infectious diseases was conducted in Tbilisi, Georgia, during 1963 and 1964 (7) and involved phages against bacterial dysentery. A total of 30,769 children (6 months to 7 years old) were included in the study. Of these, children on one side of the streets (17,044 children) were given *Shigella* phages orally (once every 7 days), and the children on the other side of the streets (13,725) did not receive phages. The children in both groups were visited on a once-a-week basis to administer phages and monitor their overall status. Fecal samples from all children having gastrointestinal disorders were tested for the presence of *Shigella* spp. and other, unspecified diarrhea-causing bacteria. Based on clinical diagnosis, the incidence of dysentery was 3.8-fold higher in the placebo group than in the phage-treated group (6.7 and 1.76 per 1,000 children, respectively) during the 109-day study period; based on the culture-confirmed cases, the incidence of dysentery was 2.6-fold higher in the placebo group than in the phage-treated group (1.82 and 0.7, respectively) (Fig. 1). The phage effectiveness index (disease incidence per 1,000 children in the placebo group divided by the corresponding number in the phage-treated group) was highest in children between 6 months and 1 year of age and was lowest in children 5 to 7 years of age. An interesting outcome of the study was that there was an overall reduction (2.3-fold) in diarrheal diseases of unknown origin among children treated with phages compared to the children in the placebo group. This may have been observed because some dysentery cases were not diagnosed as such (but were prevented with the *Shigella* phage preparation) or because the phage preparation, although developed specifically against *Shigella* species, was also active against some additional gastrointestinal pathogens.

Controlled trial: phage therapy was used in conjunction with bitilisin-5 and resulted in reduction of incidence of respiratory infection, tonsillitis and other respiratory diseases in subjects.