Human monoclonal antibodies against antibiotic-resistant bacteria

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Siena

The Siena cathedral: “memorial” to the plague

Plan for building larger cathedral in the XIV century

Plague in 1348 → socio-economic **crisis** → construction works interrupted and never resumed

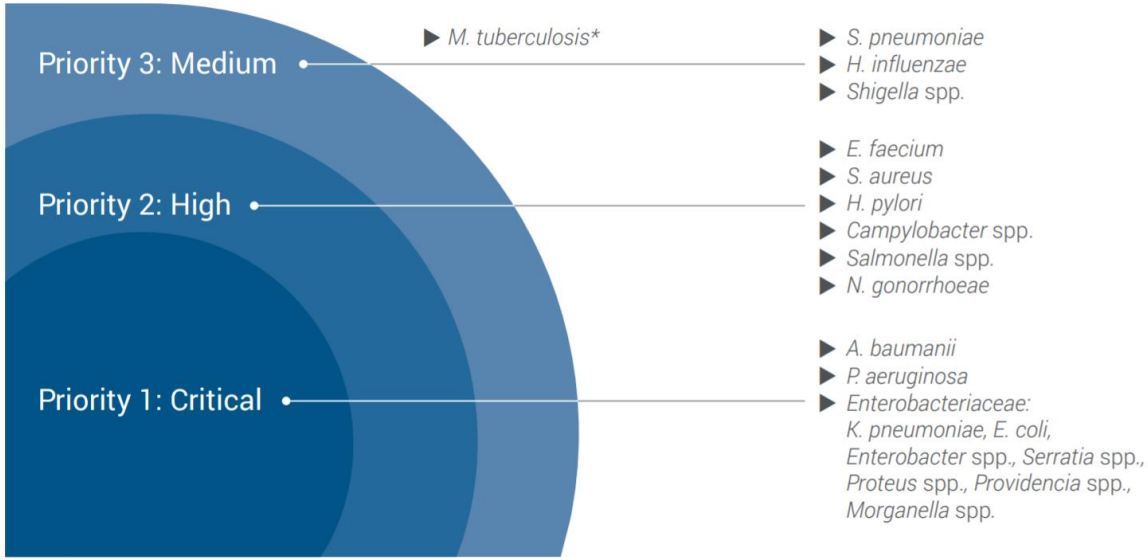
Warning against epidemics and pandemics

Today's silent pandemic: antimicrobial resistance (AMR)

AMR kills **5 million people per year**

35,000 EU/EEA citizens (ECDC, 2022)

More than HIV and TB combined



What can we do when antibiotics are useless?

Discover new antibiotics

Develop vaccines

Explore new avenues:

monoclonal antibodies, anti-virulence compounds,

host-directed therapies

mAbs & passive immunization

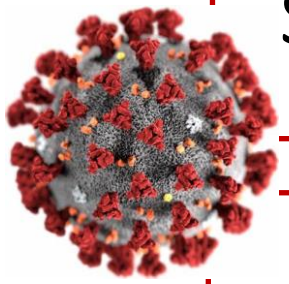
- Passive immunization with horse serum as an effective treatment against diphtheria and tetanus
- mAbs today: passive immunization and therapy vs. infectious diseases

Emil von Behring (1854 – 1917)
1901 Nobel prize in Physiology and Medicine

mAbs against infectious diseases

Advantages	Obstacles/open questions
Specificity (spare microbiota)	Accurate animal models for testing?
Only option for immunocompromised patients	Antigenic heterogeneity of pathogens
Enormous technological progress (cloning and expression)	Capsular layers may mask important antigens
Engineered mAbs → improved penetration, effector functions, conjugation to drugs	Precise timing for administration? Prophylaxis? Therapy?

MAD Lab ongoing projects overview



SARS-CoV-2

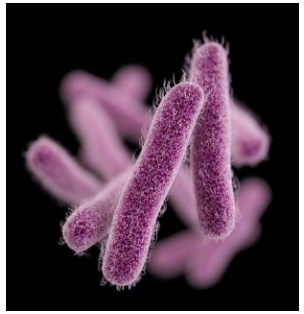
Monkeypox

Neisseria gonorrhoeae

Klebsiella pneumoniae

Shigella spp.

mRNA-mAbs



DaScH Lab

Advanced Data Analytics & Bioinformatics

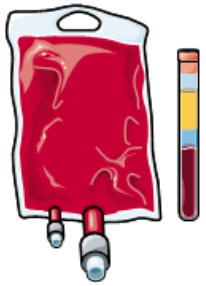
- mAbs for prophylaxis/therapy
- mAbs for antigen discovery → rational vaccine design

mAb cloning pipeline

Blood collection & PBMC isolation

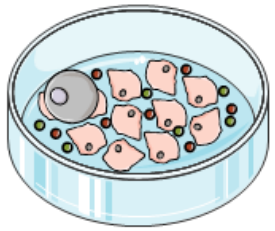
B cell proliferation *in vitro*

Production of mAbs



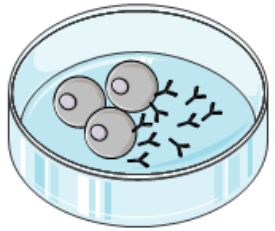
Memory B cells

sorting

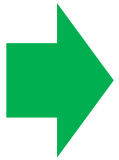


In vitro

mAb secretion

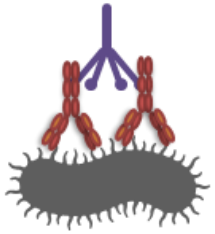


mAbs are released into ≈ 50µL



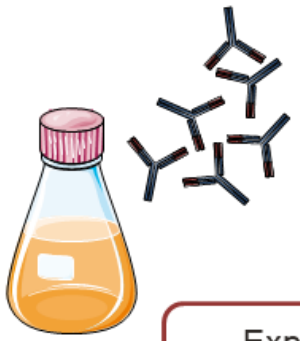
No need to take blood from patients anymore

Antigen binding ELISA assays



Assessment of activity

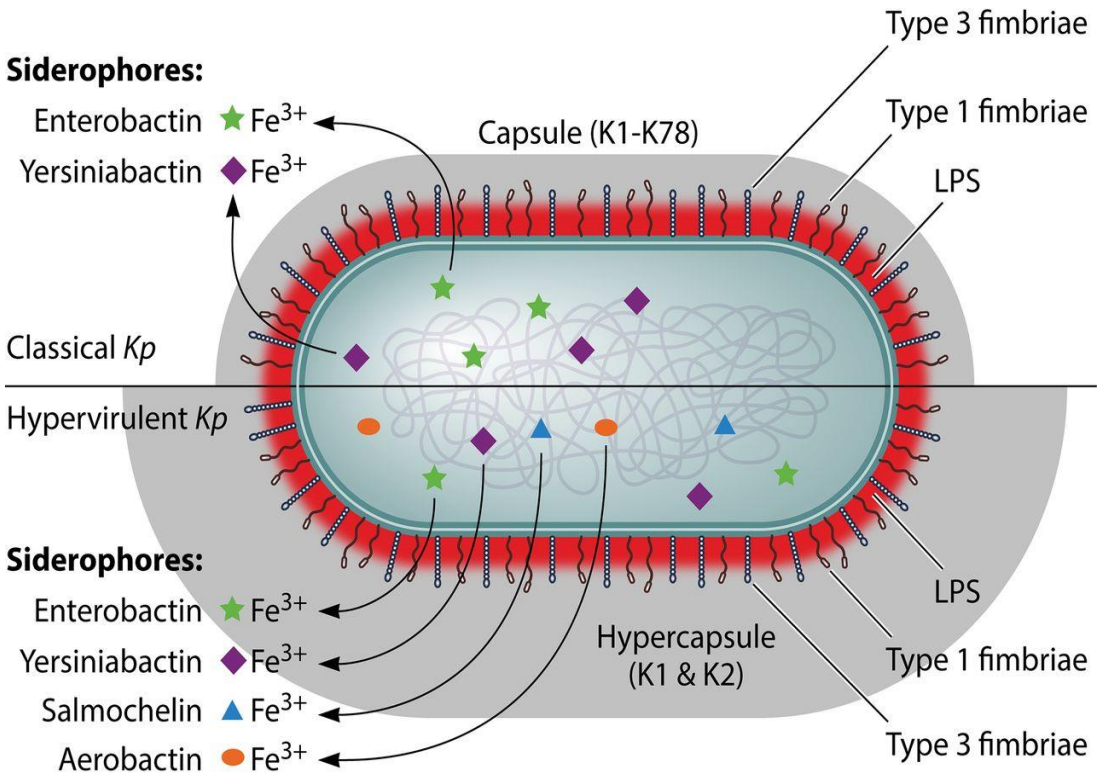
Sequence recovery



Expression as recombinant proteins

mAbs vs. *Klebsiella pneumoniae*

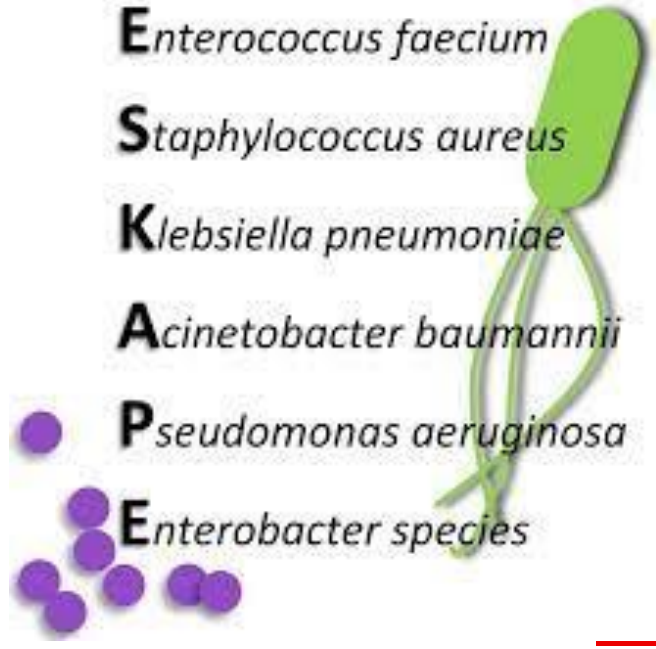
Klebsiella pneumoniae: overview



→ Gram-negative, **encapsulated**, non-motile, **opportunistic** pathogen

→ Leading cause of **hospital-acquired infections** (i.e., pneumonia, UTI, bloodstream infections)

→ Kp acquired **resistance** to most classes of antibiotics, including carbapenems



New Delhi metallo-beta-lactamase (NDM) - producing *K. pneumoniae*

Global concern

Global spread of hypervirulent and pandrug-resistant ST147

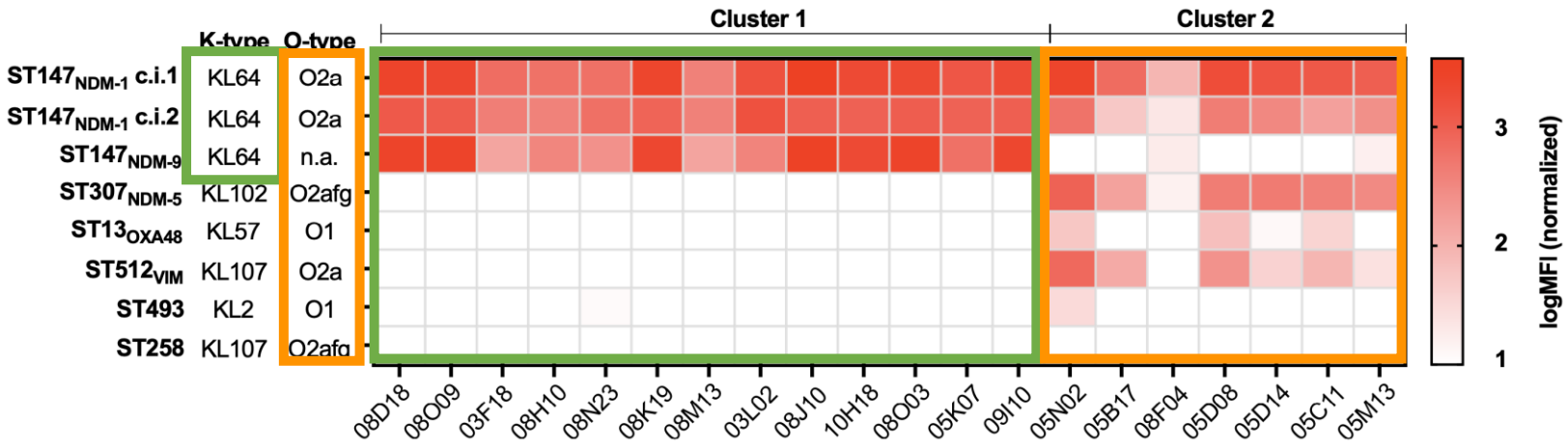
1,933 ST147 isolates
(6% of Kp genomes uploaded in PathogenWatch)

Persistent nosocomial outbreak of ST147 Kp in Tuscany

- Colonization to bloodstream infection
- 499 blood infection cases (2018-2022) with 22.7% lethality
- Extensive AMR profile, genetically evolving

mAb discovery in convalescent patients

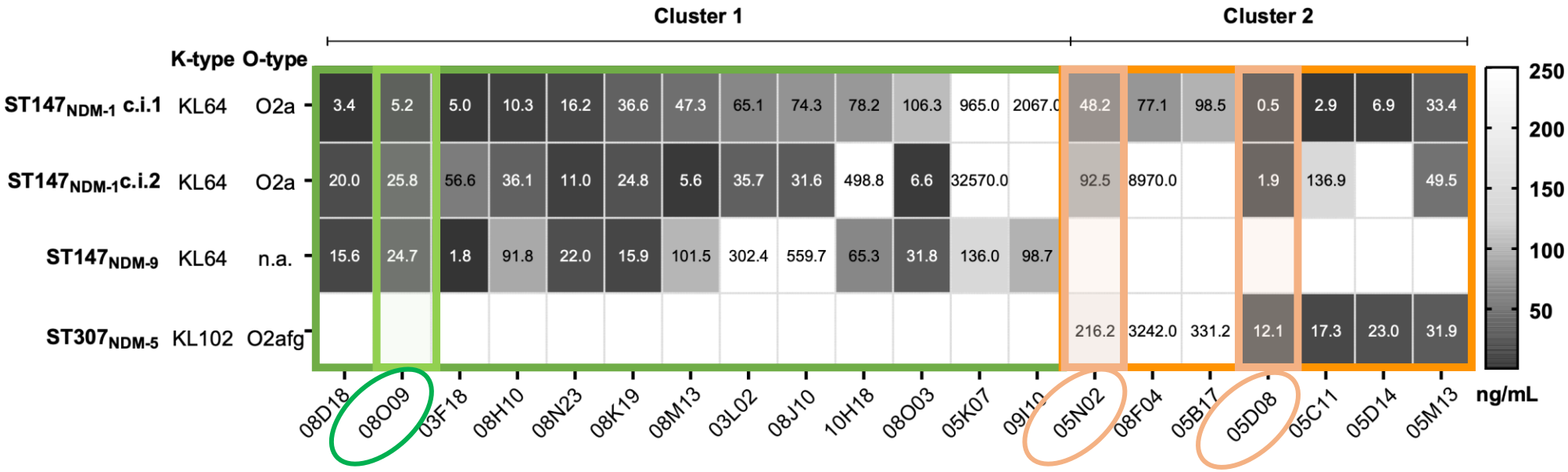
Two mAb clusters targeting capsule and O-antigen with ng/mL bactericidal activity



FACS Binding

ELISA Binding

Capsule
O-antigen

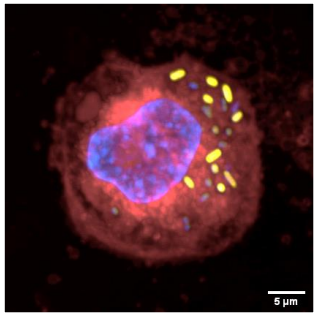


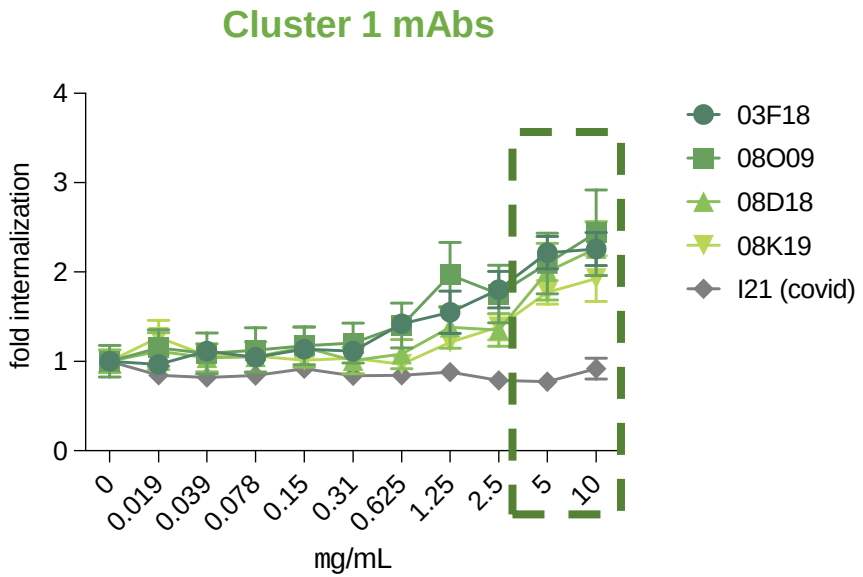
Complement-dependent killing

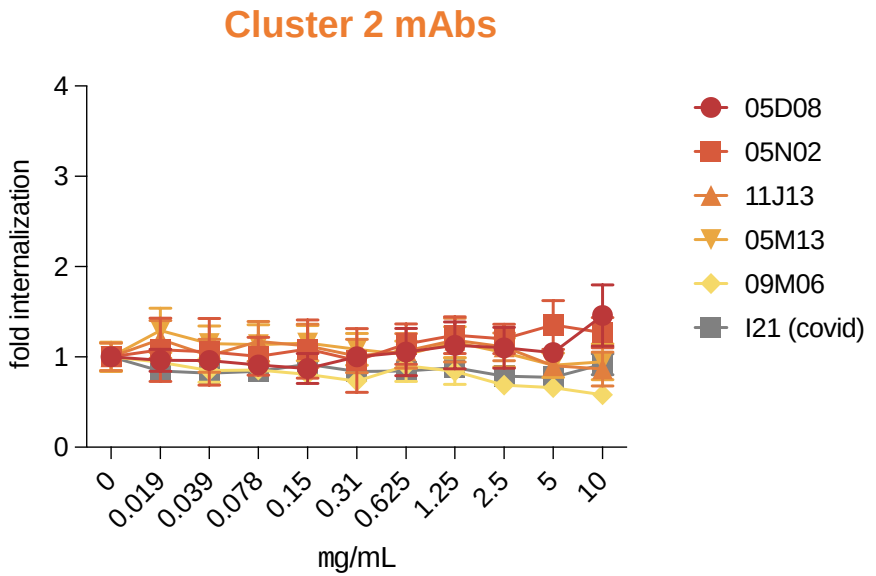
Roscioli et al, submitted

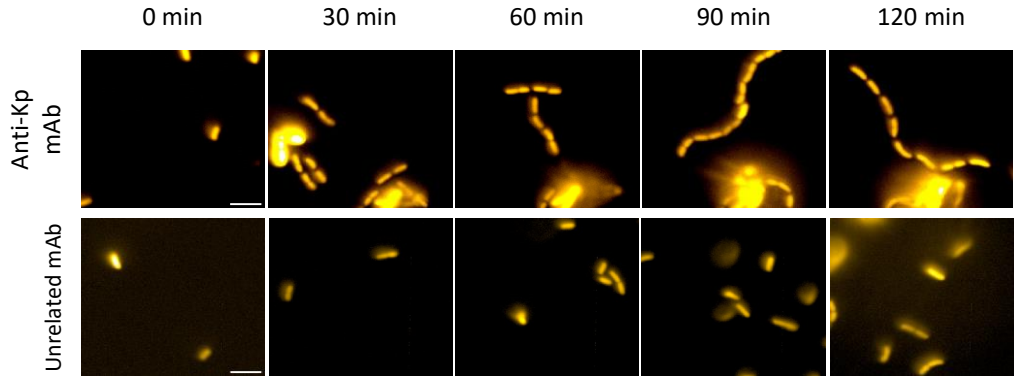
Cluster 1 mAbs promote opsonophagocytosis and enchained growth

THP-1 cells

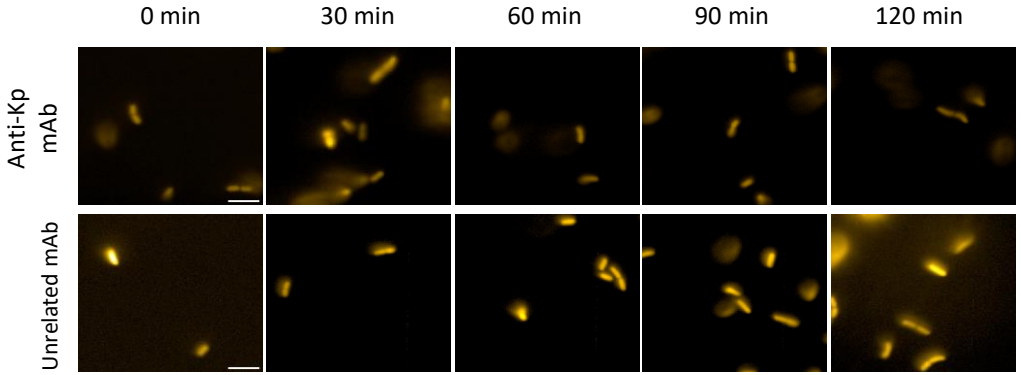








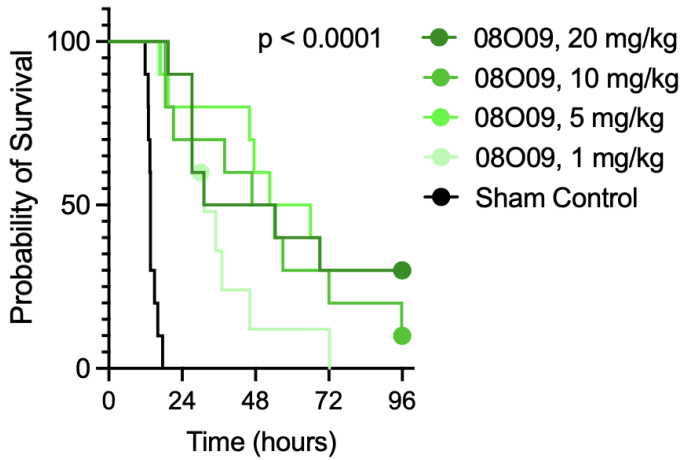
Cluster 1 mAb
10-100 µg/mL

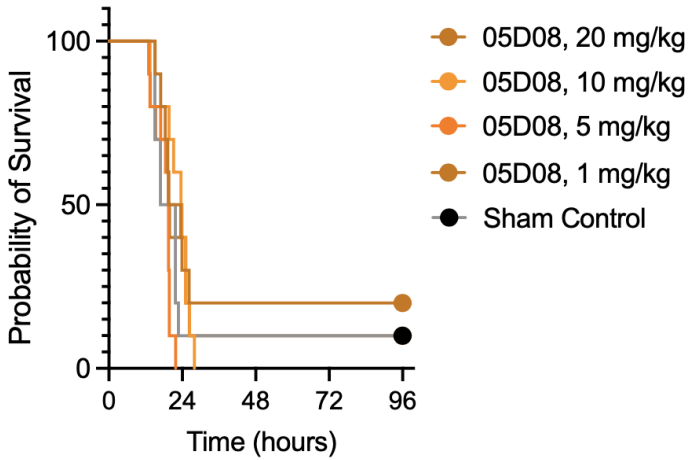


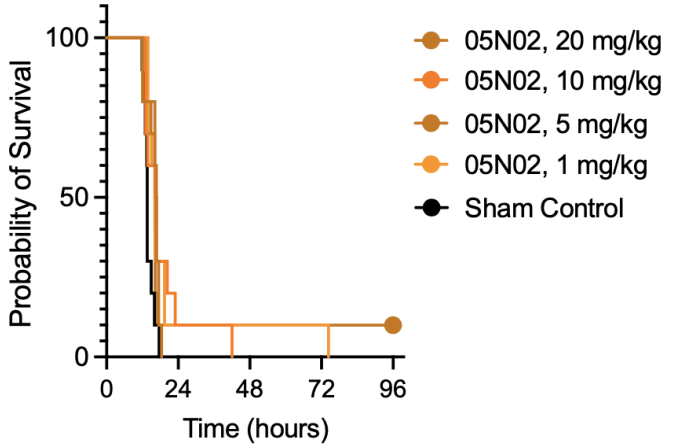
Cluster 2 mAb
10-100 µg/mL

Roscioli et al, submitted

Cluster 1 mAbs protect from bacterial challenge *in vivo*



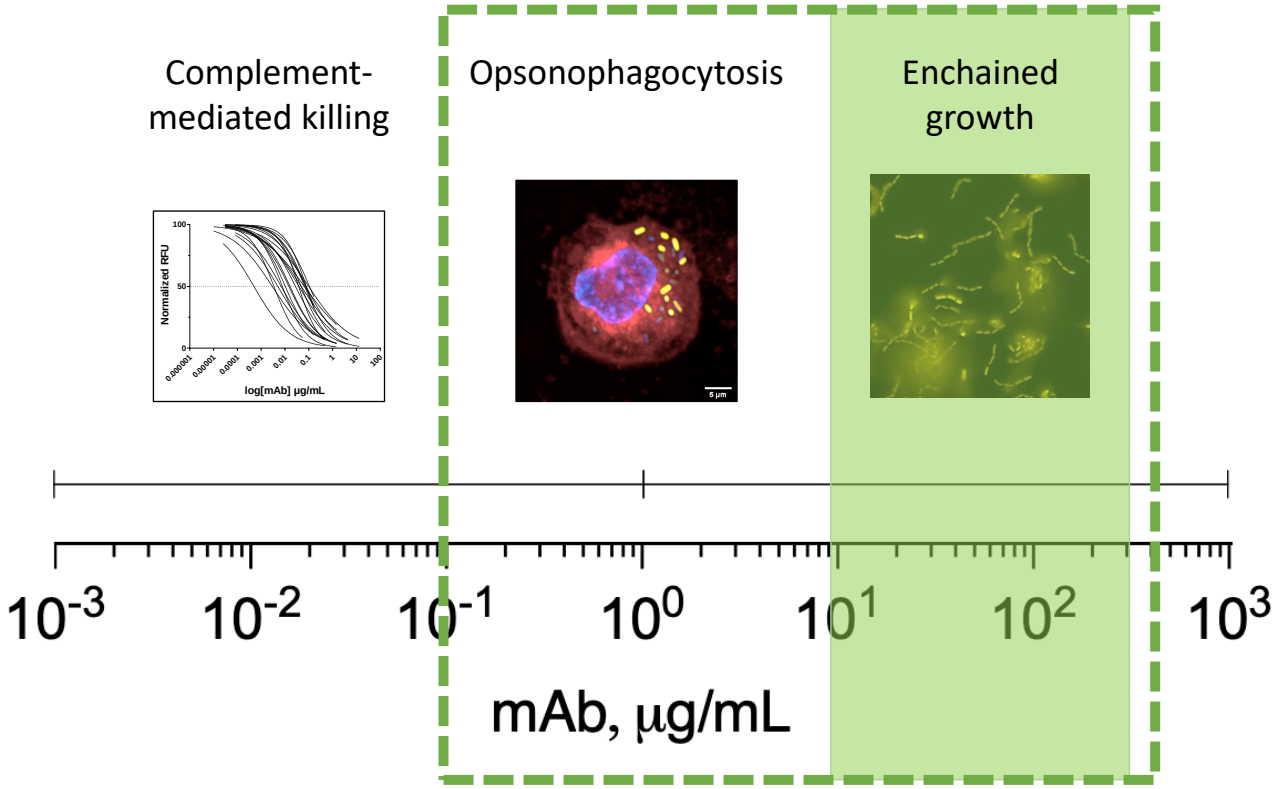




Serum mAb concentration 24h hours post ip injection*:* 50-100 μg/mL

Roscioli et al, submitted

Protection against pandrug-resistant Kp correlates with mAb poly-functionality



Protective serum mAb concentration *in vivo*

1. Multi-functionality is important *in vivo,* right assays are important *in vitro*
2. Complement-based killing is not predictive of protection
3. KL64 shields O-antigen (and other antigens)

Roscioli et al, submitted

mAbs vs. *Shigella sonnei*

Shigella: AMR emergency

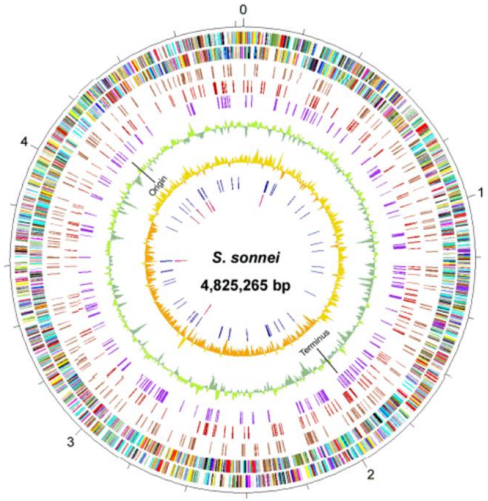
- ***Shigella***: Gram-negative bacterium with more than **50 different serotypes**

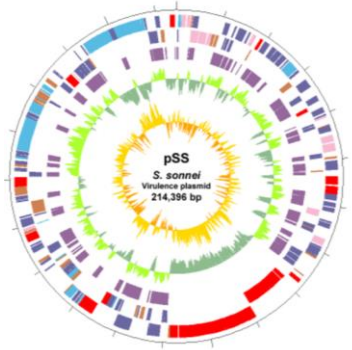
Global health problem in **low-income countries** (children < 5)

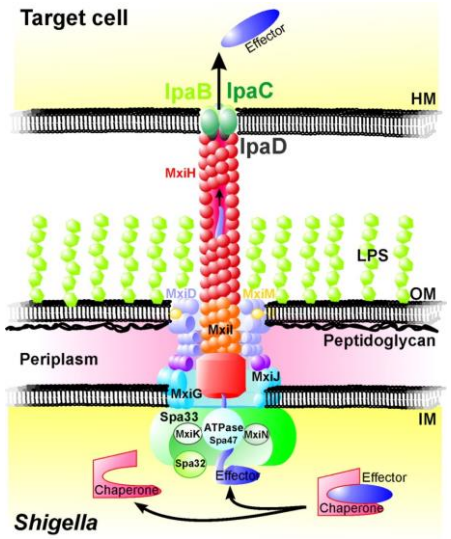
AMR strains isolated in Europe, UK, US

No approved **vaccine** exists

"*Shigella* is *E. coli* with a plasmid" → **plasmid is essential for virulence (T3SS)**



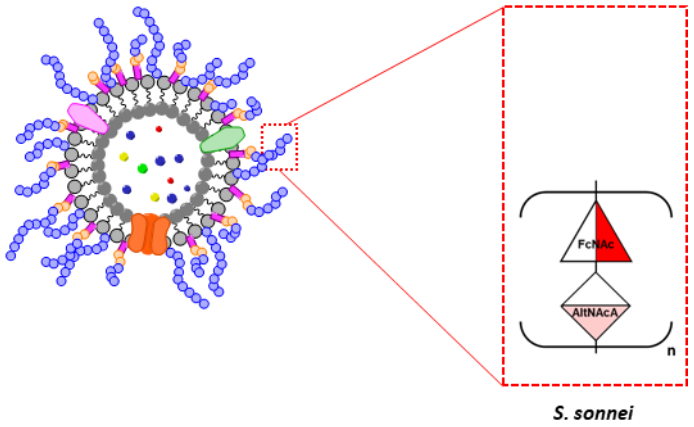




http://www.mgc.ac.cn/ShiBASE/

Anti-*S. sonnei* mAbs from vaccinated + challenged volunteers

Study	Samples	Immunization schedule	Challenge	Sample collection
Cincinnati	GMMA vaccination (*S. sonnei*) or placebo followed by challenge (*S. sonnei*)	Day 1 and 29	Yes (day 57)	~2 years later



Generalized Modules for Membrane Antigens (**GMMA**):

- outer membrane **vesicles** derived from Gram-negative bacteria
- attractive **platform** for **vaccine** design
- **delivery** system for O-antigen and protein **antigens**
- Immunogenic, present antigens in natural conformation, self-adjuvanticity

mAb discovery pipeline → Top candidate: **mAb1**

Target: **O-Antigen**

Conclusions & next challenges

Conclusions

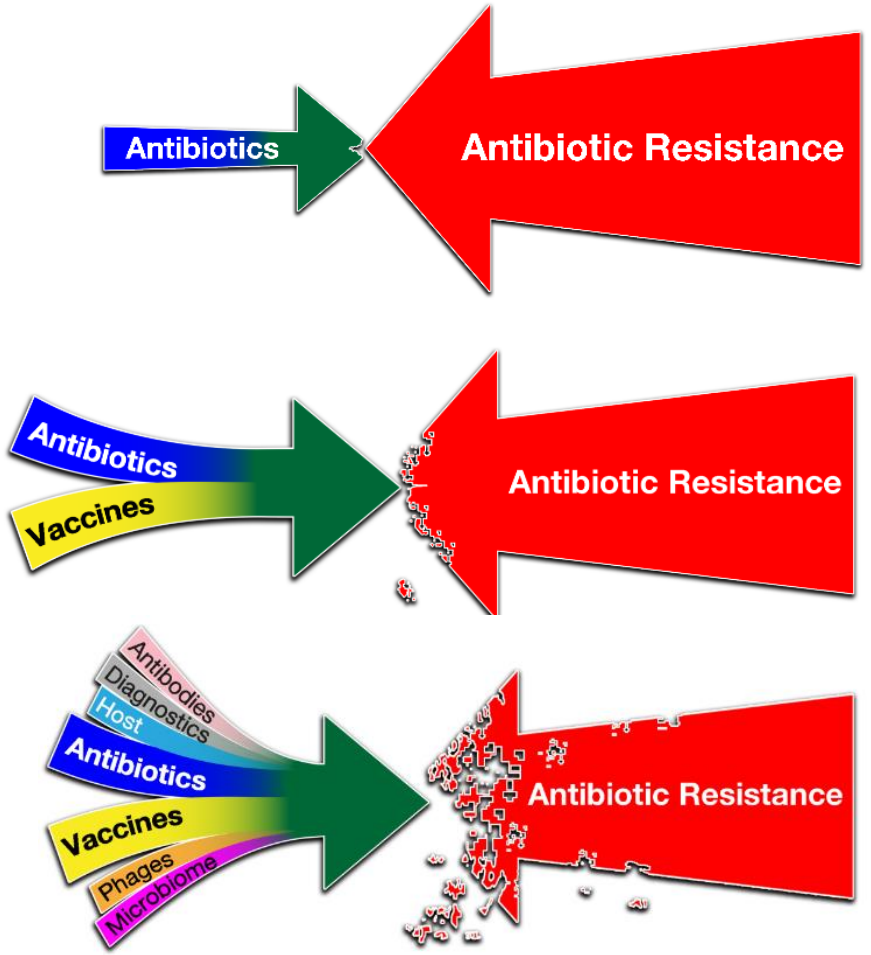
- mAbs for tackling **health challenges**
- mAbs for addressing **pandemic preparedness**
- mAbs for developing new **research tools**

Next challenges

- Deliver **mRNA-encoded mAbs**
- Bring mAbs to **those in need**
- Promote **equitable access to mAbs**
- mAbs for **defining correlates of protection and assist vaccine design**

Tackling AMR requires a joint effort

- AMR is a hard challenge for antibiotics alone
- Vaccines and Antibiotics together have a better chance to control AMR
- By joining forces we can control AMR



Source: Bloom et al, Antimicrobial resistance and the role of vaccines. PNAS 2018 115:51 12869. Adapted with permission of the authors.

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