The Silent War – Wound Infections: past, present, and future

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Defense Health • Global Health

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Research was conducted under an IACUC-approved animal use protocol in an AAALAC International-accredited facility with a Public Health Services Animal Welfare Assurance and in compliance with the Animal Welfare Act and other federal statutes and regulations relating to laboratory animals.

Throughout history, war has decided the fate of civilizations. Decided borders, provided advancements, but caring for the wounded and remediating infection could have impacted outcomes

Journey through time

- Even the earliest humans knew about the importance of wound care and keeping skin intact. Once a breach, early scientists must have tried anything they could.

- Archaeologic sites showed that Neanderthals and early *homo sapiens* used herbs to treat injury and fever

Hamilton 2017

Ancient Medicine – India, China, Mesopotamia, and others

Animals	Plants	Minerals
Bile	Bark	Alum
Blood	Dyes	Antimony
Butter	Fruits	Arsenic
Cobwebs	Herbs	Copper salts
Egg whites	Honey	Lead salts
Lard/Grease	Leaves	Mercury salts
Meat	Oils	Potassium salts
Milk	Resins	Tar
	Sap	Zinc
	Sugar	
	Turpentine	
	Wine/Vinegar	

Forrest 1982

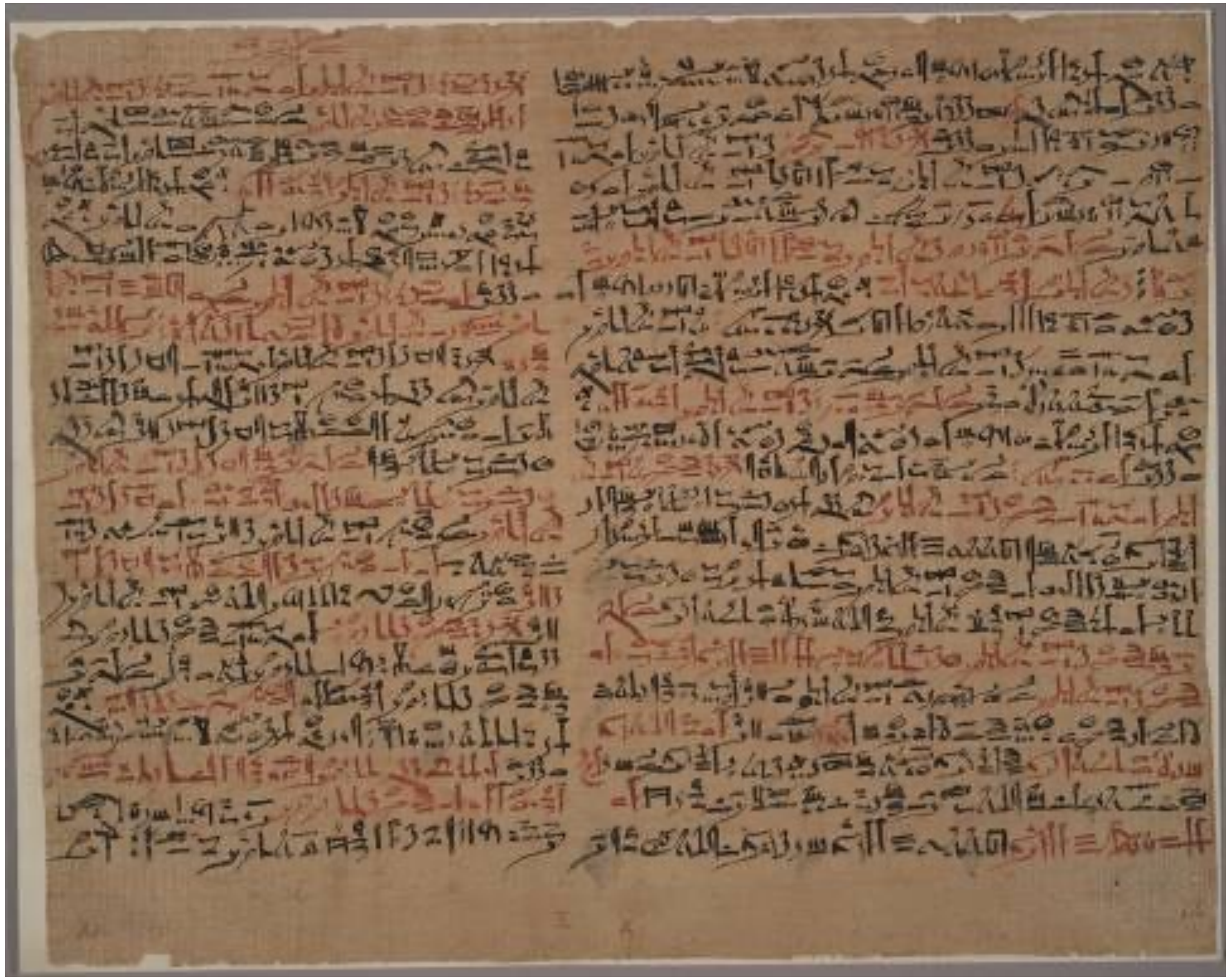
Egyptians

- Pressure sores and dressings/evidence of treatment have been found on 5,000 year old mummies.
- The ancient Egyptians often used honey as a wound treatment.
- The 1700 B.C. Edwin Smith Surgical Papyrus – a dressing over "fresh meat" – add powdered alum and honey.
- A later document (Ebers Papyrus, 1550 B.C.) relates the further use of various concoctions and dressings containing honey (antibacterial properties), lint (absorbent properties), and grease (barrier) for treating wounds.

Bhattacharya 2012
Hobson et al. 2016

Earliest known written record: The Smith Papyrus

van Middendorp 2010



- Sold to Edwin Smith, antiquity dealer, in Egypt in1862.
- Dated to 1700 B.C.
- Outlines 48 separate injury/trauma cases.
- Seven specific cases discuss wound care.

One Translation:

Treatment: You have to bind it over fresh meat the first day. Afterward, you should treat with (powdered) alum and honey every day until he recovers.

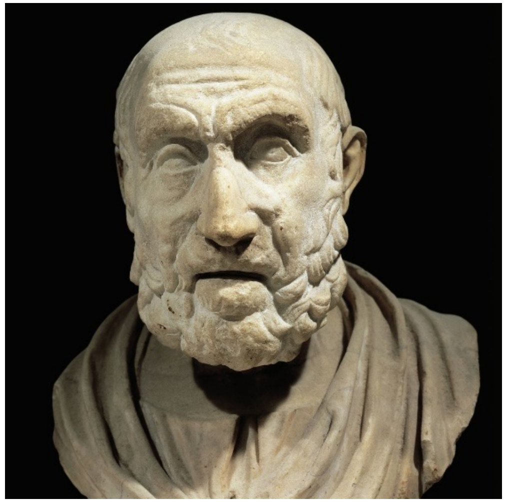
Greeks

- First writings of Homer in the Odyssey and Iliad (~800 B.C.) described very detailed injuries and treatments. Soldiers themselves used herbs such as yarrow root and even copper filings from a sword.

- Hippocrates, a Greek physician and surgeon, 460-377 BC, known as the father of medicine, used vinegar to irrigate open wounds and wrapped dressings around wounds to prevent further injury.

- He also washed ulcers with wine and after having softened them with olive oil. Then, he dressed them with fig leaves and herbs.

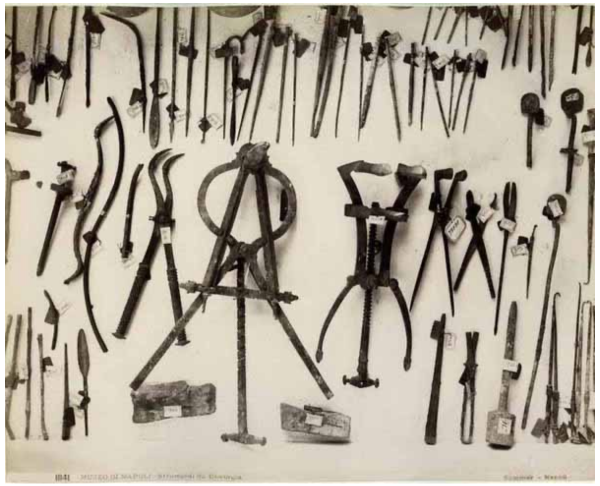
- He establish the four cardinal signs of inflammation: redness, swelling, heat and pain.



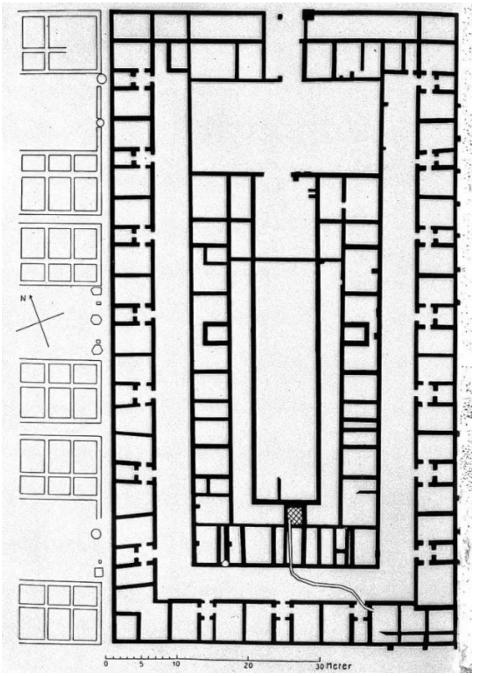
Bhattacharya 2012
Hobson et al. 2016
Minakakis 2016

Jalowiecki 2004

Romans



The Romans “industrialized” war and had the first practical wound care. Had the first, medics called the “medicus”, with different ranks (based on experience). Also, had the first, war hospitals, logistics/supply chain, and evacuation chain.



- **Galen**, a notable Roman surgeon, was first to recognize that pus from wounds inflicted by the gladiators led sometimes to poor outcome, but it preceded wound healing too.

- **Plinio** used mineral remedies such as lead and silver, Galen also used spice ointments. Some of their approaches were based on other methods of Hippocrates and Celsus.

- The goal was to get experienced legionnaires back to the field of battle. The Romans invested much money and time training their best. Wanted to keep the most experienced at the front.

Romans – A path to healing (100-400 AD)

- Series of medics/doctors – from the battlefield to the *valetudinarium* – the "battle hospital".
  - *Capsarii* were "first contact" medics.
    - They dressed wounds
    - Moved boxes with bandages (the so-called *capsa*),
    - Transported the injured to the hospital. Some soldiers volunteered to bring wound from the battlefield on horses.
  - *Medicus* more senior medic who directly managed the care specific the legion/army/camp.
    - *Medici ordinarii* – often Greek slave with medical training.
    - *Medicus duplicarius* ("double wage medic")
    - *Milites sesquiplicarii* ("soldier's 1.5 times wage") - legionary medics
  - *Others*: Hospital administrators (*optiovaletudinarii*), secretaries (*librarii*), instructors (*discentes*), magicians and herpetologists (*marsus*), vets (*pequarius*), vets for e.g. foals (*pollio*), vets for camels (*ad/cum camellos*).
- Hospitals could accommodate ~5-10% of 5000-10000 legionnaires (two accounts had ~400 wounded/Tacitus). Hospitals were always secluded, separate from the main camps and near a clean water source. They were aware of "invisible little creatures" that could contaminate water and wounds. They boiled water and planted/grew herbs outside the hospital to be used for wound care.

Jalowiecki 2004, Dougherty 2020, Forrest 1982

Romans – the path to healing (100-400 AD)

Patient Care:

1) Surgical instruments were cleaned with boiling water.
2) Drug the patient with poppy opium or black henbane seeds.
3) Remove any foreign material and slow bleeding (cobwebs/honey/vinegar), but not completely stop.
4) Debridement (remove any non-viable, not red tissue)
5) Clean and irrigate with wine or vinegar.
6) Suture in ”soft” areas of the body, otherwise leave open. If it wound opens, keep it open.
7) Dress the wound with wool/cotton/linen/flax on and area around wound. Ointments/treatments applied at regular intervals.
8) If an infection develops (gangrene), amputation may be necessary, but be sure to have some flesh cover the remaining bone.
9) Massage wound area to improve blood flow.

Jalowiecki 2004
Dougherty 2020
Forrest 1982

Romans – Wound treatment

Wound treatment salves:

1) Wine/Vinegar/honey – Initial treatment, often boiled.
2) Combination of (often diluted in wine):
   a) Salts – copper sulfate, copper oxide, lead oxide, silver
   b) More vinegar
   c) Nuts
   d) Oils – Olive or Rose
   e) Herbs
   f) Flowers
   g) Garlic
   h) Grease (lard)
   i) Fragrance – myrrh/frankincense – both bactericidal and fragrant
3) Dressing changed once or twice a day with reapplication. Kept moist until healing.

Jalowiecki 2004
Dougherty 2020
Forrest 1982

American Civil War: 1861-1865

- General Ulysses S. Grant once famously demanded that onions be sent to him before he would move his army.
  - At the time, they were used to treat powder burns.
  - Garlic also used for infections.
  - Present - antimicrobial agents such as **ajoene** and **allicin** found in garlic and onions.

- Francis Porcher, a botanist, was commissioned to find and catalogue plants native to the southeastern US that could be used as medicines in their place.
  - Porcher compiled a book of his findings, including 37 plant species to be used as antiseptics
  - Treating gangrene and other infections.

- Samuel Moore, the Confederate Surgeon General, published a field guide of native plant medicines to be used by battlefield physicians.
  - Including methods of collection, preparation, and administration.
  - Infection was a leading cause of death for soldiers in the Civil War and was often treated with amputation.

Dettweiler et al. 2019

The Lister effect – Sterility matters!

- Joseph Lister, a Professor of Surgery in London, recognized that antiseptics could prevent infection.

- Lister placed carbolic acid into open fractures to sterilize the wound and prevent sepsis.

- Changes were also made to sterilize the surroundings of a wounded patient.

- Hand washing prior to care along with sterilization of instruments as well as wearing of gowns, masks and gloves began in 1880s.

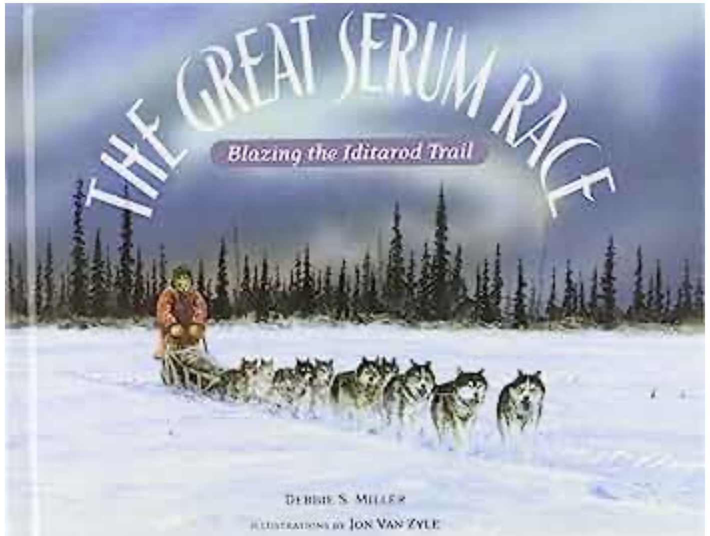
- Germs are the cause of everything!

Van May 2016

Pre-WW1 and the roaring 20's

Two major modes to fight bacterial infection:

- Bacteriophage
- Serum – antibodies (anti-toxins) made primarily in horses.



Balto

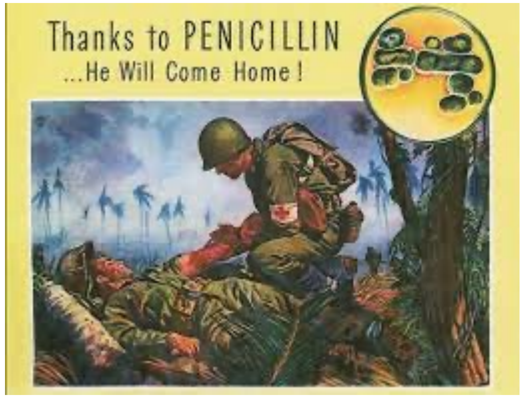
Togo

Zurawski and McClendon 2020

WW1/WW2/Korea/Vietnam

- Evacuation times protracted in WW1 and WW2.
- Gangrene becomes #1 killer in WW1 because of trench warfare.
- *Clostridium* and other Gram-positive organisms primary problem.

- **Alexander Fleming** discovers penicillin in 1935, takes 10 years to commercialize it. However, has a role in preventing infection during WW2. First time bacterial infection isn't the #1 killer during war.

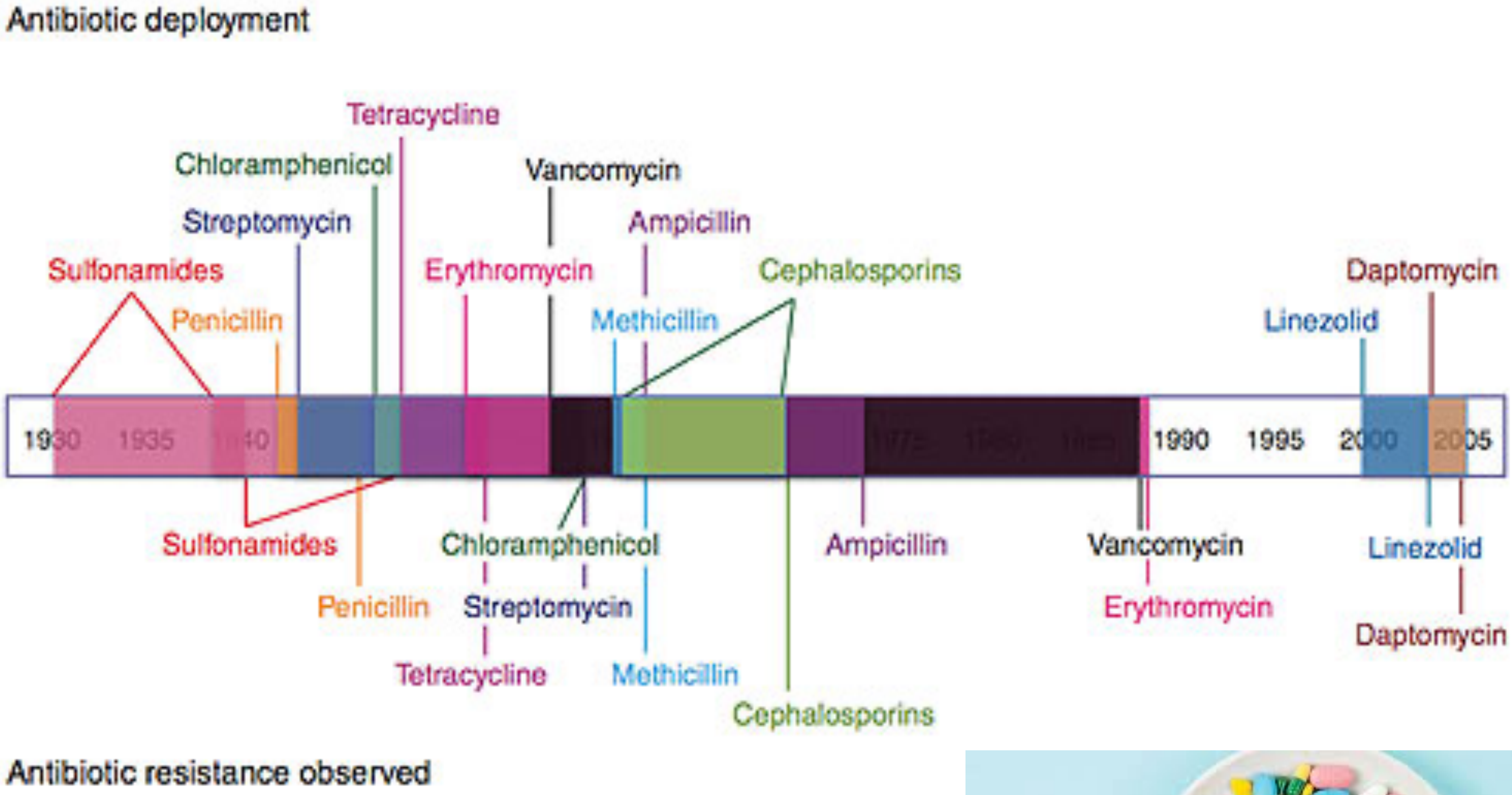


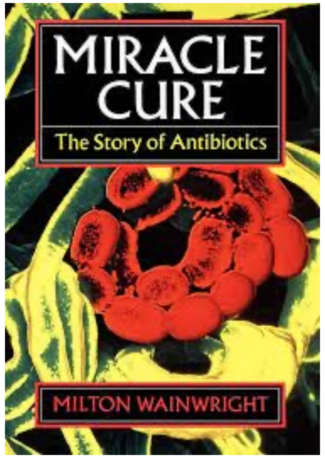
- **1950 – 1965 – Golden Age of Antibiotics**
  - Over 20 new classes of antibiotics found and brought to patients
  - Infection rates plummet, but also start to see first signs of resistance.

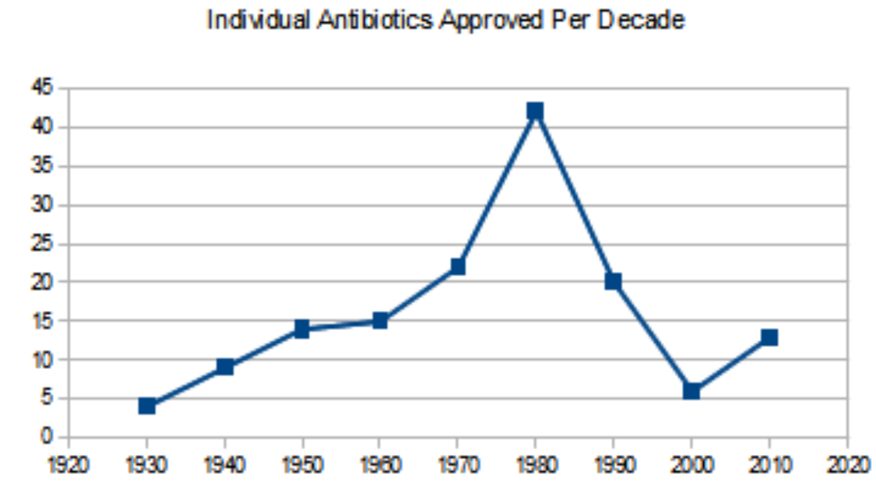
- **Vietnam** – Sepsis still major killer (38%) after 24 hours of injury/polytrauma. More of an emergence of Gram negatives in wound infection to include *Pseudomonas* aeruginosa and *Acinetobacter baumannii*

Blyth et al. 2015
Lewis 2013

Golden Age of Antibiotics – 1950 - 1965

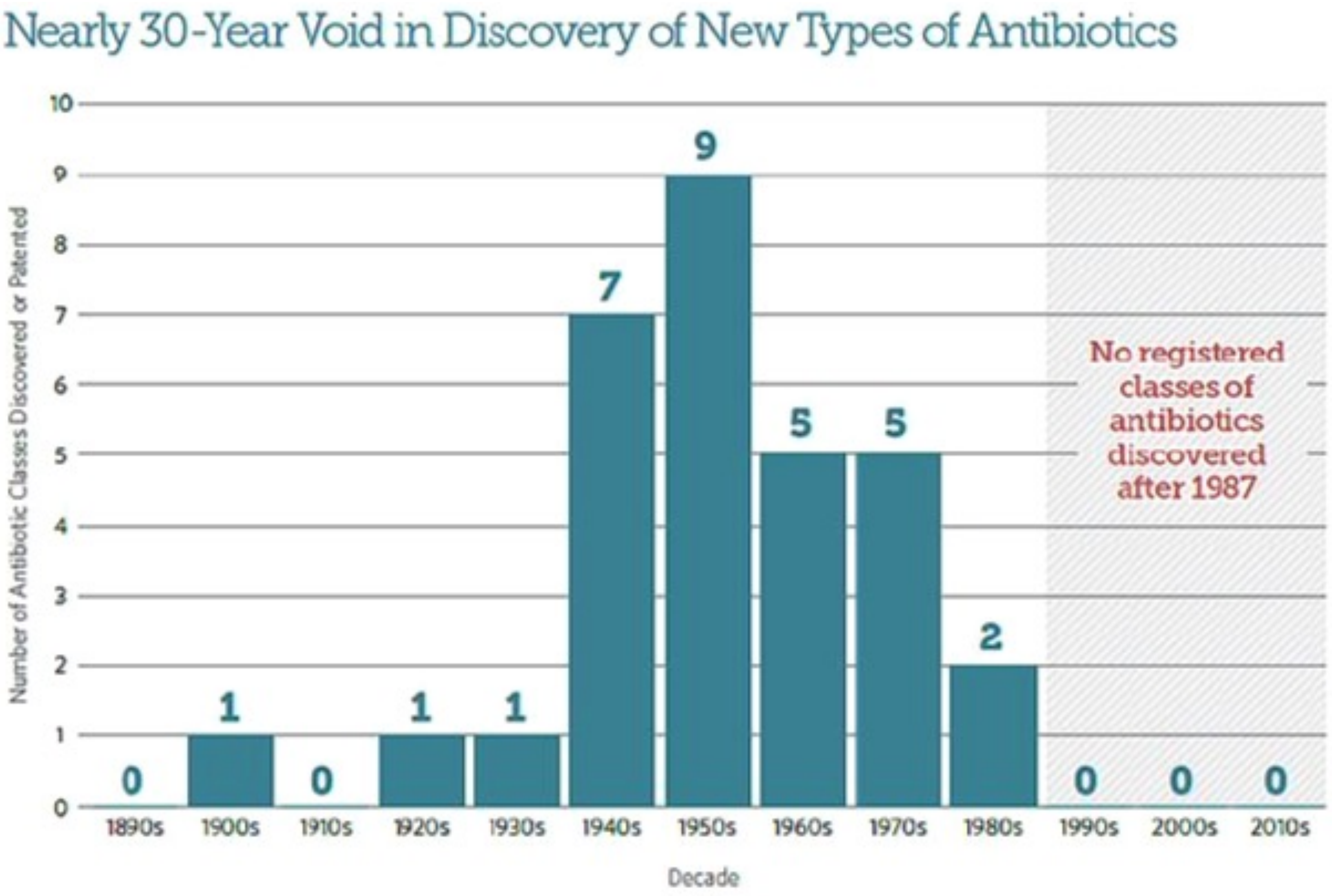






Matt Cooper 2012
Scott Alexander 2014

Present Day – The AMR Crisis

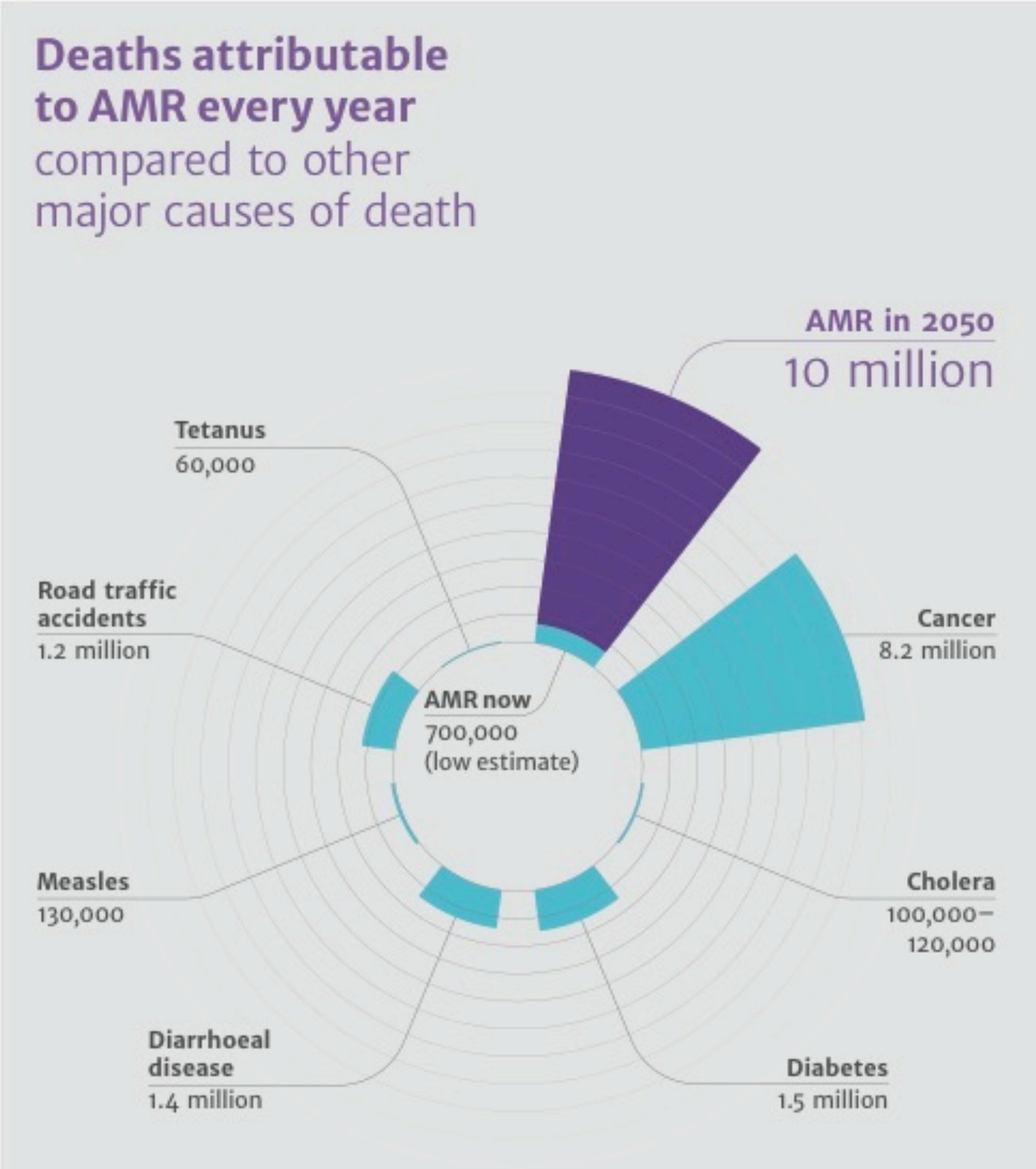


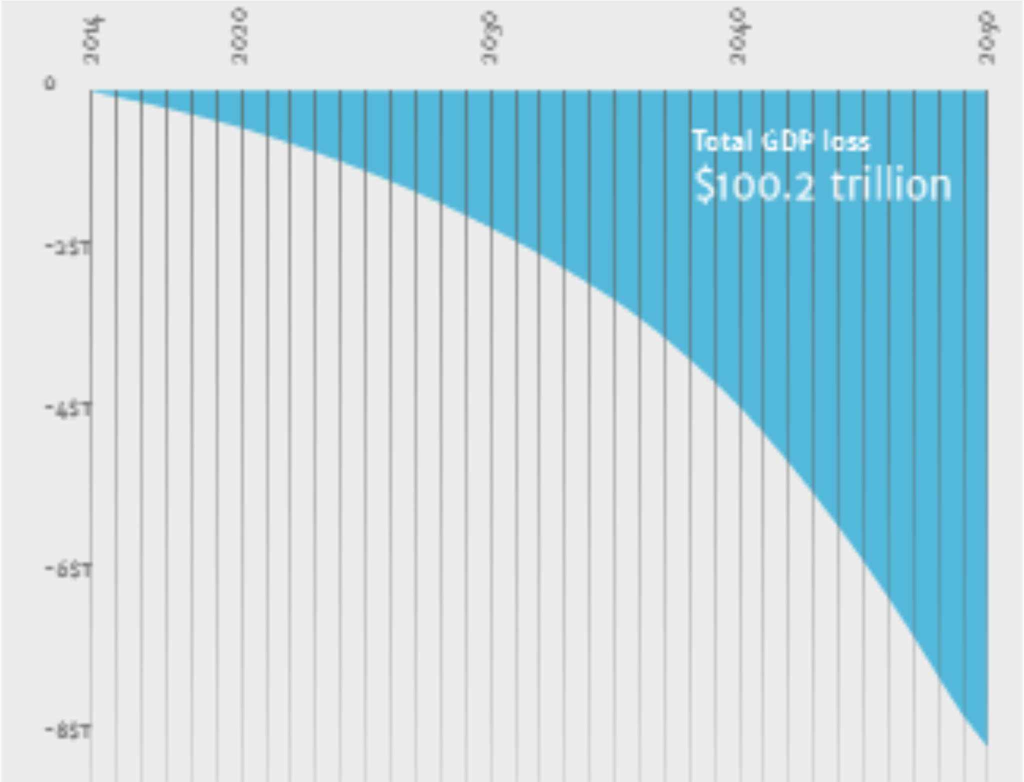
Resistance is on the rise with every major class of antibiotics, and

there are pan-resistant strains, and new antibiotics are not being developed at a quick enough pace.

Pew Charitable Trust, 2016

ESKAPEE Pathogens – World wide problem



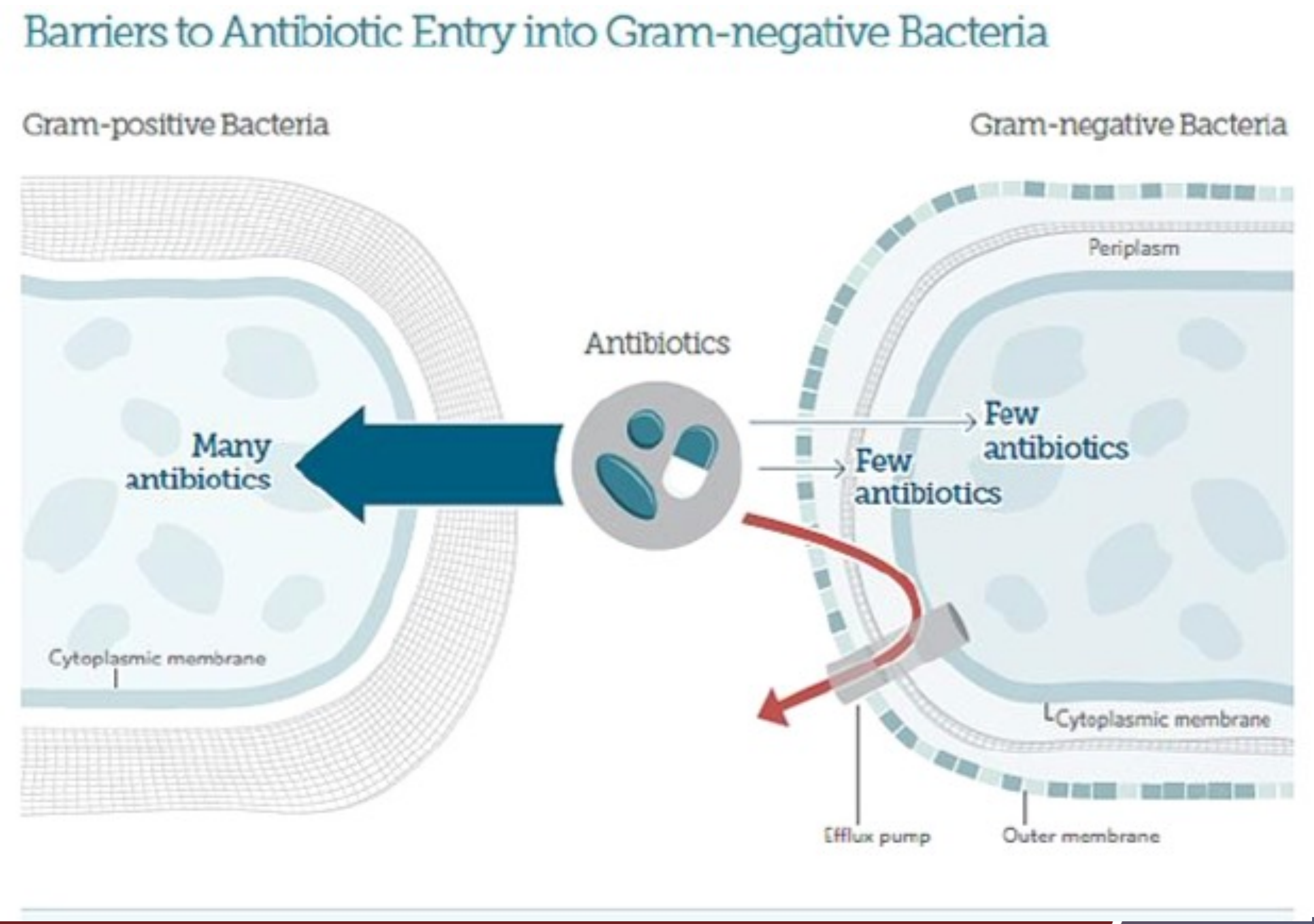


- *CDC estimates (2019)*
  38,000 deaths/year - AMR
  75,000 - 100,000 deaths - sepsis

- *Another recent report suggests: > 10M infections/year – 4.5 million deaths worldwide (Lancet 2023)*

Slides from AMR 2014 - Sir Jim O'Neill

Gram-negative bacteria are tougher to target



Pew Charitable Trust, 2016

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

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Research Letter

Six Extensively Drug-Resistant Bacteria in an Injured Soldier, Ukraine

Patrick T. Mc Gann, Francois Lebreton, Brendan T. Jones, Henry D. Dao, Melissa J. Martin, Messiah J. Nelson, Ting Luo, Andrew C. Wyatt, Jason R. Smedberg, Joanna M. Kettlewell, Brain M. Cohee, Joshua S. Hawley-Molloy, and Jason W. Bennett

Author affiliations: Multidrug-Resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA (P.T. Mc Gann, F. LeBreton, B.T. Jones, H.D. Dao, M.J. Martin, M.J. Nelson, T. Luo, J.W. Bennett); Landstuhl Regional Medical Center, Landstuhl, Germany (A.C. Wyatt, J.R. Smedberg, J.M. Kettlewell, J.S. Hawley-Malloy); 512th Field Hospital, Rhine Ordinance Barracks, Germany (B.M. Cohee)

Suggested citation for this article

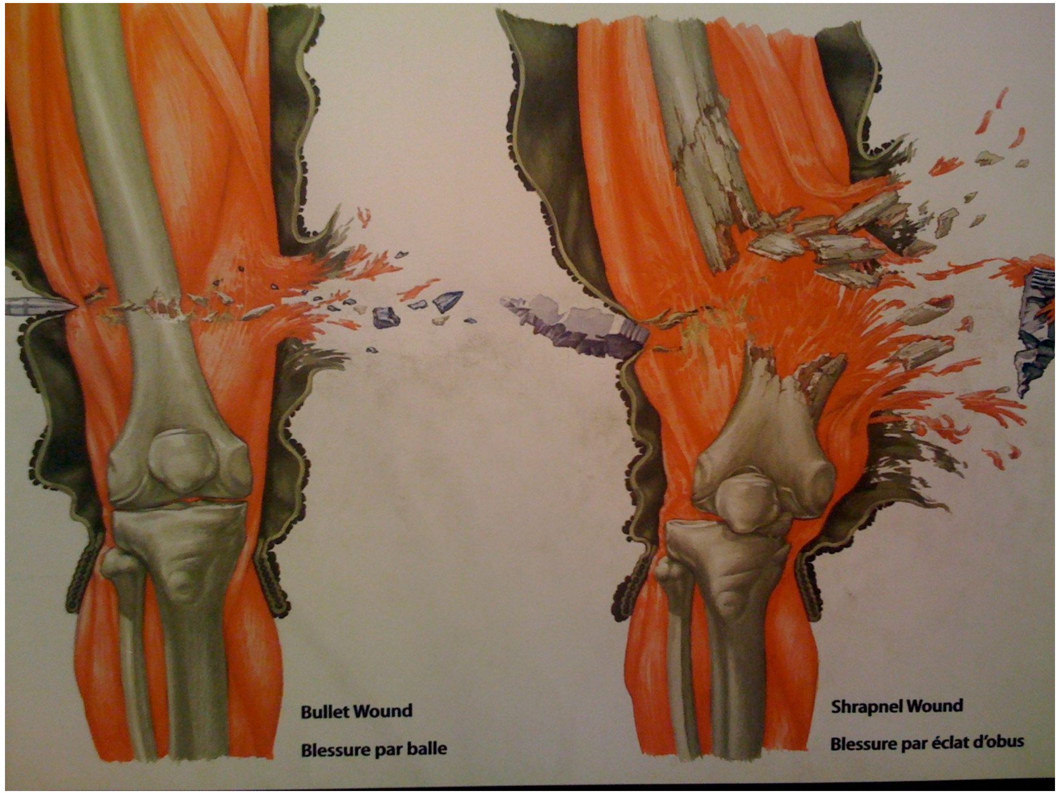
Abstract

Blood and surveillance cultures from an injured service member from Ukraine grew *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterococcus faecium*, and 3 distinct *Pseudomonas aeruginosa*

Combat Trauma

Characterized by:

- Penetrating trauma
- Foreign body inocula
  - (metal fragments, rocks, dirt, etc)
- Bone and soft tissue disruption
- Nerve damage
- Localized ischemia
  - (Tourniquet / edema)
- Blood loss
  - (often severe, >10U) - 1:1:1 – pRBCs, plasma, Plts
- Devitalized tissue
- Systemically disturbed physiology
  - Immune system dysfunction



http://onlyhdwallpapers.com/military/science-war-ammunition-drawings-bullet-shrapnel-wound-drawing-desktop-hd/

Combat Wound Infection

Combat-related wound infections

- Unique to military
- Clinically challenging
- Can have unique microbiota
- Enduring threat – any conflict
- Main pathogens same as Civilian

ESKAPEE bacterial pathogens

Enterobacter cloacae
Staphylococcus aureus
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterococcus faecium
Escherichia coli

- **Estimated 34% / ~15,000 wound infections**

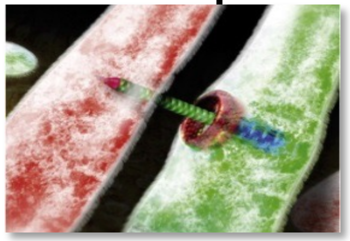
Wounded Numbers in OIF + OEF +OND +OIR +OFS (2016)	
WIA	52,536
Deaths from Wounds	5381
Amputees	1645

Sources:
DCAS website
Fisher et al. 2015
Tribble et al. 2019

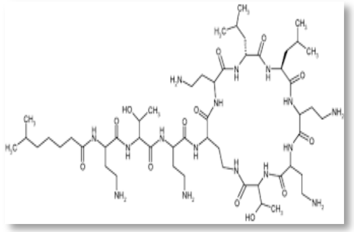
Wound Infections Dept. (WID/WRAIR)

Mission

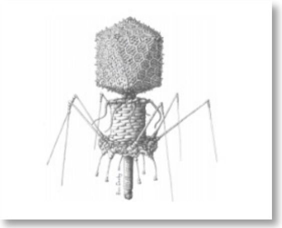
Defeat combat-related wound infections



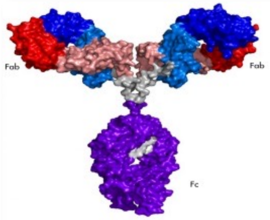
Host Immunology



Small molecules

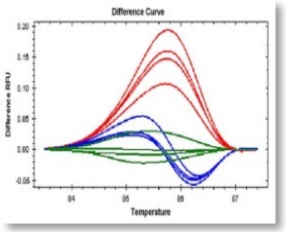


Phage

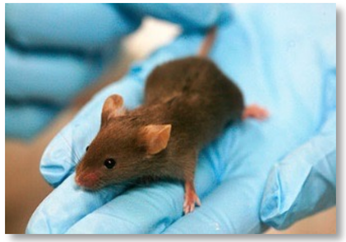


Monoclonal antibodies

Everything else



Diagnostics



Animal Models

Structured around ***lines of effort***

Modern Lens – Three Pillars: Three main antibacterial approaches

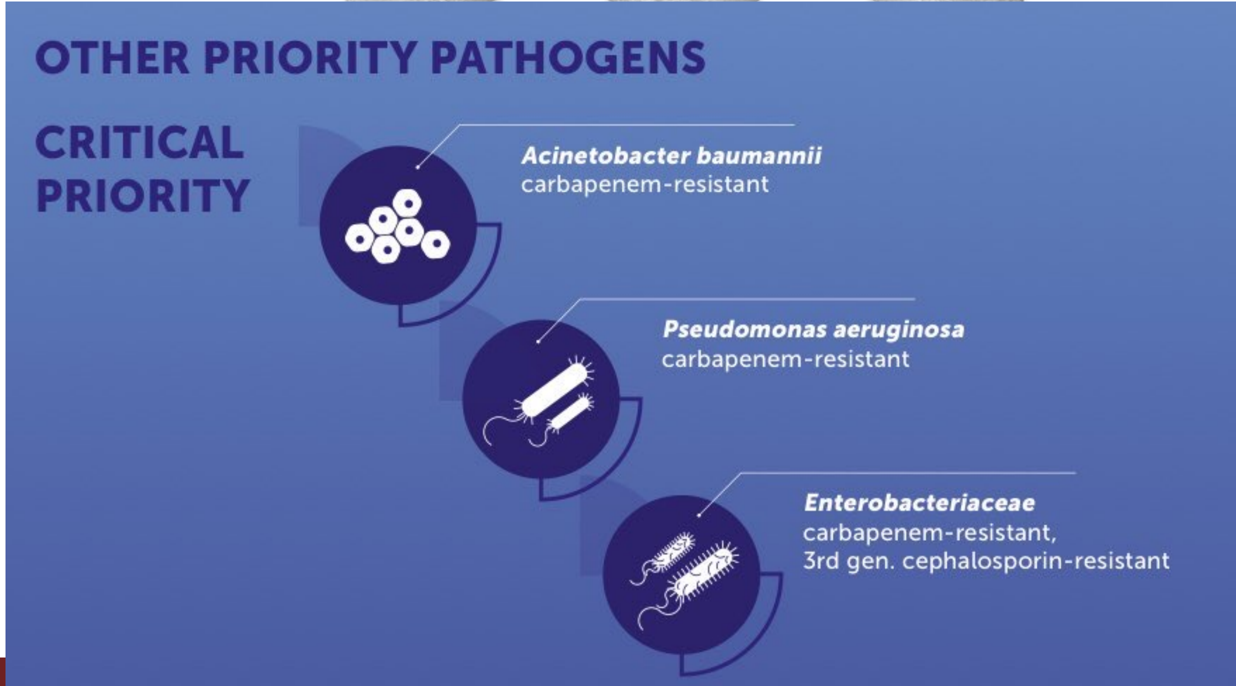
The countermeasures:

1. **Small molecules & antibiotic adjuvants**

2. **Monoclonal antibodies**

3. **Bacteriophage**

All approaches/testing systems use *ESKAPEE* pathogens as the model organisms



WHO Priority Pathogens List

"Iraqibacter"… *Acinetobacter baumannii*

Superbug brought back by Iraq war casualties
By Colin Brown , Deputy Political Editor
Wednesday 08 November 2006

Forbes.com
Military Chases Mystery Infection

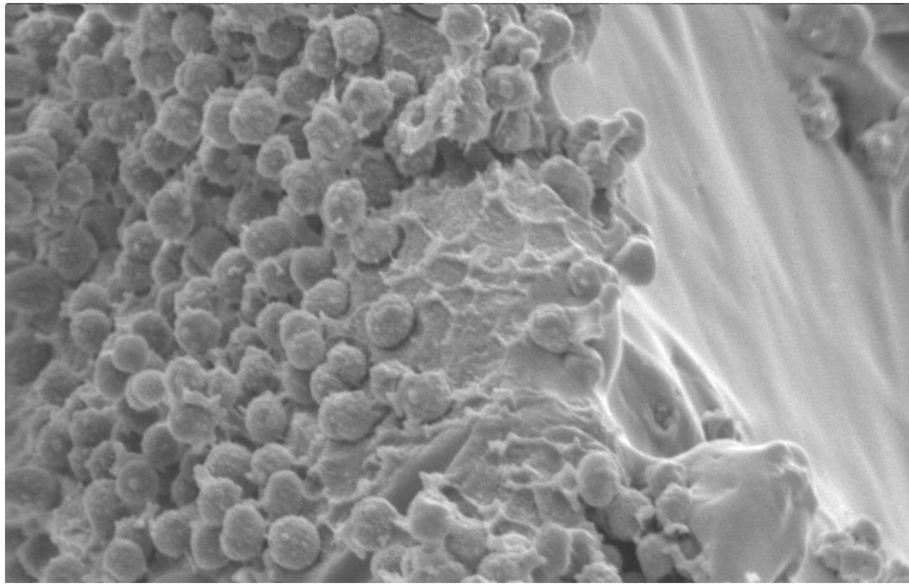
epi Medical News & Exposé
The Acinetobacter threat

Pentagon to Troop-Killing Superbugs: Resistance Is Futile

What makes a Superbug.....Super?

No matter species, it comes down to just two, often linked, attributes:

- **Antibiotic resistance**
  - Wide variety – intrinsic and acquired resistance
  - Resistance genes move from bacteria to bacteria via plasmids (horizontal transfer)
  - Intrinsic mechanisms – efflux pumps, porins, biofilms
  - Often linked to virulence genes.
- **Virulence**
  - Genetic insertions that provide extraordinary benefits in the host environment.
  - Virulence can vary from strain to strain.
  - Virulence factors are also shared via horizontal transfer.
  - Examples – toxins, immune system evasion, proteins and polysaccharide required for biofilm.



Zurawski 2012

Drug Resistance Increases

- Last two decades - Multidrug-resistant (MDR) grows:
  - Almost always resistant to most 1st Generation drugs: cephalosporins, fluoroquinolones, and aminoglycosides.
  - >50% resistant rate to carbapenems (Peleg *et al*., 2008)
    Multidrug-resistant (MDR)
  - >80% resistant rate to carbapenems and 2nd/3rd Gen aminoglycosides and cephalosporins (Potron et al., 2015)
    extensively drug resistant (XDR)
  - Recent strains even resistant to colistin (pandrug-resistant) (Moffat *et al*., 2010; Srinivas and Rivard, 2017)

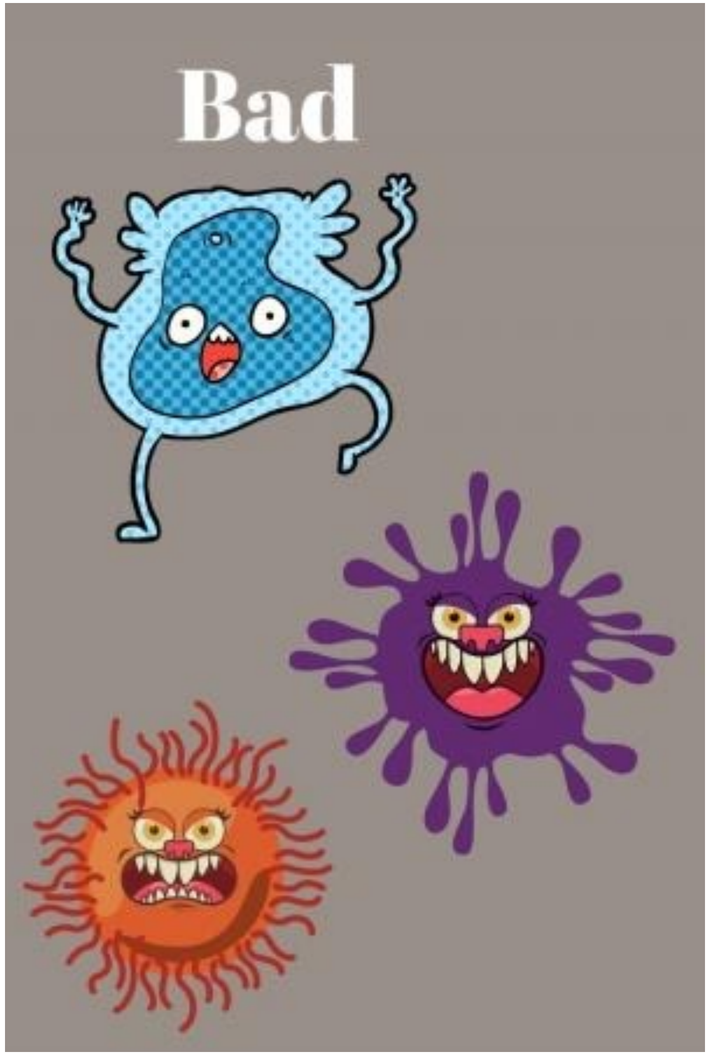
MDR

↓

XDR

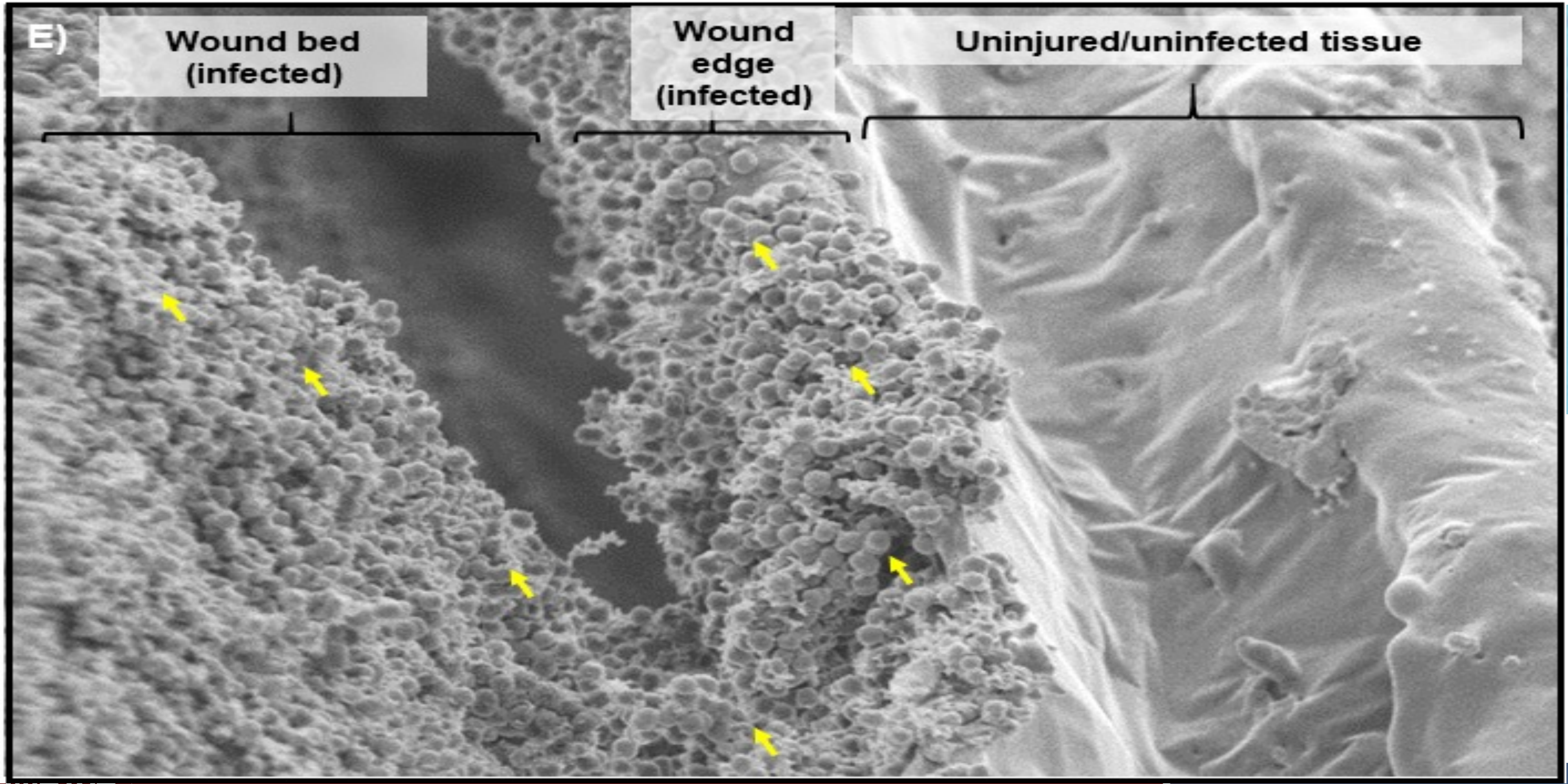
↓

PDR



To really, really, really bad….

A. baumannii is also problem with civilians

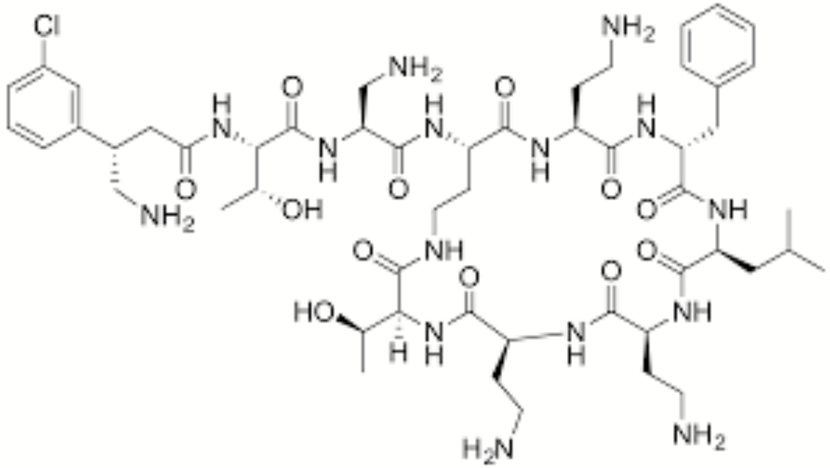


Small Molecules

- **Novel Antibiotics**
  - Combatting Antibiotic Resistant Bacteria (CARB)
    - Presidential National Action Plan (2015)
    - Novel small molecule screening – Experimental Therapeutics (ET)
    - Work with pharmaceutical companies with promising new antibiotics

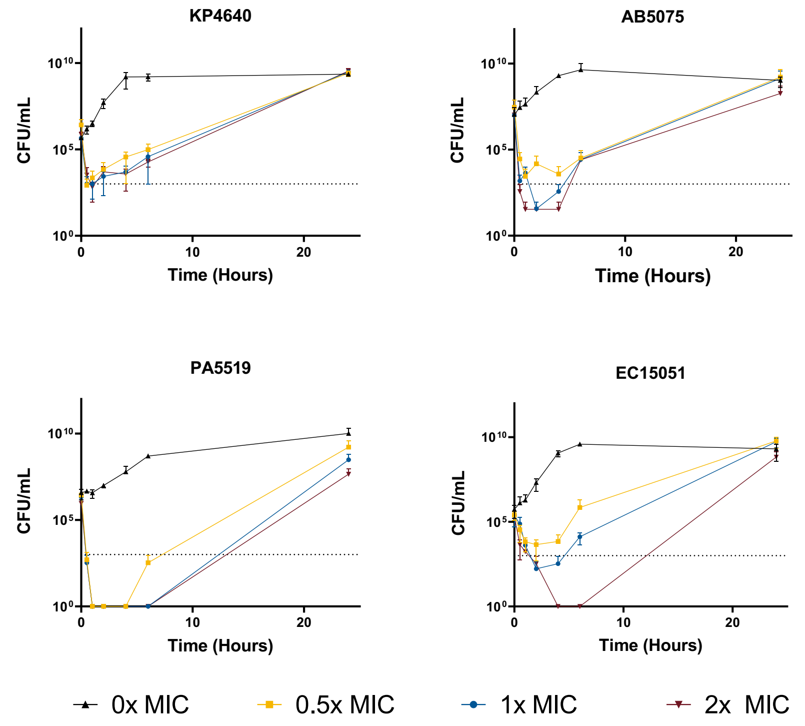
- **Antibiotic adjuncts**
  - Molecules that enhance efficacy current FDA-approved antibiotics or resensitize bacteria to Abx they were resistant to

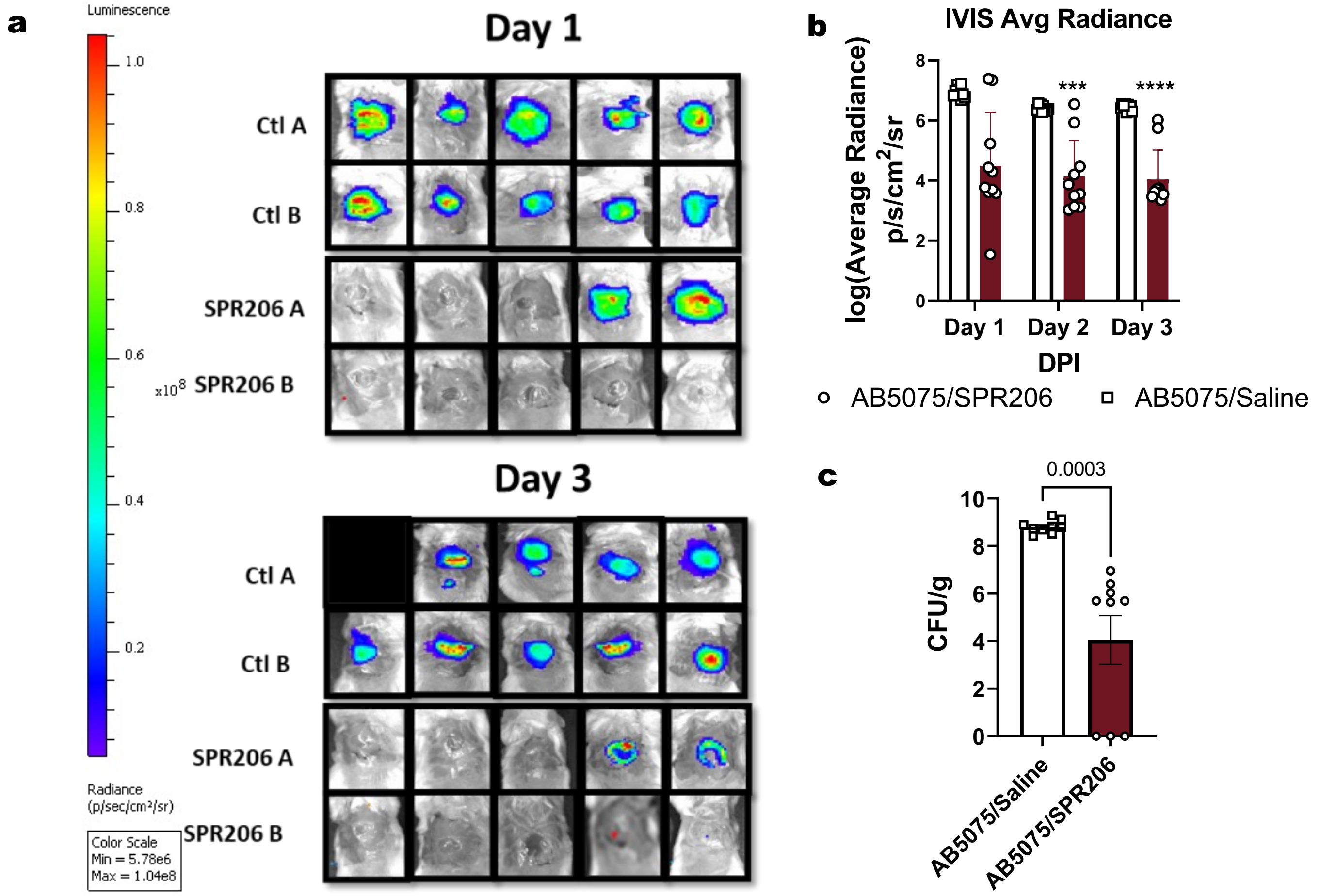
- **Spero Therapeutics, Inc.**
  - SPR206 – Novel polymyxin (no nephrotoxicity)
  - Disrupts cell membrane of gram-negative bacteria
  - Going into Phase 2 Clinical Trial in the fall.



SPR206 Data

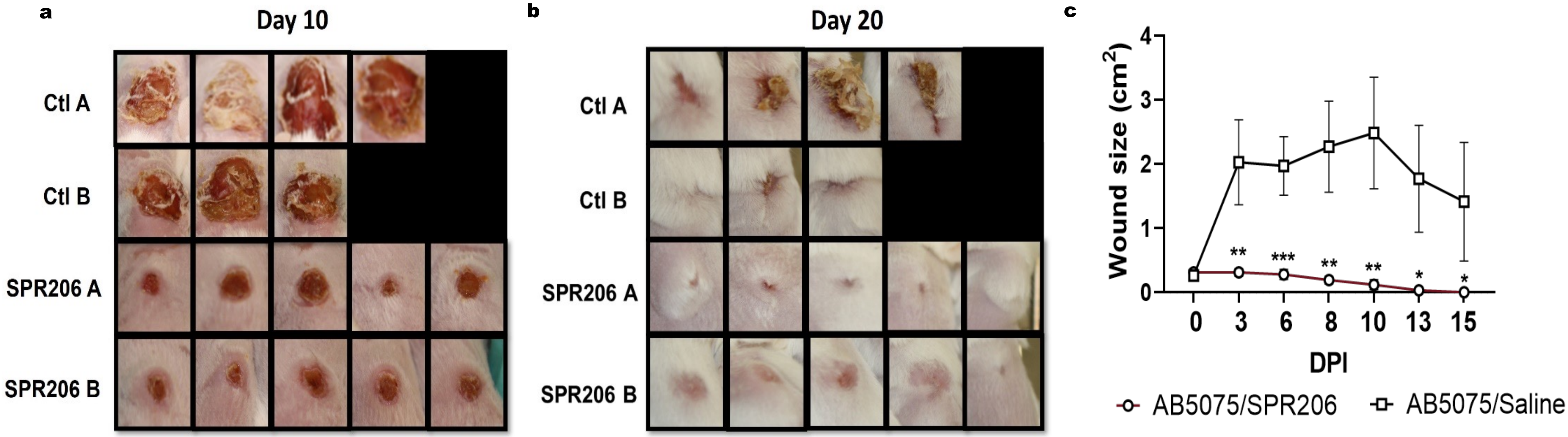
Bacterial Species	MIC_{50}	MIC_{90}
K. pneumoniae	0.063 µg/mL	2.0 µg/mL
A. baumannii	0.063 µg/mL	0.125 µg/mL
P. aeruginosa	0.5 µg/mL	2.0 µg/mL
E. coli	0.031 µg/mL	0.063 µg/mL





Fitzgerald *et al*. 2023, in review at *NPG Antimicrobials and Resistance*

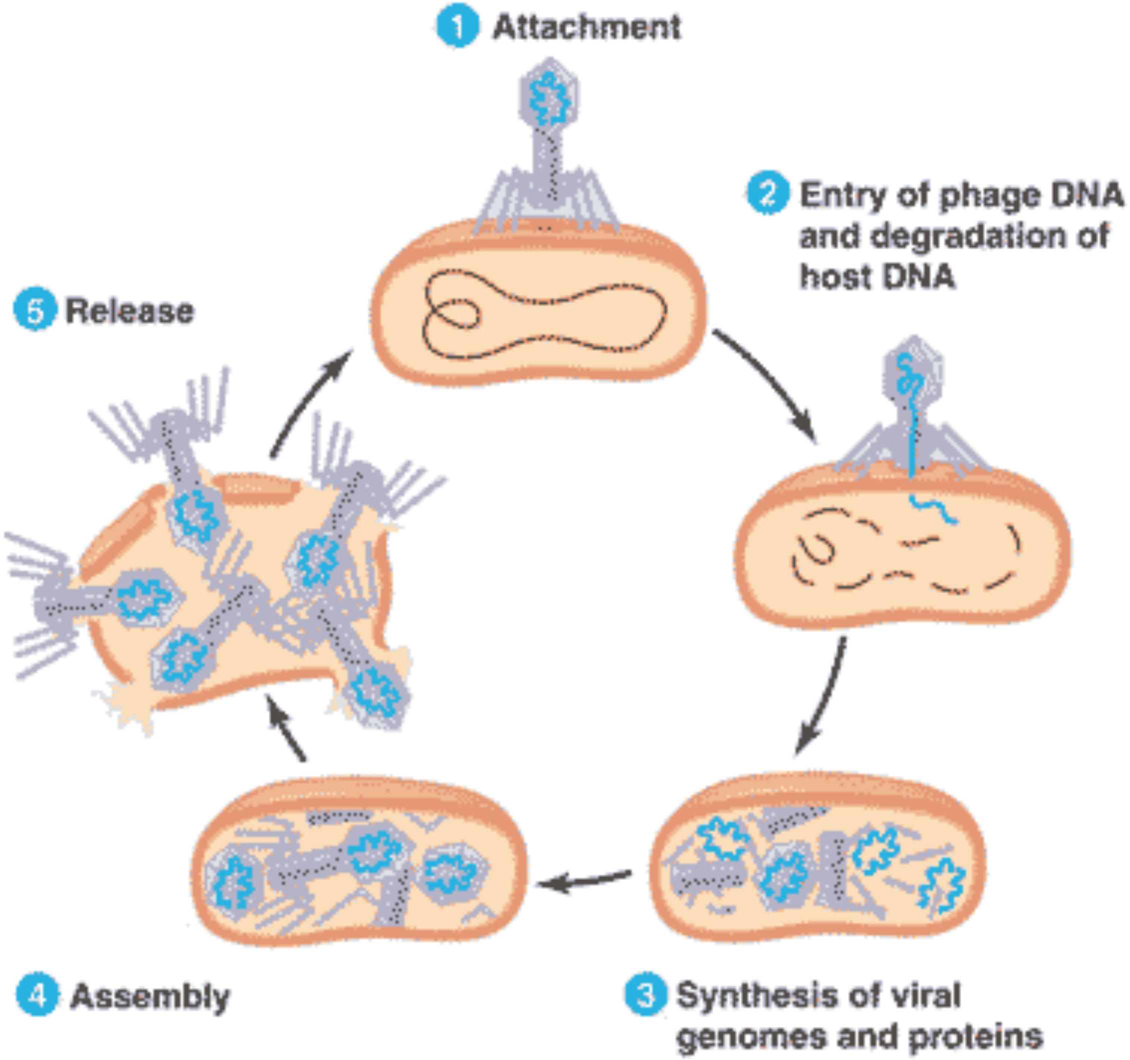
SPR206 Data



Fitzgerald *et al*. 2023, in review at *NPG Antimicrobials and Resistance*

Bacteriophages – what are they?

- Lytic phage infect, replicate inside, and destroy the host bacteria
- Used therapeutically in U.S. until the advent of antibiotic era
- Used in Eastern Europe and Russia, Ukraine, and Republic of Georgia

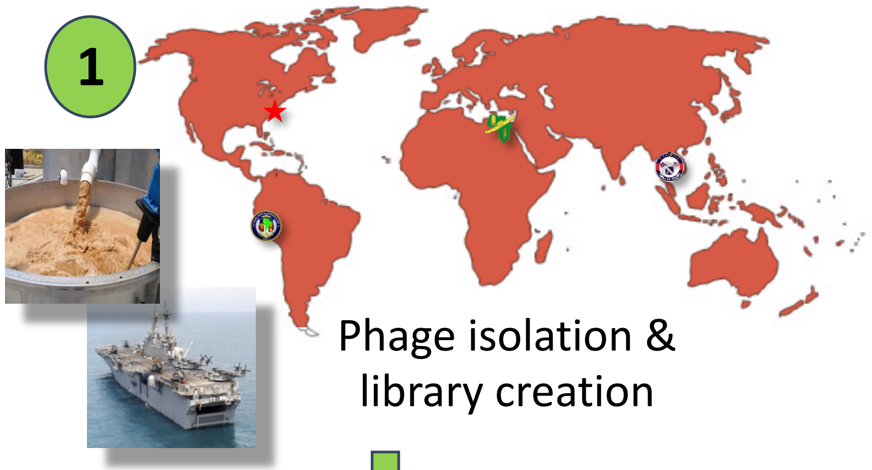


- Host range is specific to **<u>subspecies</u>** level, allowing for targeting of infectious bacteria without damage to host microbiome
- Phages can penetrate mature biofilms and cause bacterial cell lysis

https://www.quia.com/files/quia/users/lmcgee/genetics/APchapter18-Viri/phage-lytic-cycle.gif

Bacteriophage Therapeutic Pipeline

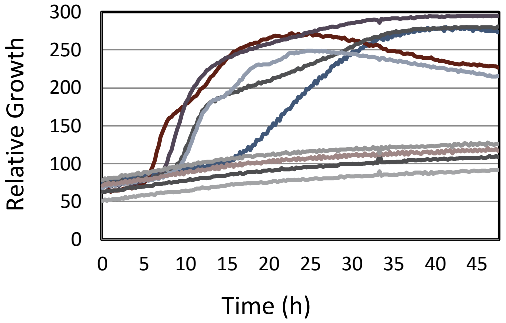
1



Phage isolation & library creation

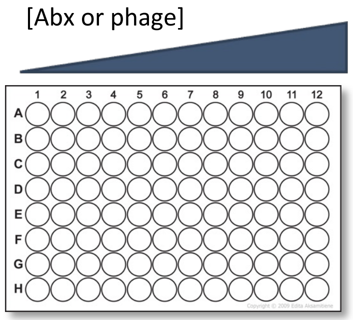
2

Library characterization & cocktail compounding



3

In vitro assessment



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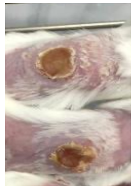
Pre-clinical proof-of-concept



Skin & Soft Tissue Infection

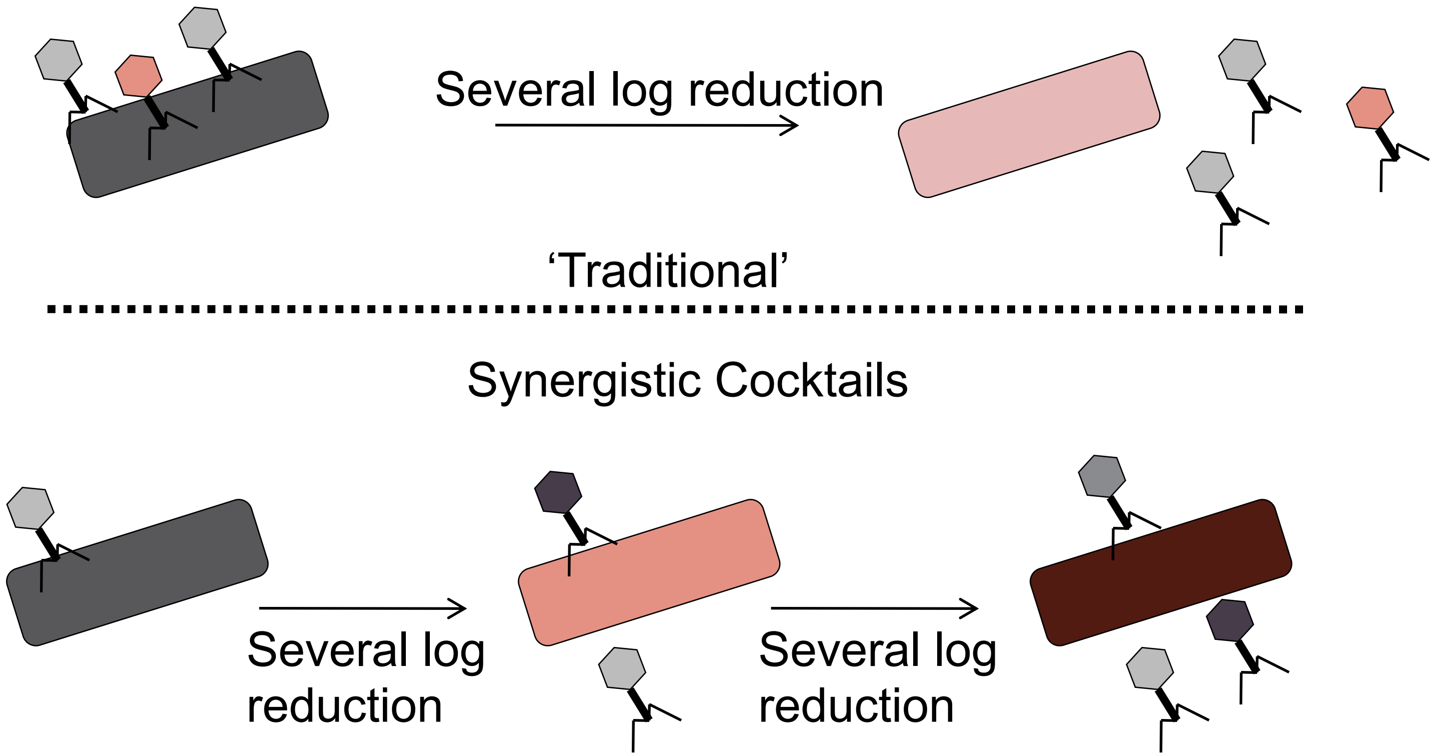
5

Pre-clinical models of clinical disease

Mouse Foreign Body Infection

Rat Femur Defect

Synergistic Phage Cocktails

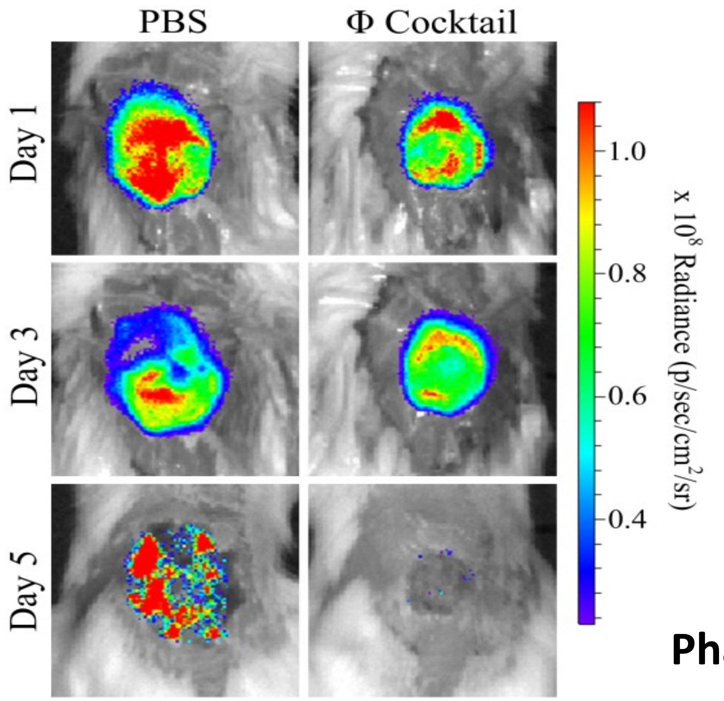


Phage resistance can alter virulence and induce re-sensitization to antibiotics.

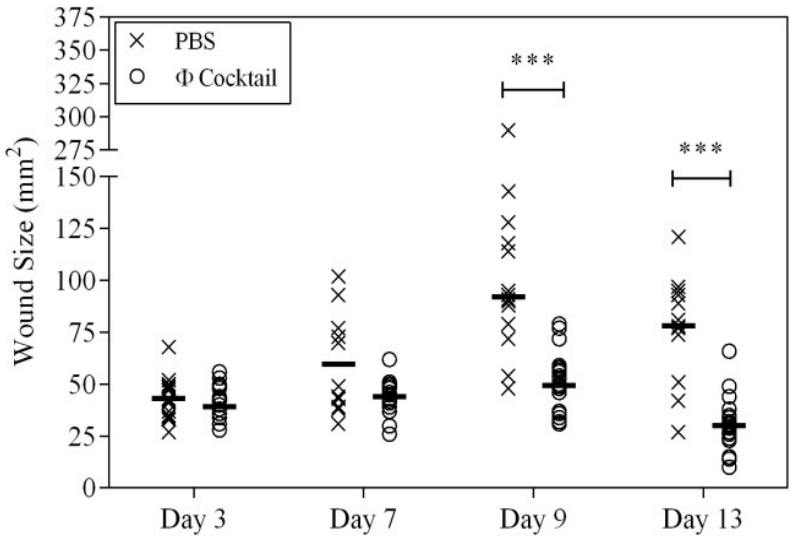
A. baumannii Phage Cocktail

5-member phage cocktail assessed in the SSTI model

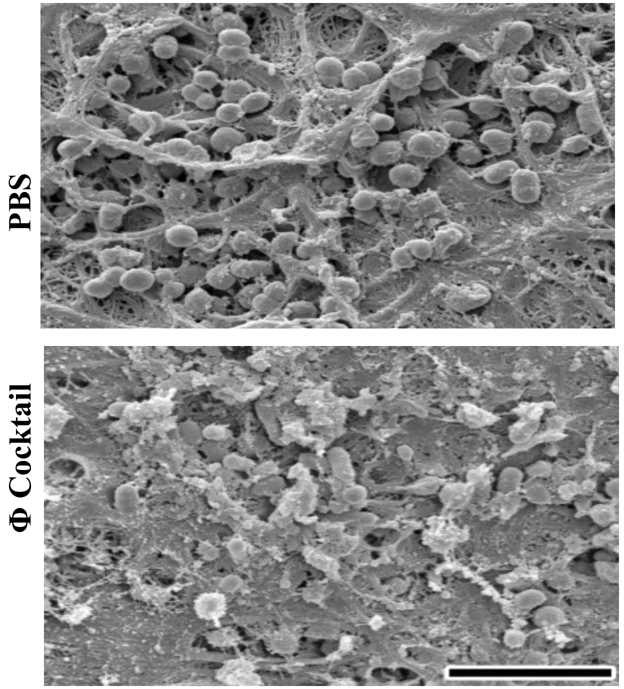
Wound Bioburden



Wound Size



Biofilm on Occlusive Dressing



Phage cocktail treatment resulted in: reduced bioburden

prevention of wound expansion

decrease in biofilm formation on wound dressing

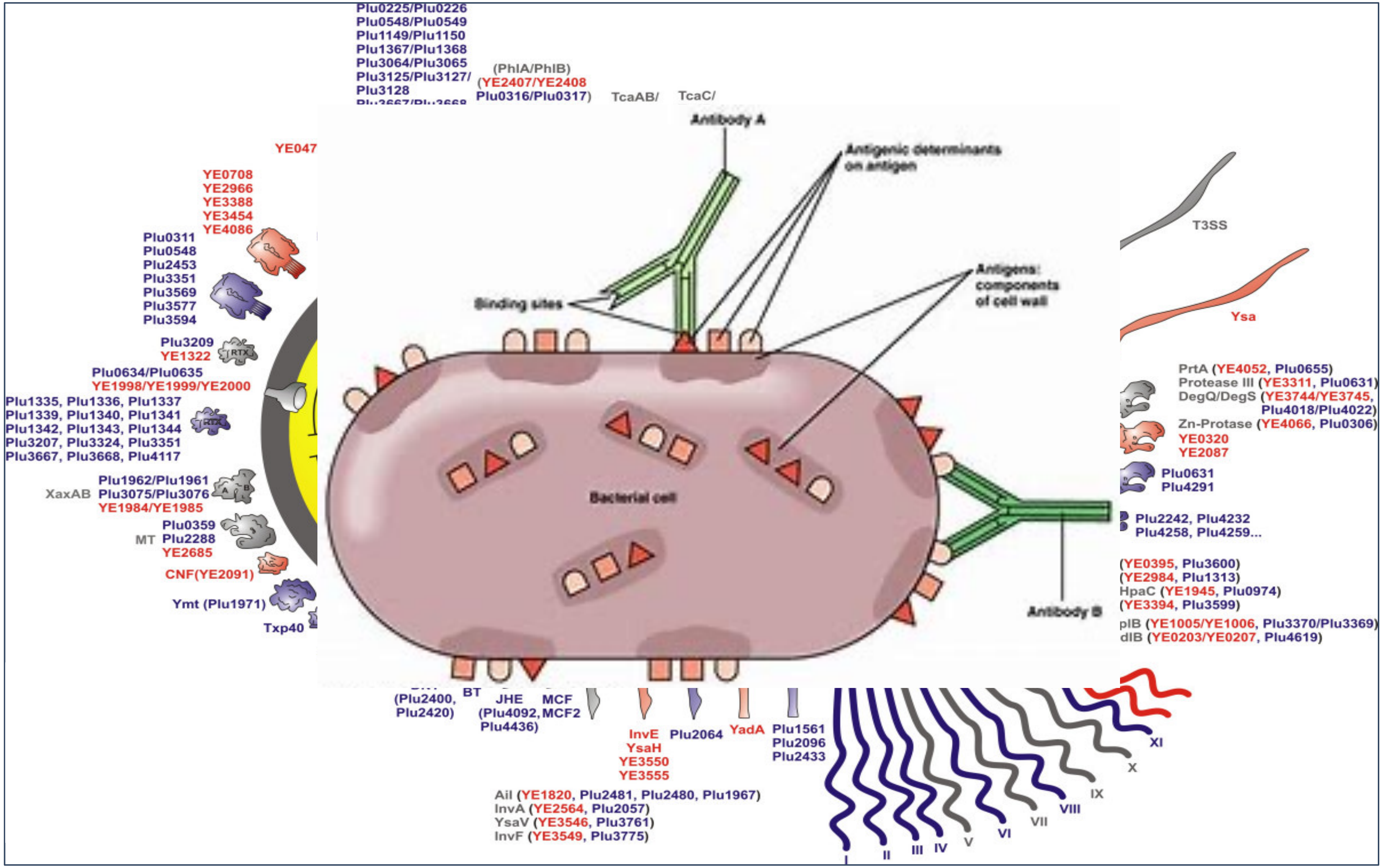
Regeimbal, Jacobs, et al. 2016, *Antimicrob Agents Chemother*

Immunotherapy (mAbs)

- **Why would we want to do this?**
  - Pre-antibiotic era – Monoclonal antibodies were in serum therapy (we know it works)
  - Currently, 7 mAb products in clinical trials for *S. aureus* and *P. aeruginosa*.
  - Used for cancer and autoimmune diseases (Over 90 FDA approved products)
  - There is no toxicity hurdle – leads to faster IND/FDA approvals. (i.e. COVID-19)
  - Can work with the standard of care (antibiotics) in synergy.
  - Can work with the immune system – macrophages/complement killing.

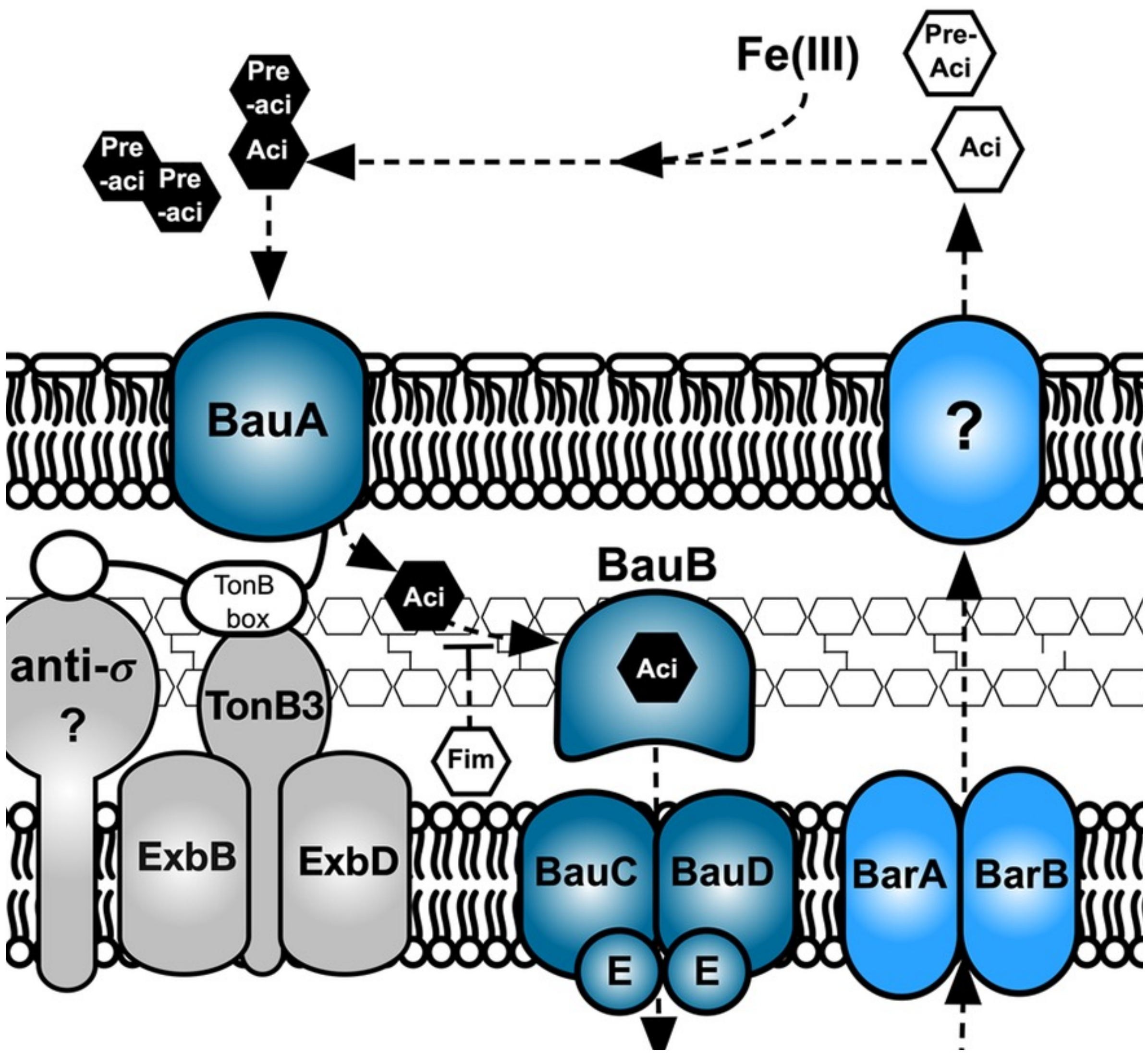
- **<u>Strategy</u>** –
  - "Broad spectrum" mAbs: hit multiple strains of same pathogen/proteins are conserved.
  - Narrow spectrum – only targets the bad bacteria.
  - Target multiple virulence targets
  - Target multiple epitopes

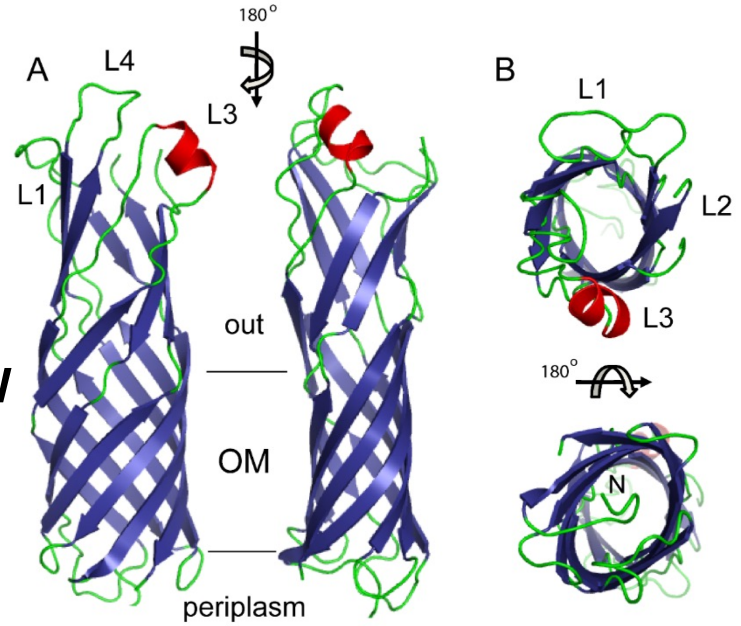
Virulence factors on the surface of bacteria

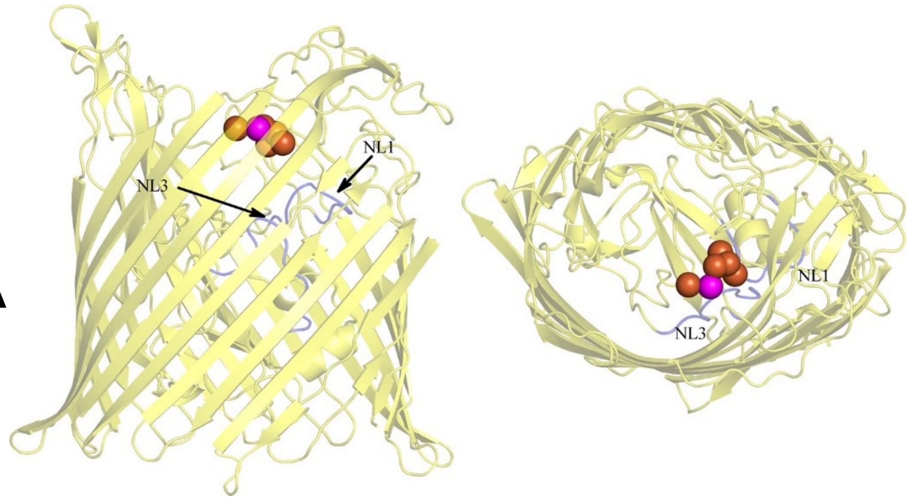


Davis et al., Microbiology, 4th ed.

OmpW and BauA capture iron from the body, but we can block this.







Jessica Sheldon and Eric Skaar, PLoS Pathogens (2020)

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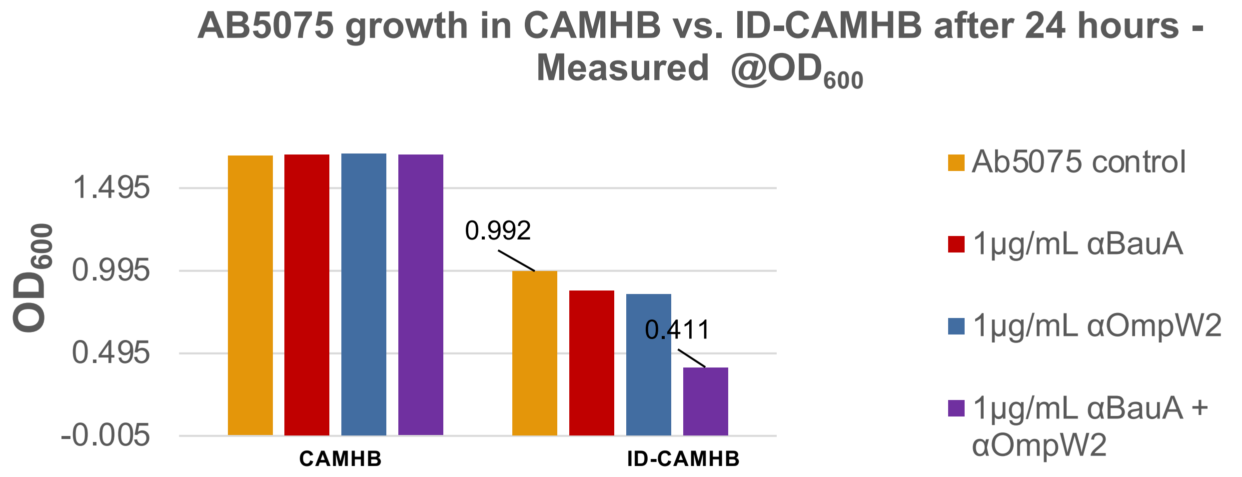
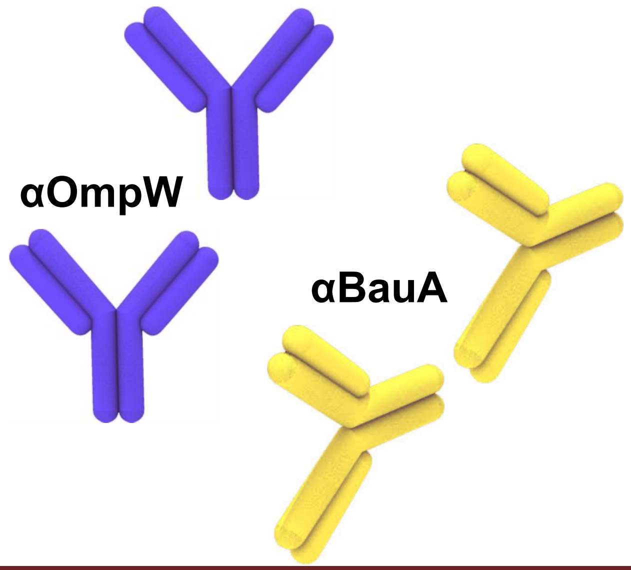
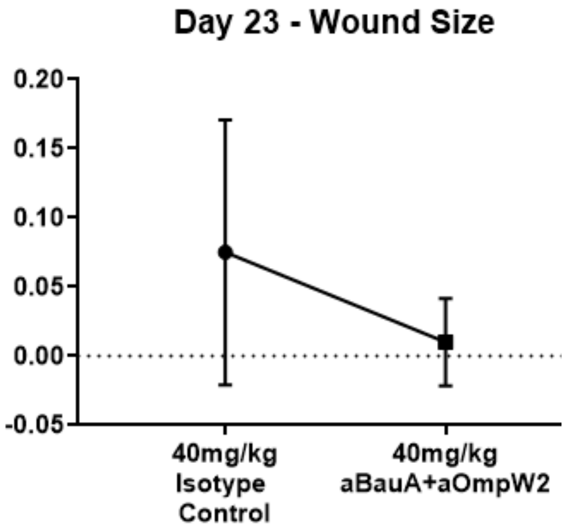
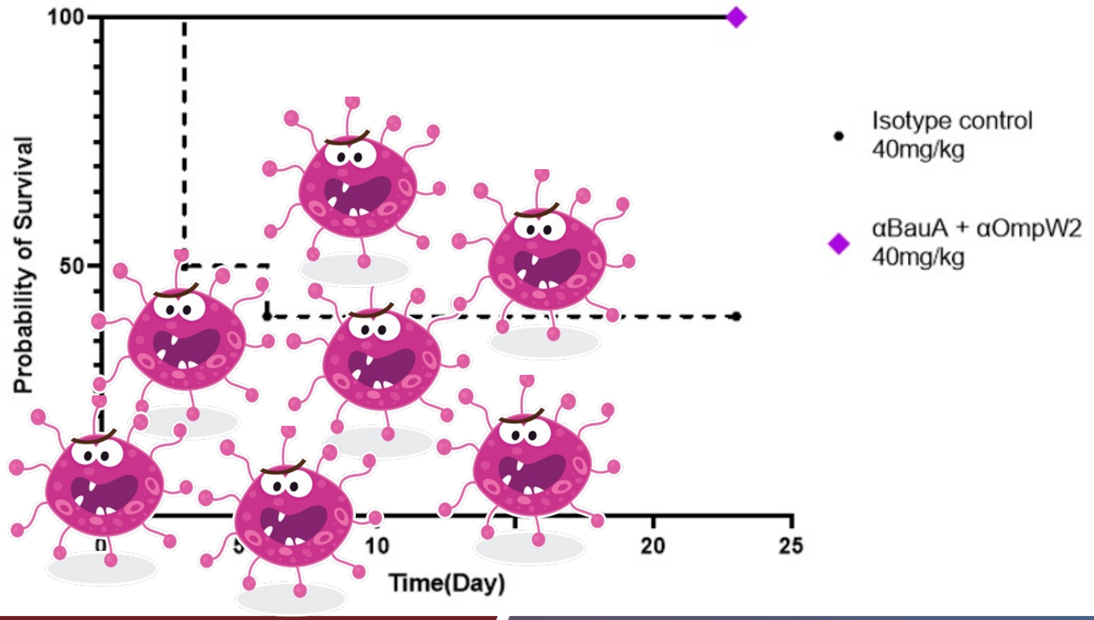




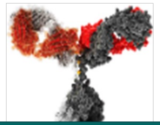




Approaches to improve outcomes

Pre-injury | Prevent

Monoclonal Antibody and Vaccine
Targeting pathogenesis factors
of MDR organisms for antibody



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SURVEILLANCE & STATE-OF-THE-ART ANAYLSIS

Proactive, worldwide surveillance via our CONUS, OCONUS labs

PREVENTIVE TREATMENT

Monoclonal antibodies and vaccines

DIAGNOSTICS FOR AUSTERE MEDICINE

Early warning/detection of infection

Identify biomarkers of early stages of sepsis

Enable precision medicine at point-of-injury

PRODUCTS FOR AUSTERE MEDICINE

Potent drugs with low toxicity

Stable phage cocktails

Polytrauma calibrated PK/PD profile of drugs

Multi-component gauze

Assess emerging tech

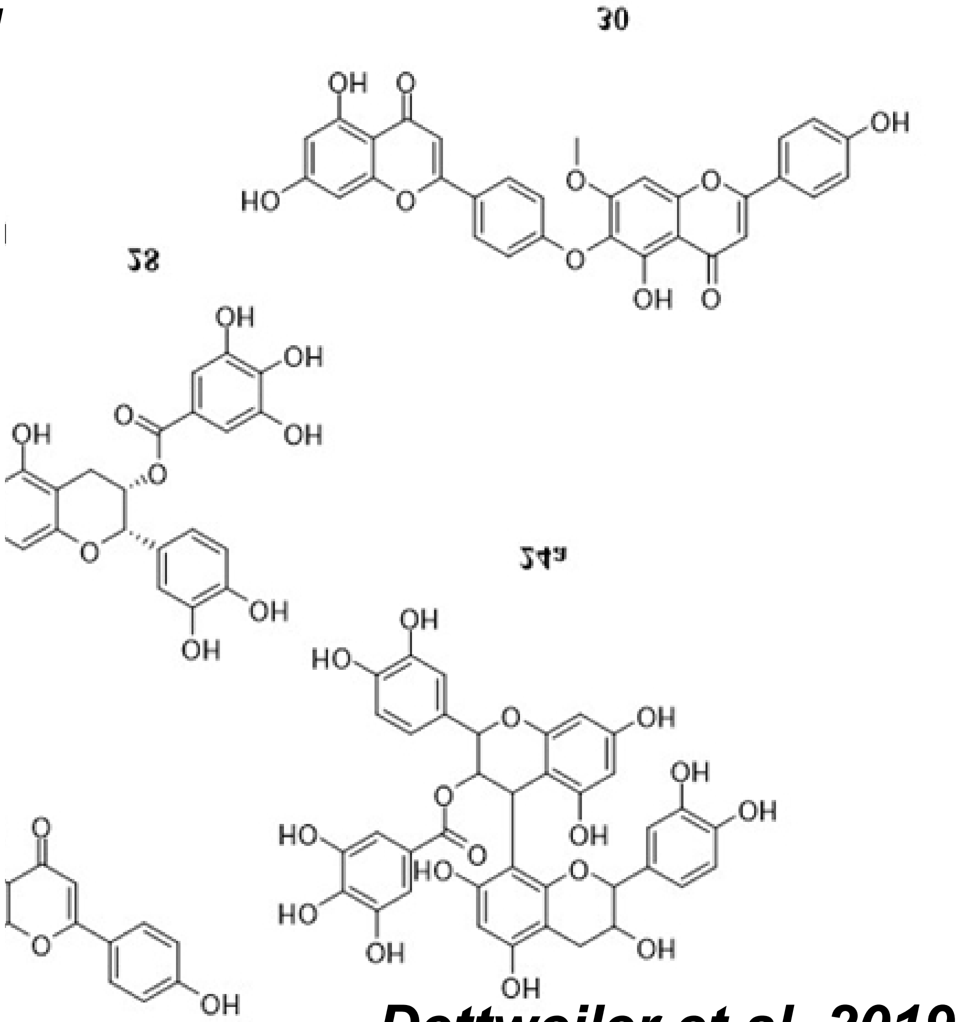
LEVERAGE FOR FUTURE SOLUTIONS

Use state-of-the-art system and repository of over 70,000 MDR samples to design new countermeasures

What does the Future hold?
A walk back through time

Natural Products – Civil War – Porcher Manual

White Oak
Tulip Tree
Devil's walking stick



Dettweiler et al. 2019

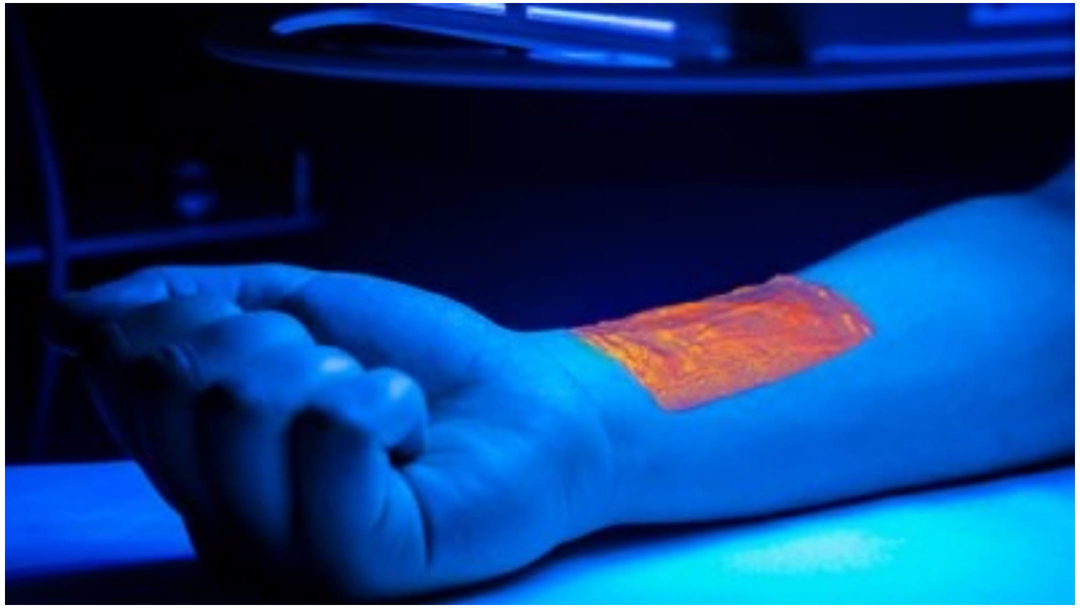
What does the Future hold?

Reactive oxygen - key ingredient
RO-101™ Gel

Matoke Holdings, Ltd.

What does the Future hold?

Activated Hydrogels

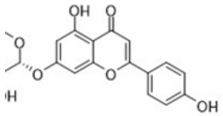


Klox Technologies, Inc.

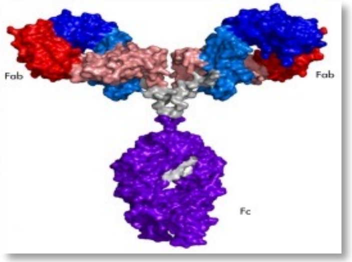
Summary – Wound Infections Past/Present/Future

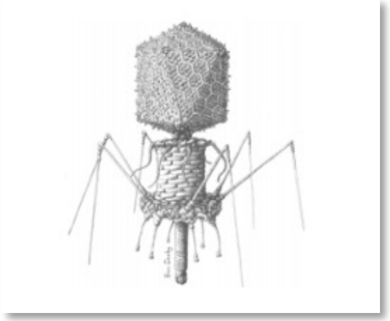
- Bacteria are complex organisms, and we need move away from monotherapies.
- Learn from recent past – COVID-19 Pandemic – Vaccines, mAbs, drugs

- We can learn from more distant past but use modern lens of science:
  - Ancient Times - Honey → Surgihoney™ → Reactive Oxygen Gel™
  - Ancient Times/Modern – herbs and garlic/oils



  - Civil War → compounds isolated from plants used at that time.





  - 1920's
    - Phage → Engineer them or use them in a cocktail
    - Serum → Engineered human antibodies

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Reese Pawlczych, Green Mountain Antibodies

Defense Medical Research and Development Program

MIDRP
Military Infectious Diseases Research Program

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