Phage Therapy for Antibiotic Resistant *Neisseria gonorrhoeae*
Gonorrhea Eradication Team

- Started in December 2012
- We are a volunteer group of experienced citizen scientists working to foster healthy communities through innovative medical research
- Public Health data acquisition, High Quality MMR, Education

PUBLIC HEALTH • EDUCATION • RESEARCH
**Neisseria gonorrhoeae**

- Gram negative, Diplococci
- Obligate human bacterial pathogen; Facultative intracellular
Neisseria gonorrhoeae

- **Polyploid:**
  - DNA is completely replicated before cell division occurs
  - Cells contain two+ copies of the genome

- Obligate human bacterial pathogen
  - Humans are the only natural reservoir
  - Dies quickly outside humans

- Facultative intracellular
Neisseria gonorrhoeae

• Transmission
  - Human to human contact
  - Requires contact with exudates (secretions) from infected individuals
  - Transmitted through sexual intercourse with an infected partner;
  - via infected birth canal of infected mothers during delivery;
  - indirectly transmitted via contaminated hands, fomites contaminated with purulent discharges;
  - directly also via oral sex
N. gonorrhoeae Virulence Factors
How does body respond to invasion?

- Enters through mucousal membranes
- Evades mucousal membranes
- Evades adaptive immunity
- Recruitment of neutrophils
- Phagocytosis occurs
- Gonorrhea exists inside cells and inhibits destruction in vacuoles
## Host Immune Response to Gonorrhea

<table>
<thead>
<tr>
<th>Postulate</th>
<th>Evidence for</th>
<th>Evidence against</th>
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<tbody>
<tr>
<td>Neisseria gonorrhoeae induces immune responses in infected subjects</td>
<td>Infected (and many uninfected) humans have serum antibodies against gonococcal antigens</td>
<td>Little or no increase in antibody levels after infection, or in subjects with previous infection</td>
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<tr>
<td>Specific antibodies are rendered ineffective by antigenic variation</td>
<td>Most major gonococcal surface molecules undergo extensive variation through:</td>
<td>Partial serovar-specific immunity reported in one study</td>
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<tr>
<td></td>
<td>• allelic polymorphism</td>
<td>Anti-Opa antibodies may be associated with resistance to salpingitis</td>
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<td></td>
<td>• genetic recombination</td>
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<td>• phase-variable expression</td>
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<td></td>
<td>• horizontal gene exchange</td>
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<tr>
<td>N. gonorrhoeae avoids complement-mediated destruction</td>
<td>N. gonorrhoeae inhibits complement activation (C4BP, RMP, LOS sialylation, factor H binding) and resists bacteriolysis</td>
<td>Serum bactericidal assay taken as an index of immunity</td>
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<tr>
<td>N. gonorrhoeae resists phagocytic destruction</td>
<td>N. gonorrhoeae invades neutrophils and partially survives within vacuoles</td>
<td>Resistance to intracellular killing is partial</td>
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<td>Hence N. gonorrhoeae can survive whatever the immune system develops against it</td>
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Gonorrhea

- Gonorrhea
  - It is an acute, inflammatory and infectious disease of the mucus membrane of the genito-urinary tracts of both men and women
  - Commonly infects the pharynx as well as the eyes
  - Incubation period is 3-5 days after contact with an infected partner.
  - More than 800k infected annually in the U.S.
GONORRHEA CAUSES BLINDNESS - ARTHRITIS INVALIDISM AND MISERY
IT CAN BE PREVENTED AND CURED

For Examination and Free Pamphlets
Go to your Doctor or Dept. of Health Clinic

ST. GEORGE — 61 STUYVESANT PLACE
STATEN ISLAND CASE FINDING PROJECT
Gonorrhea Sequelae

Urethritis
Lymphadenitis
Ophthalmia
Gonorrheal vulvovaginitis
Gonococcal pharyngitis
Anal gonorrhoea
Dysuria
Urethral discharge
Prostatitis
Inguinal lymphadenitis
Pelvic pain/Pelvic Inf. Dis.
Fever
Gonorrhea Diagnosis

- Urine sedimentation
- Swab of infected area
- Gram Stain
- Culture
- NAT
Gonorrhea Treatment

• Antibiotics
  ▪ Sulfonamides
  ▪ Penicillin
  ▪ Tetracycline
  ▪ Fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin)
  ▪ Oral Cephalosporins (ceftriaxone, cefixime)

• New Treatment
  ▪ Azithromycin + gemofloxacin
Antibiotic Resistance

It is estimated that >30% of all cases in 2013 have multi-drug resistance

ANTIBIOTIC RESISTANCE IN GONORRHEA
Antibiotic Resistance to many drugs is conferred by plasmid DNA.  
17Yr old patient sample

*N. gonorrhoeae* is readily transformable and picking up surrounding genes is natural for this organism.

Some genes encoded on the plasmid include:
- Penicillinases
- Antibiotic Pumps
- Transfer Genes
Bacteriophage Life Cycles

1. Phage attaches to host cell and injects DNA.
2. Phage DNA circularizes and enters lytic cycle or lysogenic cycle.
3A. New phage DNA and proteins are synthesized and assembled into virions.
3B. Phage DNA integrates within the bacterial chromosome by recombination, becoming a prophage.
4A. Cell lyses, releasing phage virions.
4B. Lysogenic bacterium reproduces normally.
5. Occasionally, the prophage may excise from the bacterial chromosome by another recombination event, initiating a lytic cycle.

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Bacteriophage Encoded Proteins
Bacteriophage Encoded Proteins

- Bacteriophage encode genes that allow them to:
  - Degrade, manipulate host DNA
  - Integrate their DNA into the chromosome
  - Accessory proteins to help them hack polymerase machinery
  - Structural Genes
Bacteriophage in *N. gonorrhoeae*

- No known expressed phage
- Phage associated genes (>30) in genome
  - These genes are from lysogenic phage – incorporated into the genome.
Bacteriophage as therapy?

- Isolating and characterizing phage from different strains of *N. gonorrhoeae* may result in our ability to produce a phage cocktail that can be used to treat antibiotic resistant Gonorrhea.

- But does it work?
Phage Therapy Successes

• Personalized medicine approach
• Isolate phage from patients and give back cocktails at a higher dose
• 1936 S. typhimurium
• Phage Therapy Center in Rep. Of Georgia
# Phage Therapy vs. Antibiotics

<table>
<thead>
<tr>
<th>Bacteriophages</th>
<th>Antibiotics</th>
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<tr>
<td>Very specific (i.e., usually affect only the targeted bacterial species); therefore, dysbiosis and chances of developing secondary infections are avoided (15).</td>
<td>Antibiotics target both pathogenic microorganisms and normal microflora. This affects the microbial balance in the patient, which may lead to serious secondary infections.</td>
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<tr>
<td>Replicate at the site of infection and are thus available where they are most needed (59).</td>
<td>They are metabolized and eliminated from the body and do not necessarily concentrate at the site of infection.</td>
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<td>No serious side effects have been described.</td>
<td>Multiple side effects, including intestinal disorders, allergies, and secondary infections (e.g., yeast infections) have been reported (76).</td>
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<td>Phage-resistant bacteria remain susceptible to other phages having a similar target range.</td>
<td>Resistance to antibiotics is not limited to targeted bacteria.</td>
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<td>Selecting new phages (e.g., against phage-resistant bacteria) is a relatively rapid process that can frequently be accomplished in days or weeks.</td>
<td>Developing a new antibiotic (e.g., against antibiotic-resistant bacteria) is a time-consuming process and may take several years (16, 51).</td>
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Isolation and Characterization of Phage derived from *Neisseria gonorrhoeae*
Pilot Program (BSL-2)
**10 Patients**

Can we grow Gonorrhea?

What strains are they? Antibiotic resistant?

Are there phage associated with these strain?

Molecular weight of genome? Proteins? Genes?

Do they infect and lyse hosts?

- Isolate clinical samples of *N. gonorrhoeae*
- Characterize clinical isolates of *N. gonorrhoeae*
- Isolate Gonorrhea-associated bacteriophage
- Characterize Gonorrhea-associated bacteriophage
- Conduct *in vitro* proof-of-concept experiments
Can we grow Gonorrhea?

Clinic collaboration: Obtaining patient samples

Requirements: CO2 incubator, BSL-2 facility, Modified Thayer Martin Medium, Tryptic Soy Broth
Strain Characterization (BSL-2)

**PCR:** Amplifying Antibiotic Resistant cassettes, Virulence Factors (Opa)

**Sugar Metabolism:** Glucose+, Maltose(-), Lactose(-)

**Requirements:** CO2 incubator, BSL-2 facility, Cystine trypticase broth/agar with sugars, Thermocycler, Gel-rig, Primers
Are there Phage present (BSL-2)?

**PCR:** Amplifying Phage associated genes from DNA preps of concentrated culture supernatants generated via filtration (size exclusion)
- based on known sequences in N. gon. Genome

**SDS-PAGE on Supernatants**

**Requirements:** CO2 incubator, BSL-2 facility, Tryptic Soy Broth Thermocycler, Gel-rig, Primers, SDS-PAGE gel and rig, positive control proteins?
Characterize Phage (BSL-1, 2)

**PCR:** Amplifying Phage associated genes from DNA preps of concentrated culture supernatants generated via filtration

**Genome evaluation:** Molecular weight, genetic sequencing?

**SDS-PAGE / Western Analysis?**

**Requirements:** CO2 incubator, Media, Gel Rigs, Antibodies to phage?
Infection Assays

Do Phage only infect N. gonorrhoeae? What about other Neisseria species? What about other gram negative bacteria?

Grow up phage from samples of 10 patients
Isolate phage
Create cocktail
Plaque Assays
Plaque Assays

1. λ phage stock
2. E. coli culture
3. Molten top agarose
4. 50°C H₂O bath
5. 37°C overnight

Multiplicity of Infection (MOI) = 0.1

Each plaque contains ~10⁶ isogenic λ phage
- LPS release: Holin/Lysin system mutation
- Antibody response to Phage (1961 study)
  - If N. gon. is evading the immune response will phage be protected in vivo?
- Phage Persistence in Presence of Bacterial Host within animal Host?
If it works, we have a potential Phage Therapy

Pre-clinical work in animals!

- Pre-clinical efficacy assays? N. gon is obligate human.

vs.

Homeopathic remedy created as a supplement to antibiotic therapies?
BSL-1 Projects

• Primer validation, DNA (wcRT)

• Amplify Phage Genes from TCDC-NG08017, clone into expression vectors, make purified Phage Proteins

• Create antibodies to Phage, N. gon proteins for use in Western Blots

• Create a Diagnostic Assay (ELISA based?)
Thank you!

• Sudo Room
• Counter Culture Labs for Hosting

GETit founding members:
  ▶ Ami Knop Ullrich
  ▶ Louis Huang
  ▶ Nina DiPrimio

Supporters