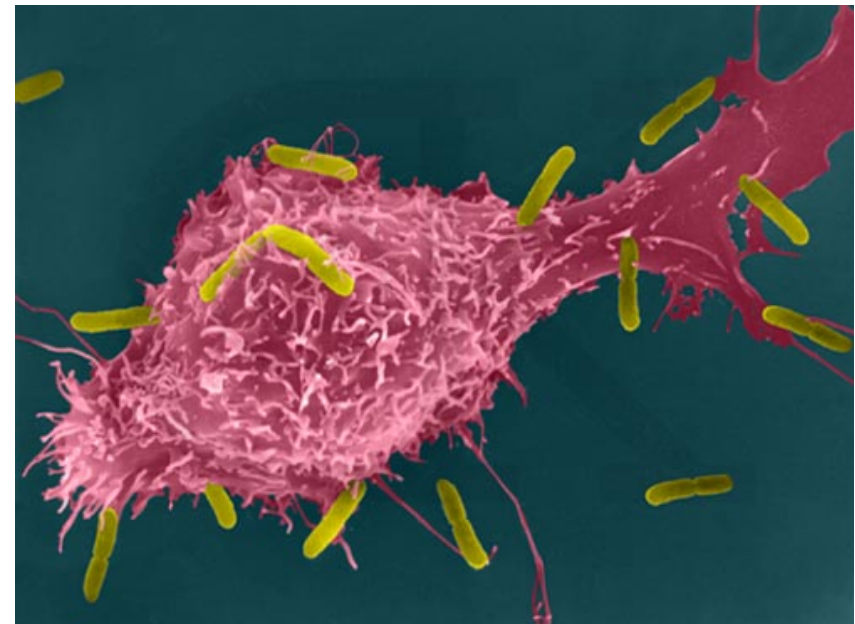


Antibiotic failure mediated by a phenotypically resistant bacterial subpopulation

David S. Weiss

Emory Antibiotic Resistance Center
Division of Infectious Diseases
Emory University School of Medicine
Emory Vaccine Center



The World Before Antibiotics



**REMEDIES FOR
CHOLERA**

As prescribed by the Edinburgh Board of Health, and approved of by the
Faculty of New-York.

— — — — —

**CAREFULLY PREPARED BY JEFFERSON B. NONES,
APOTHECARY AND CHEMIST,
NO. 644 BROADWAY, NEW-YORK.**

NO. 1. CHOLERA MIXTURE.
A table-spoonful with 60 drops of Laudanum, in half a wine-glassful of cold water. If this fail to relieve, repeat two spoonful, with 30 drops of Laudanum every half hour. Half these doses of mixture and laudanum, for children of 14. One-fourth for children of 7. Do not exceed the doses prescribed; and stop when the vomiting and cramps cease, unless you have medical advice.

NO. 2. BOTTLE OF LAUDANUM.

NO. 3. CHOLERA PILLS.
To be used if the mixture No. 1 be vomited. Two pills at first, and then one every half hour, if the first fail to relieve. Half these doses for children of 14; one-fourth for children of 7. Do not exceed the doses prescribed, and stop when the vomiting and cramp cease, unless you have medical advice.

NO. 4. CHOLERA CLYSTERS.
Inject three tea-spoonful in a wine-glassful of thin warm gruel, and retain as long as possible by pressure below with a warm cloth; if not retained, repeat immediately, but otherwise not. Half the dose for children of 14—one fourth for children of 7.

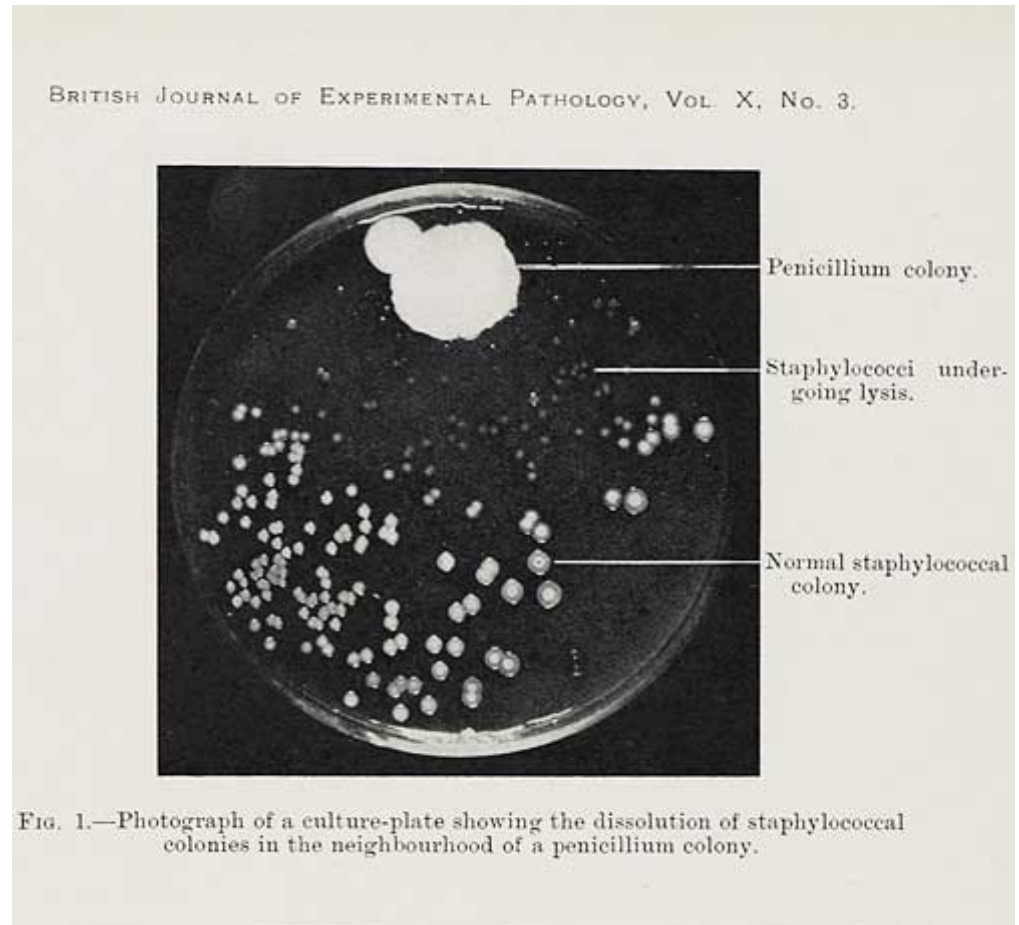
NO. 5. MUSTARD POULTICES.
A fourth part is enough for one person. Dust it thickly over porridge poultices, of which apply a large one on the belly, and others on the soles and calves. Remove when the patient complains much of the smarting.

Cornwall's Printing Office, 117 BROADWAY.

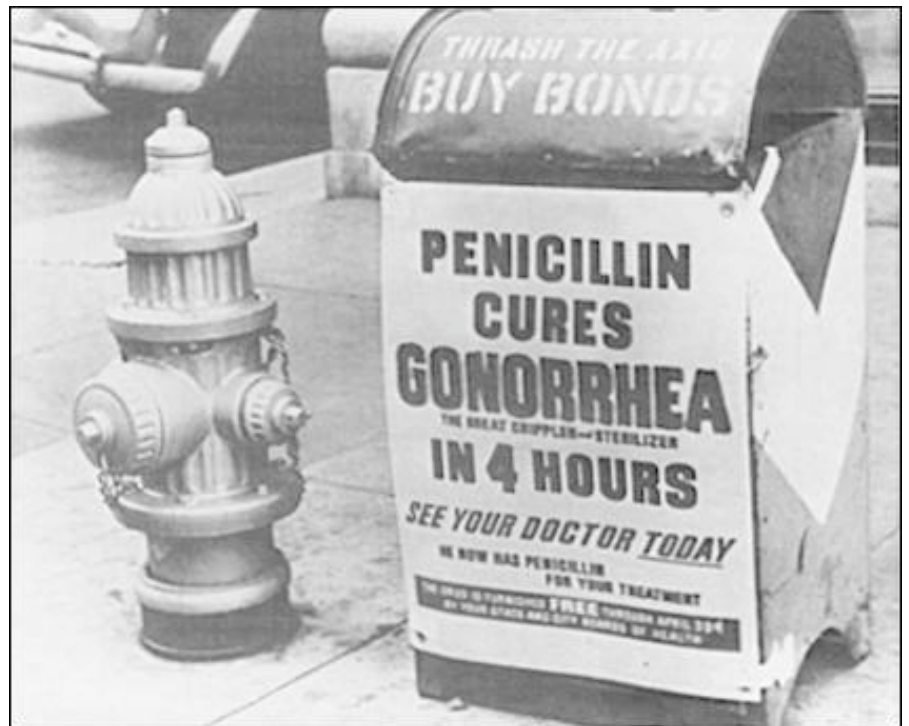
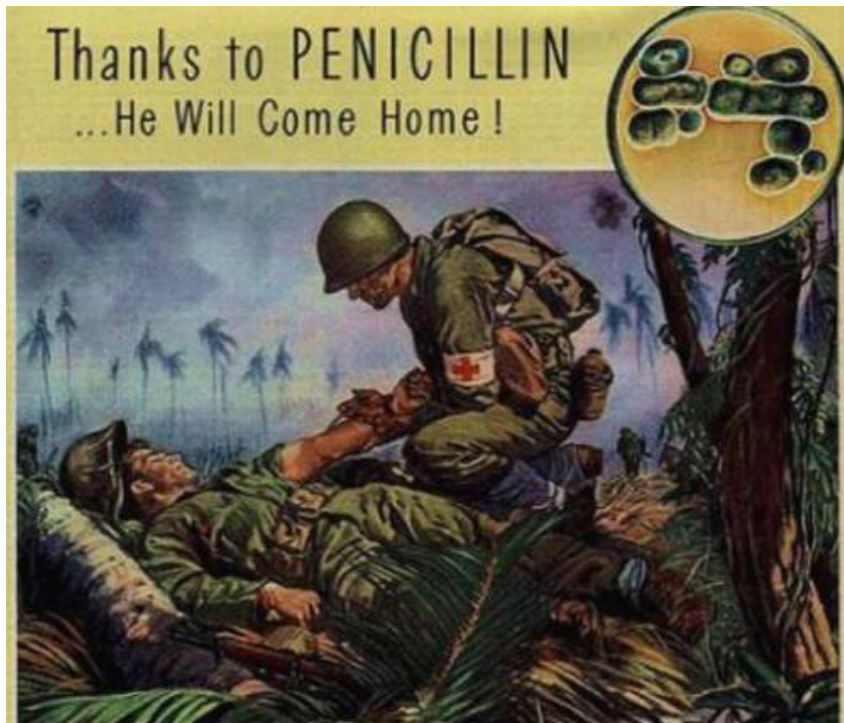


Year	Deaths	Population	Death Rate (per 100,000)
1936	277,541	129,083,333	216.3

The Discovery of Penicillin

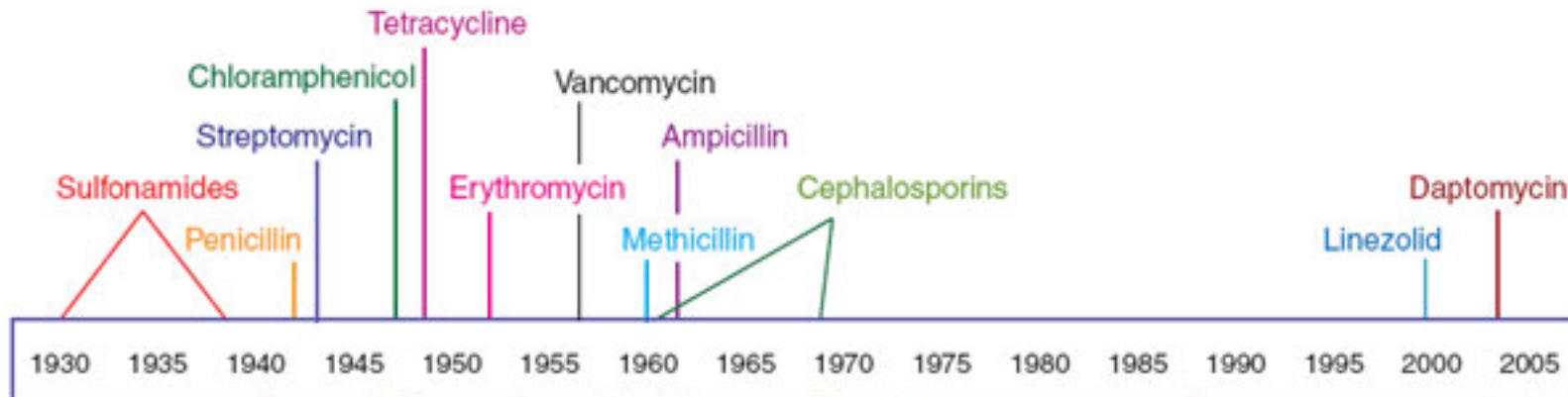


The Antibiotic Era



The Antibiotic Era

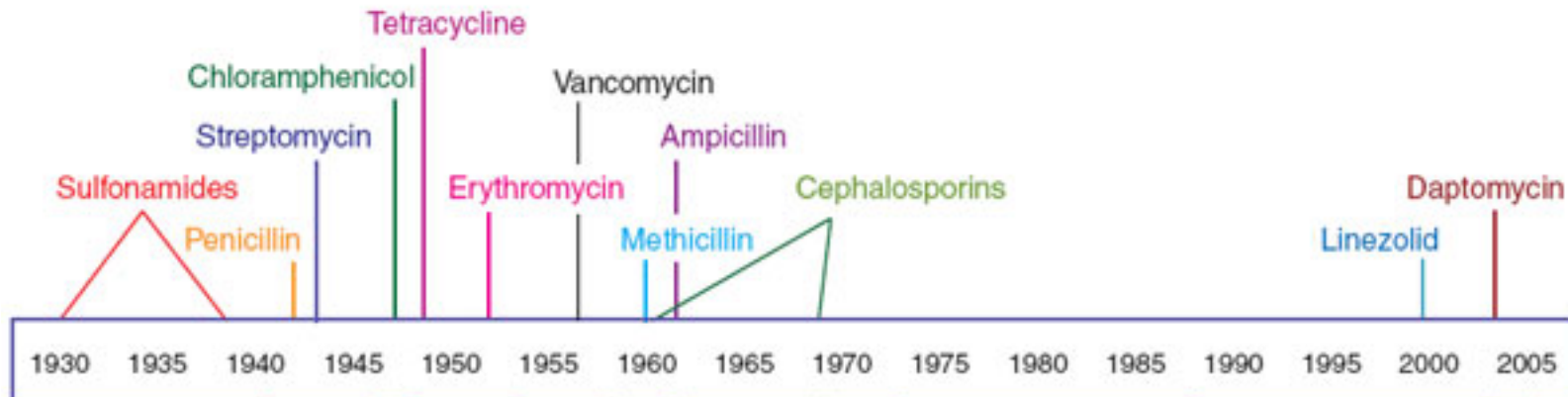
Antibiotic deployment



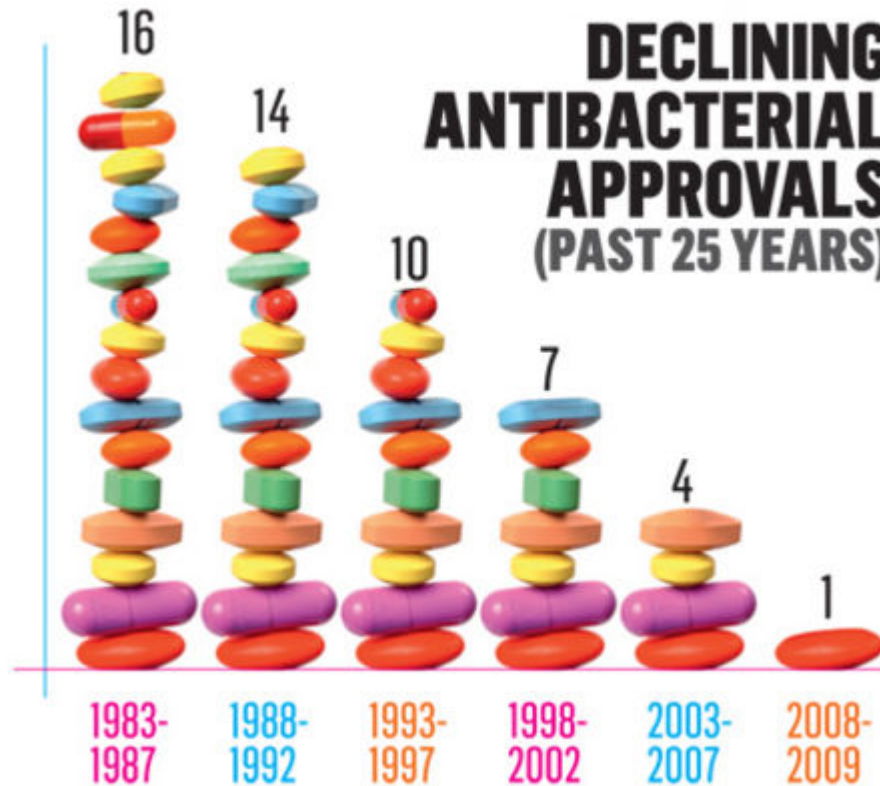
Year	Deaths	Population	Death Rate (per 100,000)
1936	277,541	129,083,333	216.3

Antibiotic Resistance

Antibiotic deployment



Dearth of new drugs



The burden of antibiotic resistant infections



“Each year in the United States, at least **2 million people become infected** with bacteria that are resistant to antibiotics and at least **23,000 people die** each year as a direct result of these infections.”

Untreatable infections

NATURE | NEWS

Totally drug-resistant TB emerges in India

CDC sounds alarm on deadly, untreatable superbugs

DEADLY BACTERIA THAT DEFY DRUGS OF LAST RESORT

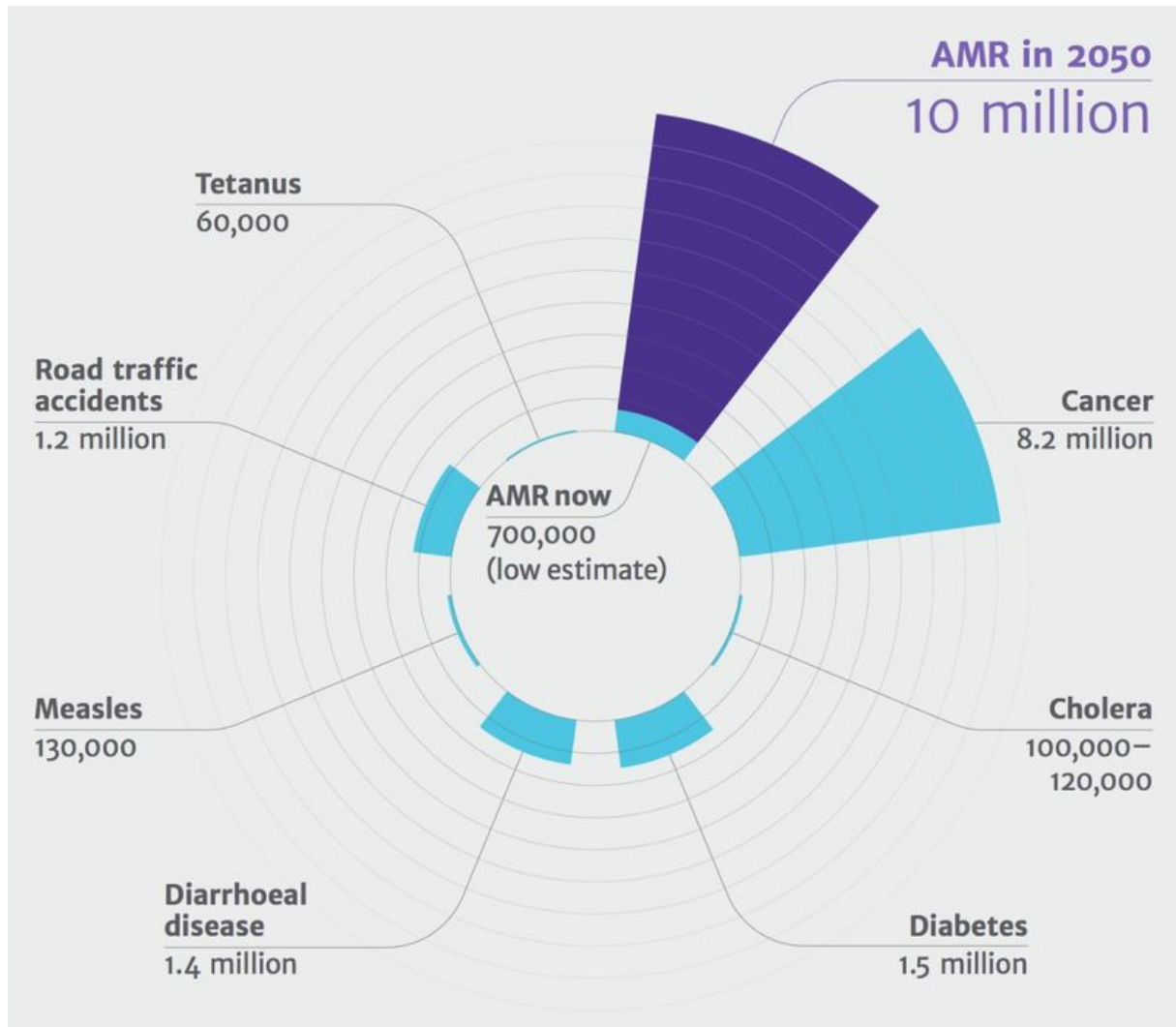


A post-antibiotic era?



“A problem so serious that it threatens the achievements of modern medicine.

The future burden of antibiotic resistant infections?





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Bringing scientists and clinicians together

Our Mission

Our mission is to better understand antibiotic resistance to combat this crisis and improve human health.

[Learn more »](#)



Featured Researcher

Jyothi Rengarajan, PhD

Mechanisms of tuberculosis (TB) pathogenesis

HIGHLIGHTS

- Call for public health funding to fight drug-resistant TB
- Super bacteria lurk in the home
- Trainees selected for ARTDTP training grant
- Sean Stowell selected for NIH Early Independence Award

UPCOMING SEMINAR

- Arjun Srinivasan, MD
Centers for Disease Control
"Antibiotic Resistance: National Priorities for Urgent Action"
Emory ARC Quarterly Meeting
August 19th, 2015 - 3 pm
- [More Seminars >>](#)

Enterobacter cloacae

Gram-negative, facultative anaerobe

Opportunistic pathogen

Causes urinary tract and respiratory tract infections in hospital patients

Often contaminates hospital equipment (ventilators and catheters)



Multi-drug resistant (MDR) *E. cloacae*

Highly resistant *Enterobacter cloacae* isolate from a kidney transplant patient at Emory Hospital

Antibiotic	colR/S
Amikacin	R
Amp/ Sulbactam	R
Ampicillin	R
Aztreonam	R
Cefazolin	R
Cefepime	R
Cefotaxime	R
Cefoxitin	R
Ceftazidime	R
Ceftriaxone	R
Cefuroxime	R
Gentamicin	S
Meropenem	S
Pip/Tazo	I
Tobramycin	I

Polymyxins

Older class of antibiotics, includes polymyxin B and colistin

Used infrequently due to nephrotoxic and neurotoxic side effects and low bioavailability

Drug of last resort to treat highly resistant bacteria

Attacks cell wall components, mechanism similar to host antimicrobial peptides



Polymyxin resistance is a global concern

A blue silhouette of a world map is centered on the slide. Four black arrows point from specific geographic regions to callout boxes containing text. The arrows point to Europe, the Middle East, East Asia, and South America.

Colistin resistance in *Klebsiella pneumoniae* infections **increased** to 12% in **two years**.

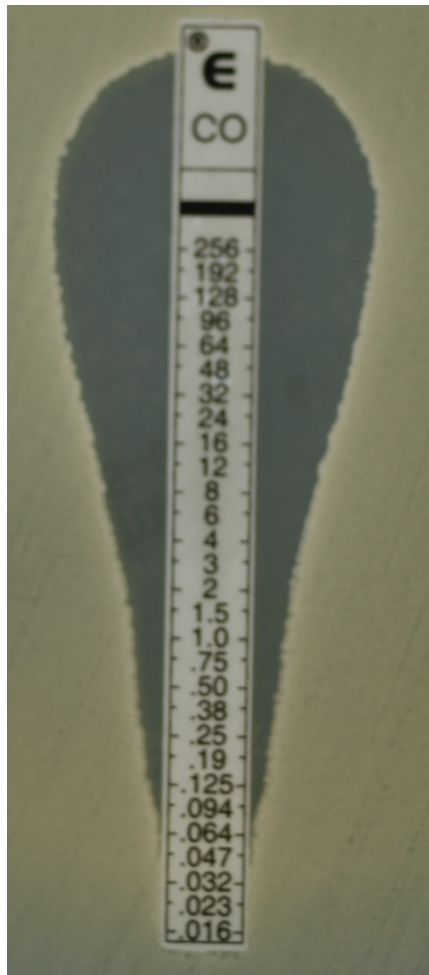
(Parisi S, 2015 BMC Infect Dis)

93-case **hospital outbreak** of colistin resistant *Klebsiella pneumoniae* due to increased colistin usage. (Gianni T, 2015 J Clin Microbiol)

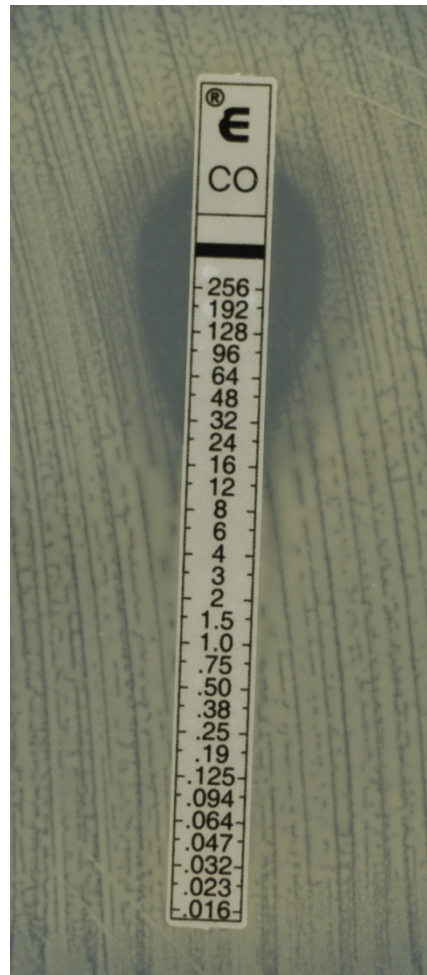
28% *Enterobacter cloacae* strains were colistin resistant. (Jean SS, 2015 J Microbiol Immunol Infect)

30% mortality due to evolution of colistin resistance in *Acinetobacter baumannii* infections treated with colistin. (Qureshi ZA, 2015 Clin Infect Dis)

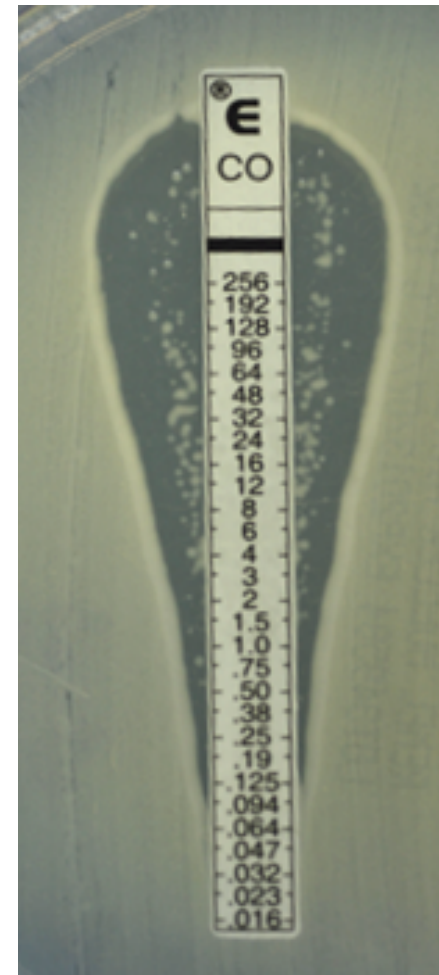
E-test to determine antibiotic susceptibility



Susceptible



Resistant



Heteroresistant

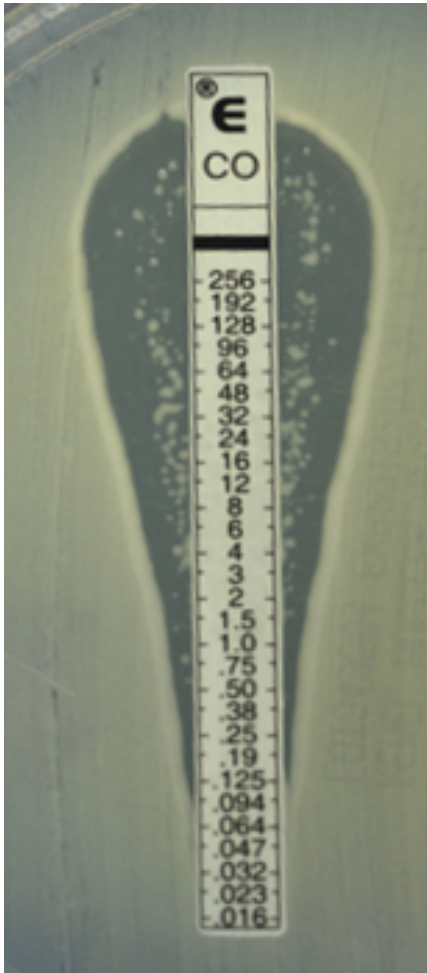
Antibiotic heteroresistance

Initially reported in 1946, but still only partially characterized

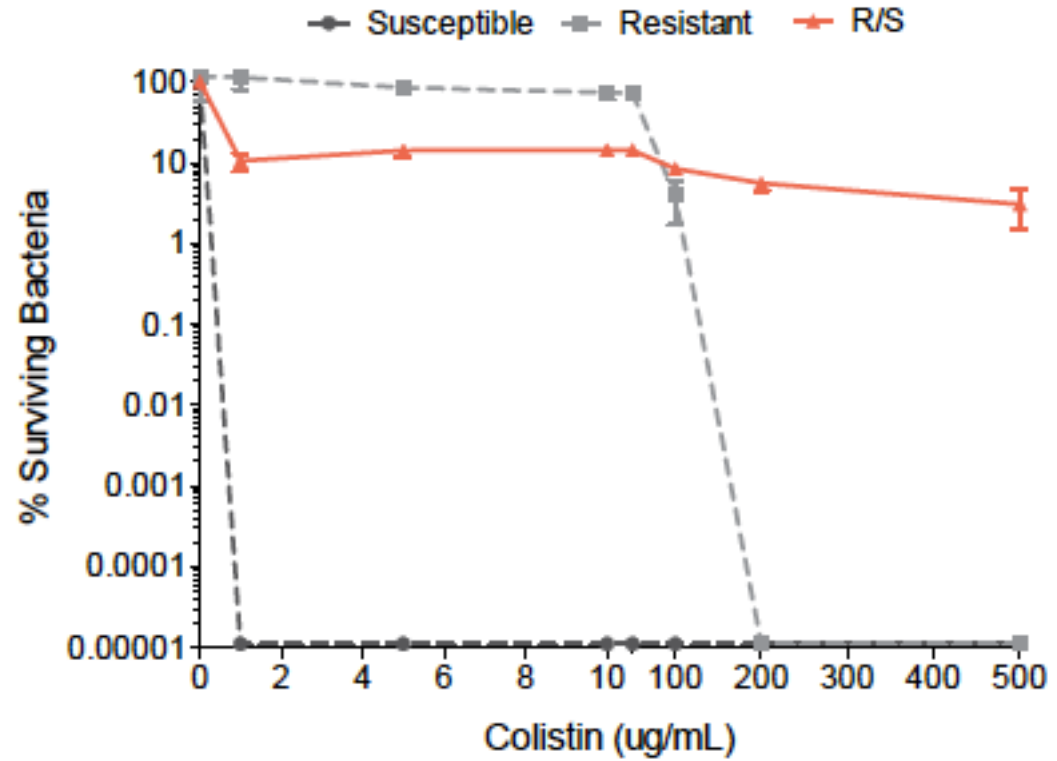
Organisms	Antibiotics
Gram Positive <ul style="list-style-type: none">- <i>Staphylococcus aureus</i>- <i>Streptococcus pneumoniae</i>	Glycopeptides
	Penicillins
Gram Negative <ul style="list-style-type: none">- <i>Escherichia coli</i>- <i>Klebsiella pneumoniae</i>- <i>Acinetobacter baumannii</i>- <i>Enterobacter cloacae</i>	Carbapenems
	Cephalosporins
	Polymyxins

The clinical significance and genetic mechanisms that underlie heteroresistance are poorly defined

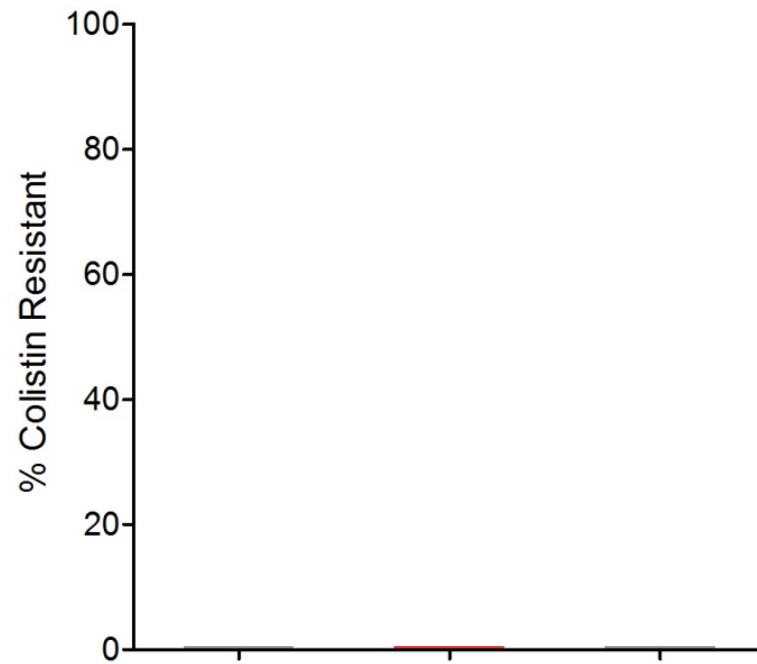
Highly resistant subpopulation



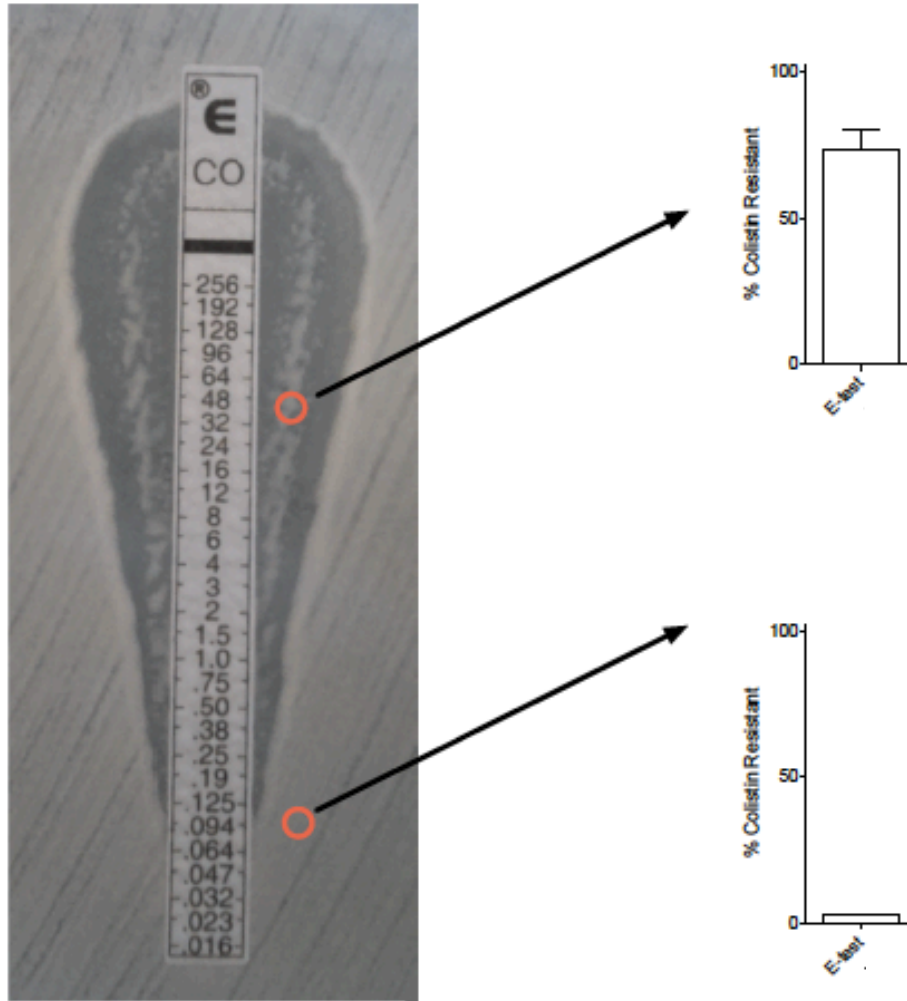
Heteroresistant



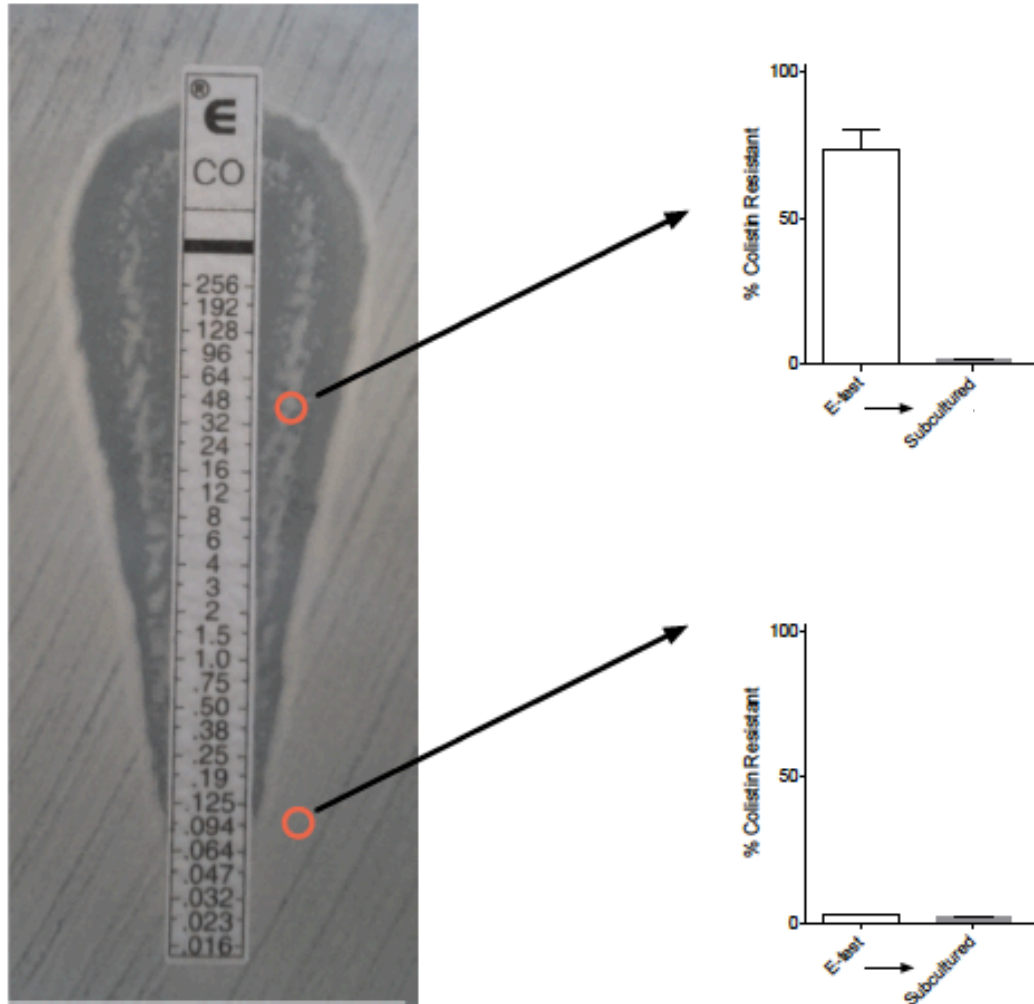
Distinct from mutational resistance



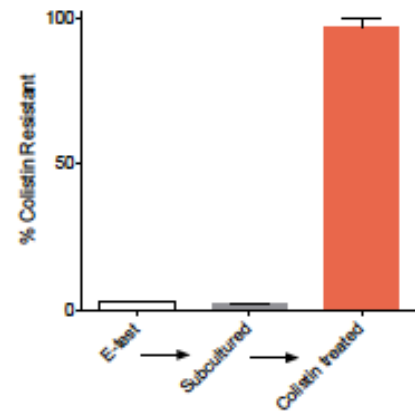
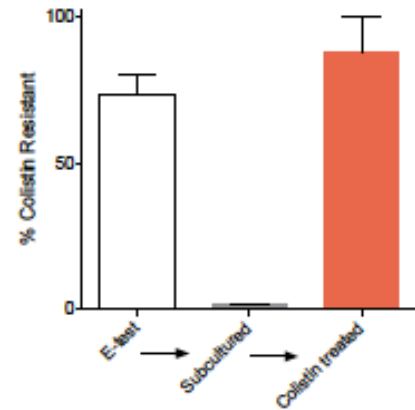
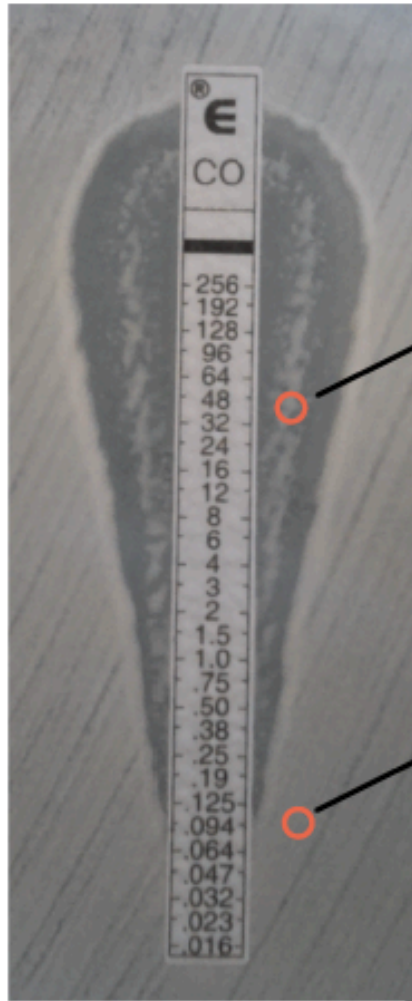
Phenotypically distinct cells behave identically after passage



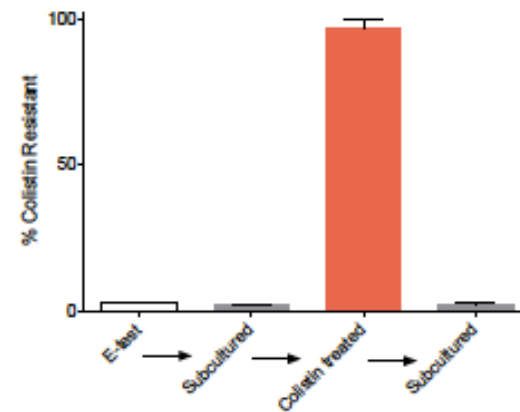
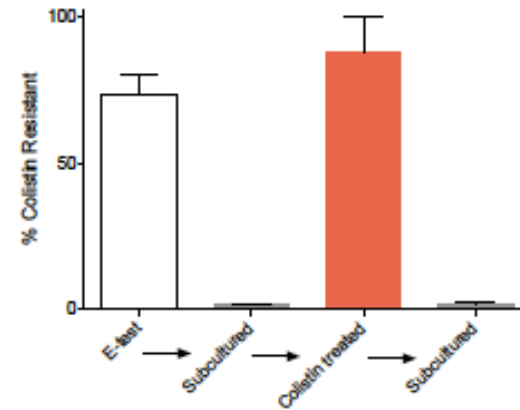
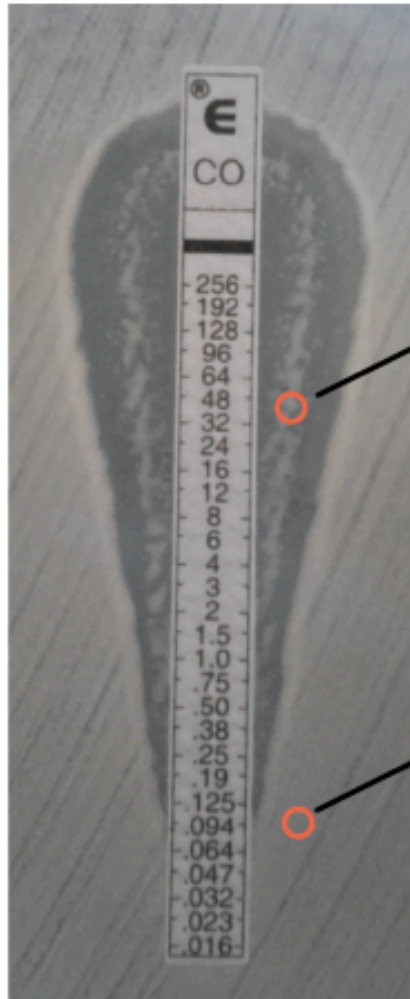
Phenotypically distinct cells behave identically after passage



Phenotypically distinct cells behave identically after passage

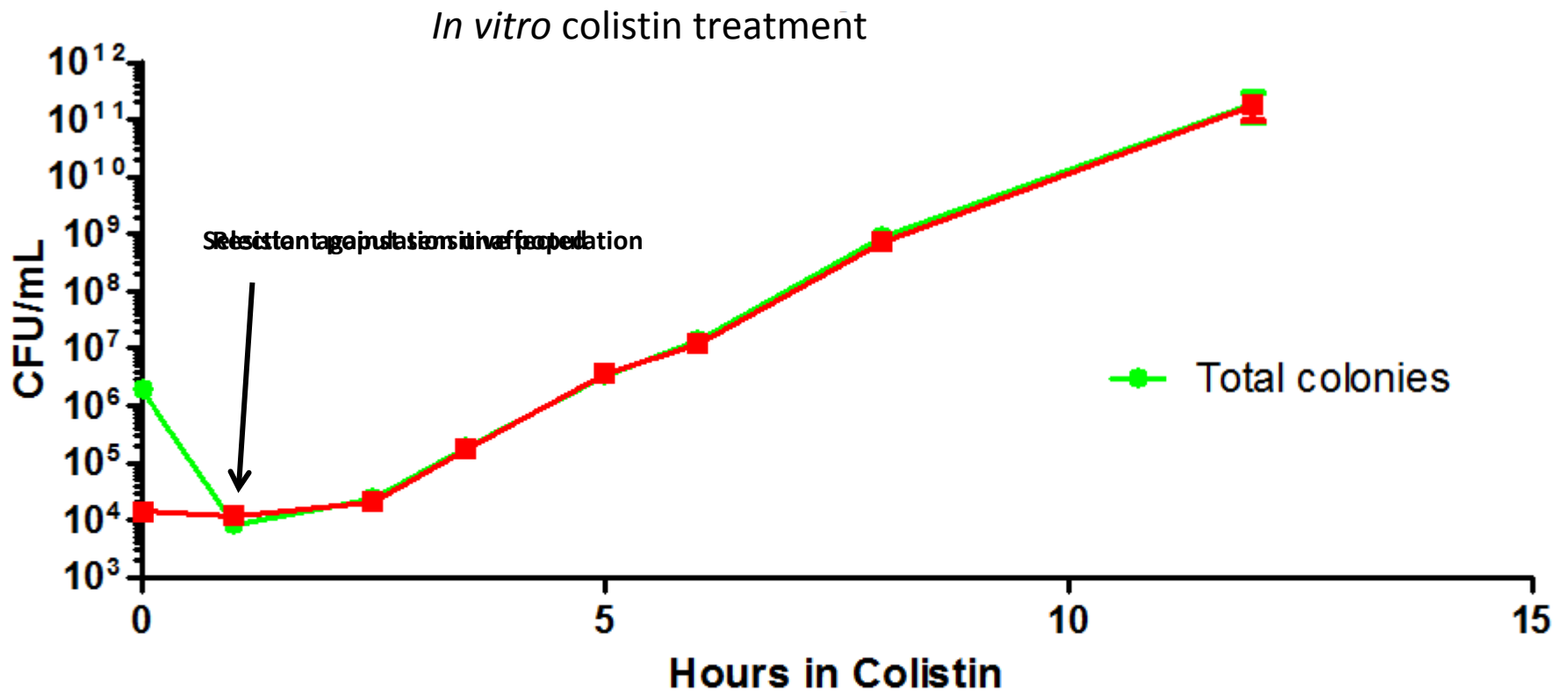


Phenotypically distinct cells behave identically after passage

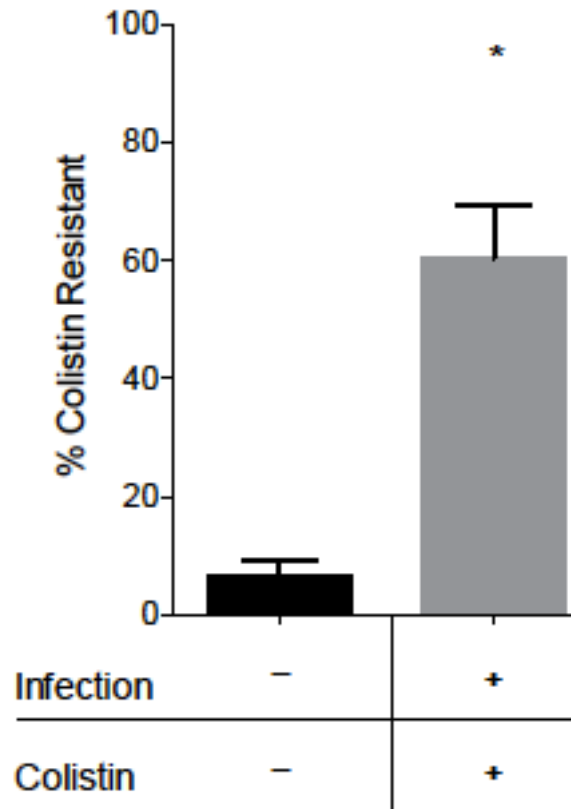


DNA sequences are identical

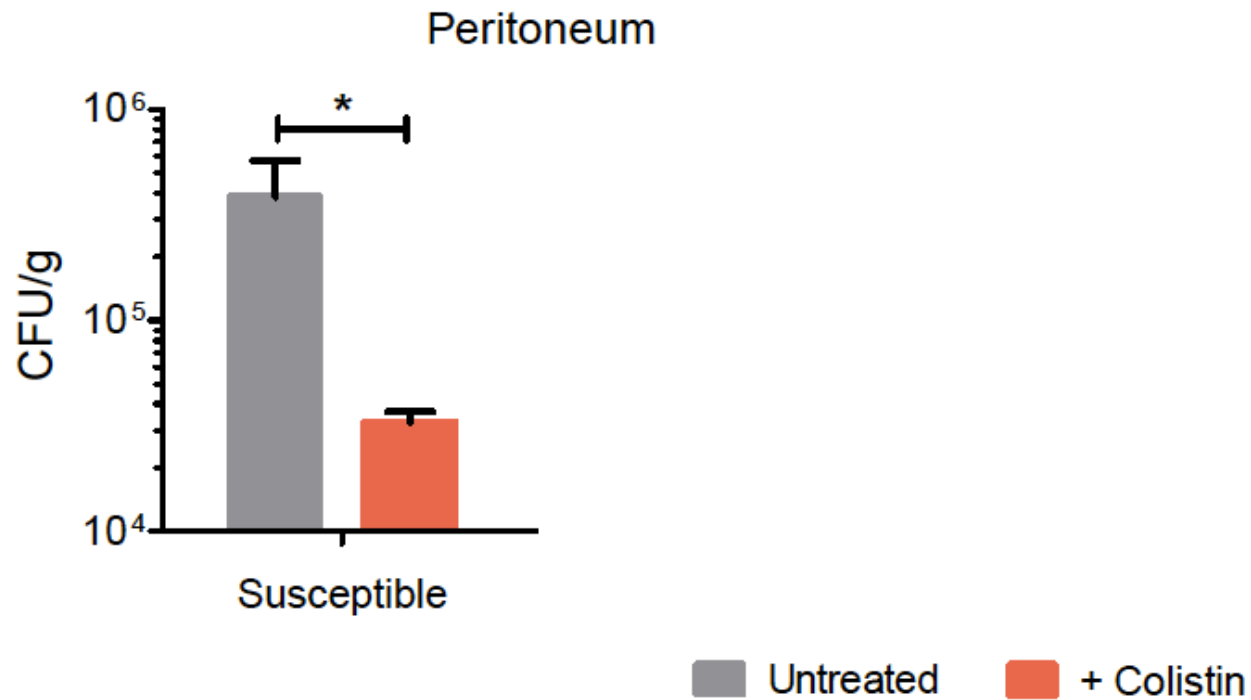
Distinct from persistence



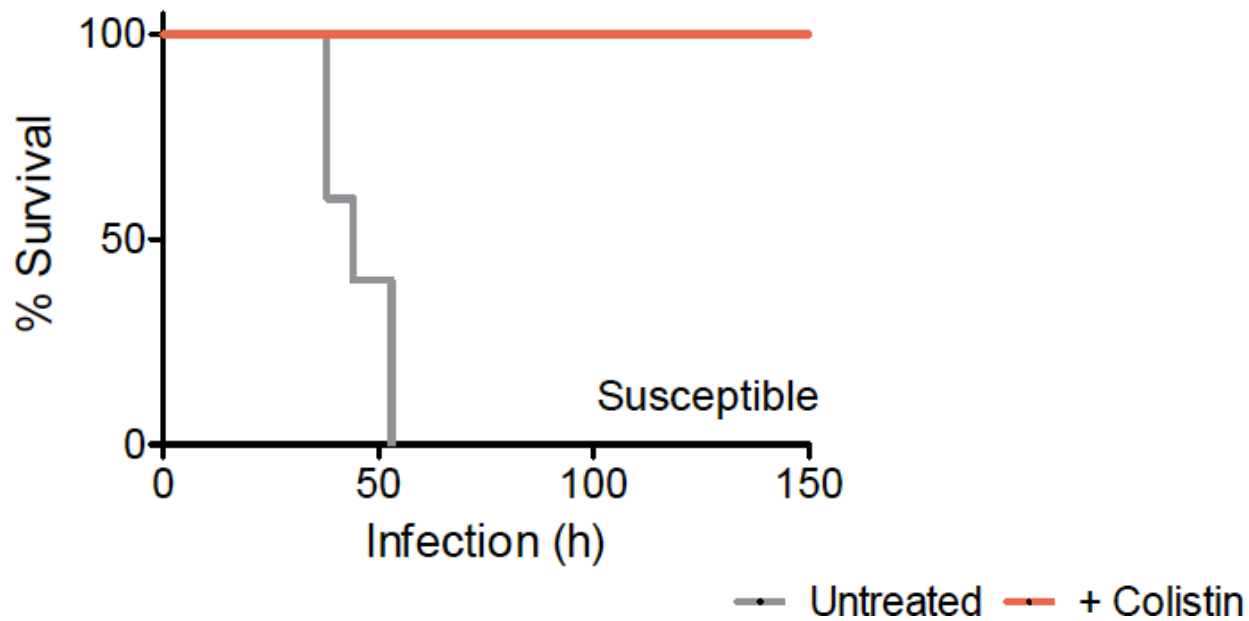
Resistant subpopulation increases in frequency during *in vivo* infection



Resistant subpopulation leads to inefficacy of colistin treatment



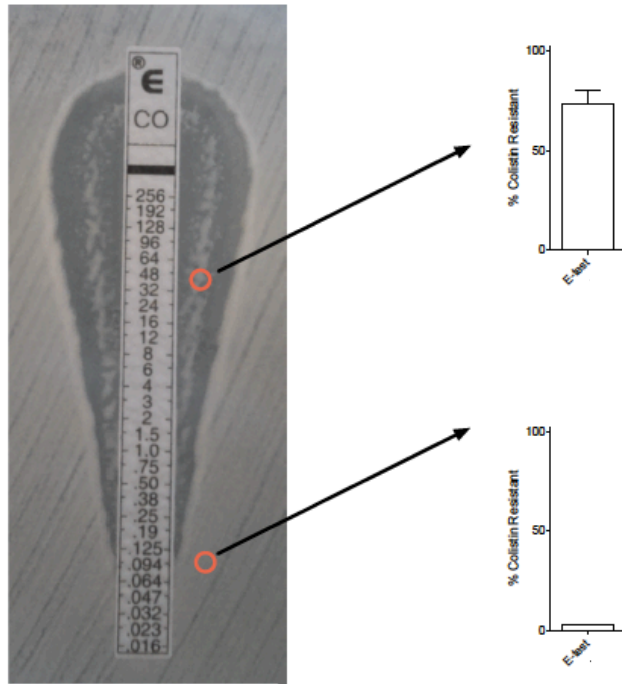
Resistant subpopulation mediates treatment failure



Summary

Genetically identical, but phenotypically distinct, subpopulation of colistin-resistant bacteria can mediate *in vivo* treatment failure

Transcriptome analysis identifies genes preferentially expressed by the resistant subpopulation

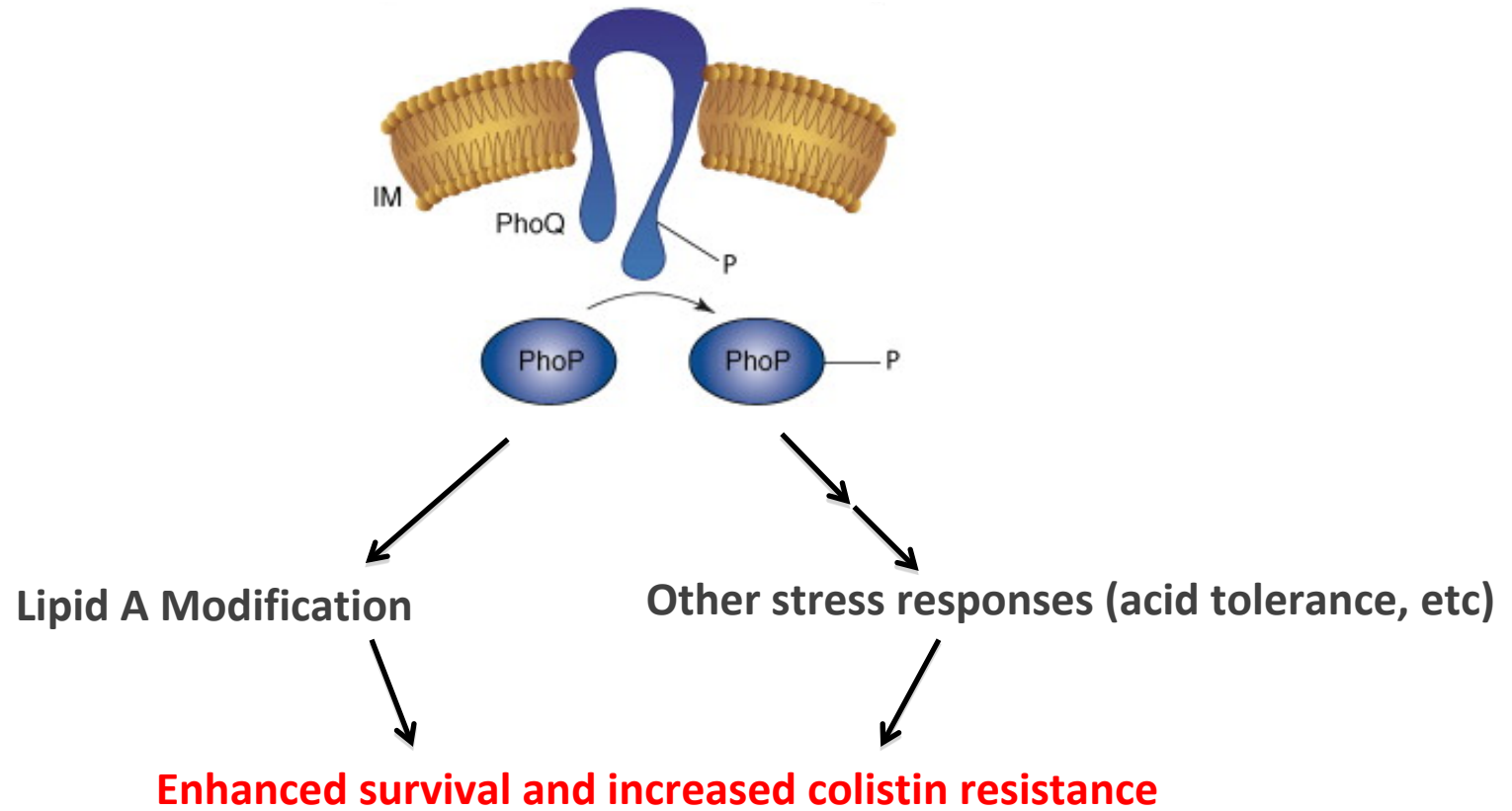


DNA sequences identical

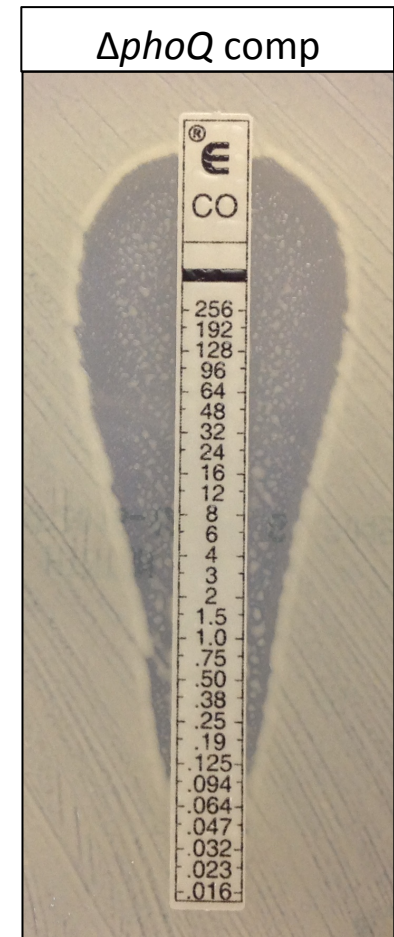
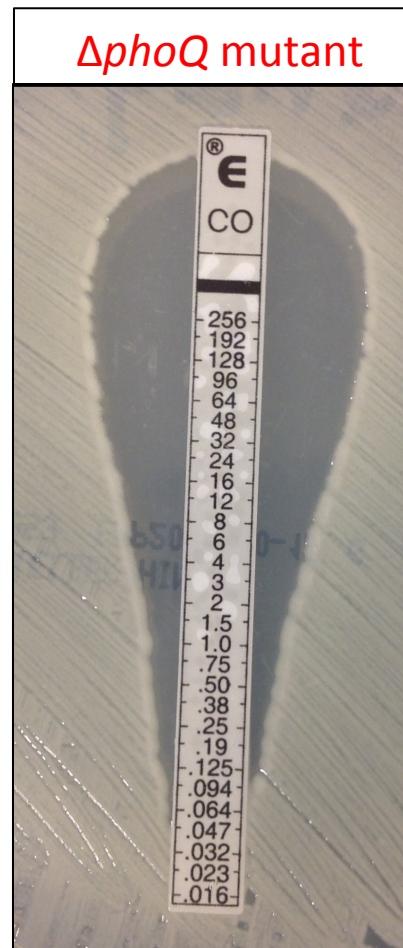
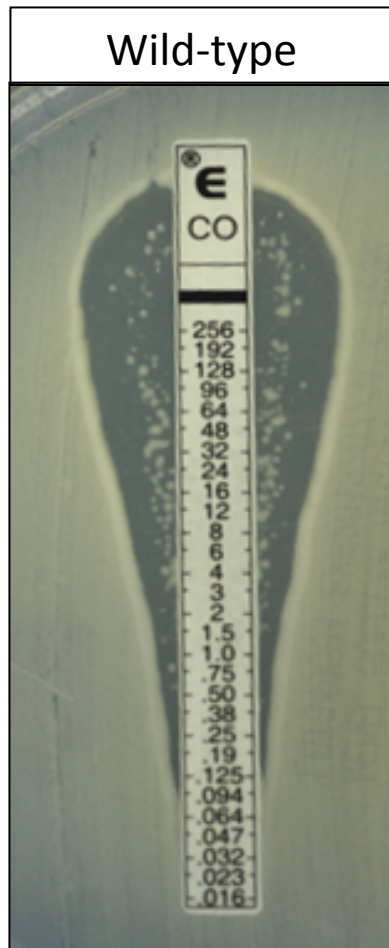
686 genes significantly differentially expressed in the resistant subpopulation
(325 up-regulated, 361 down-regulated)

PhoP/PhoQ signature

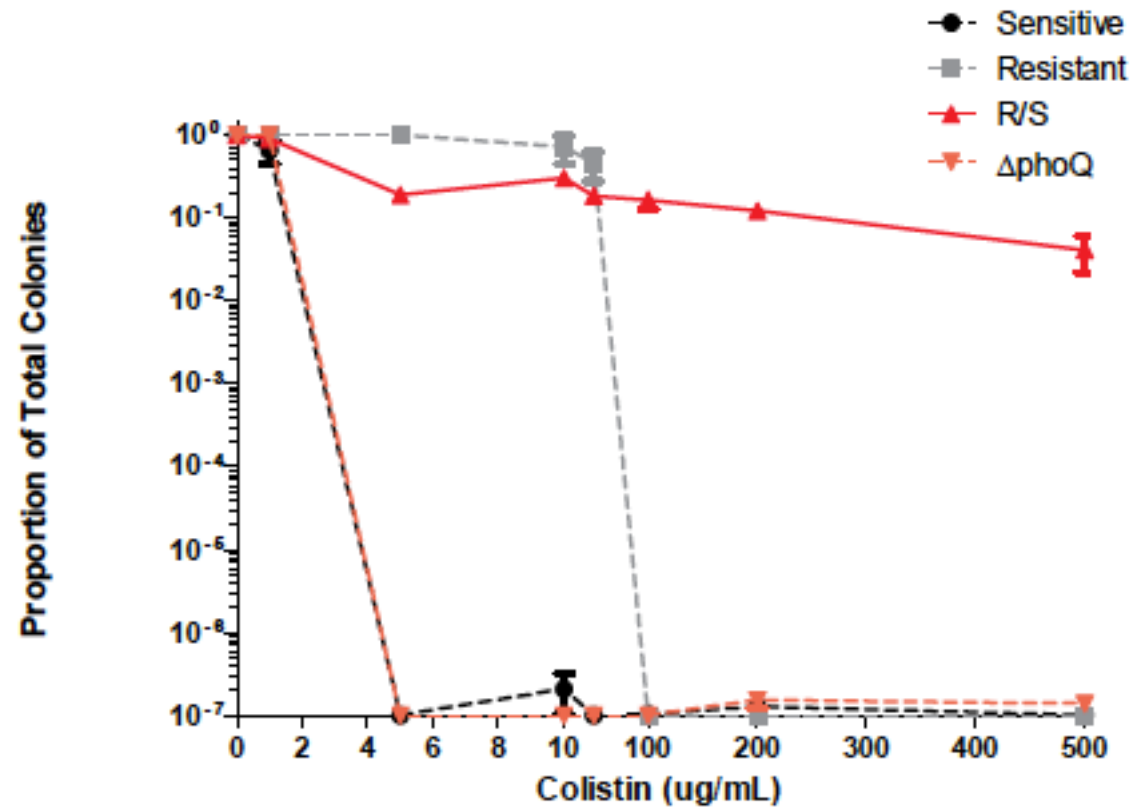
PhoP/PhoQ



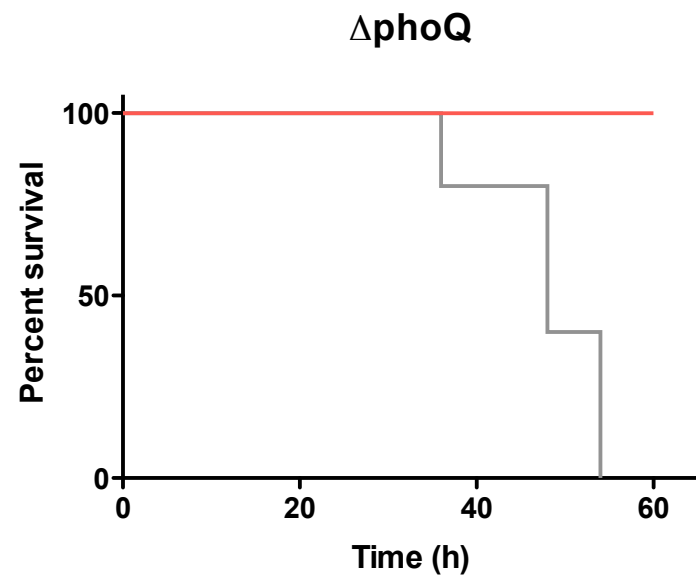
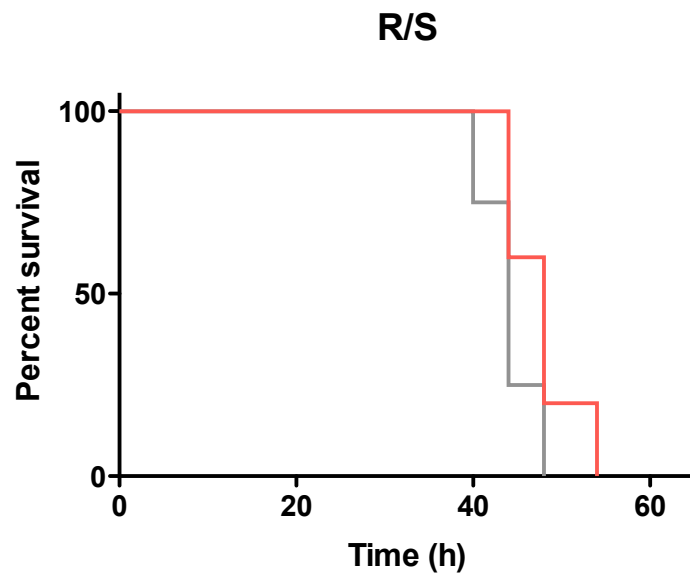
PhoQ is required for colistin heteroresistance



PhoQ is required for colistin heteroresistance



phoQ mutant is sensitive to colistin therapy

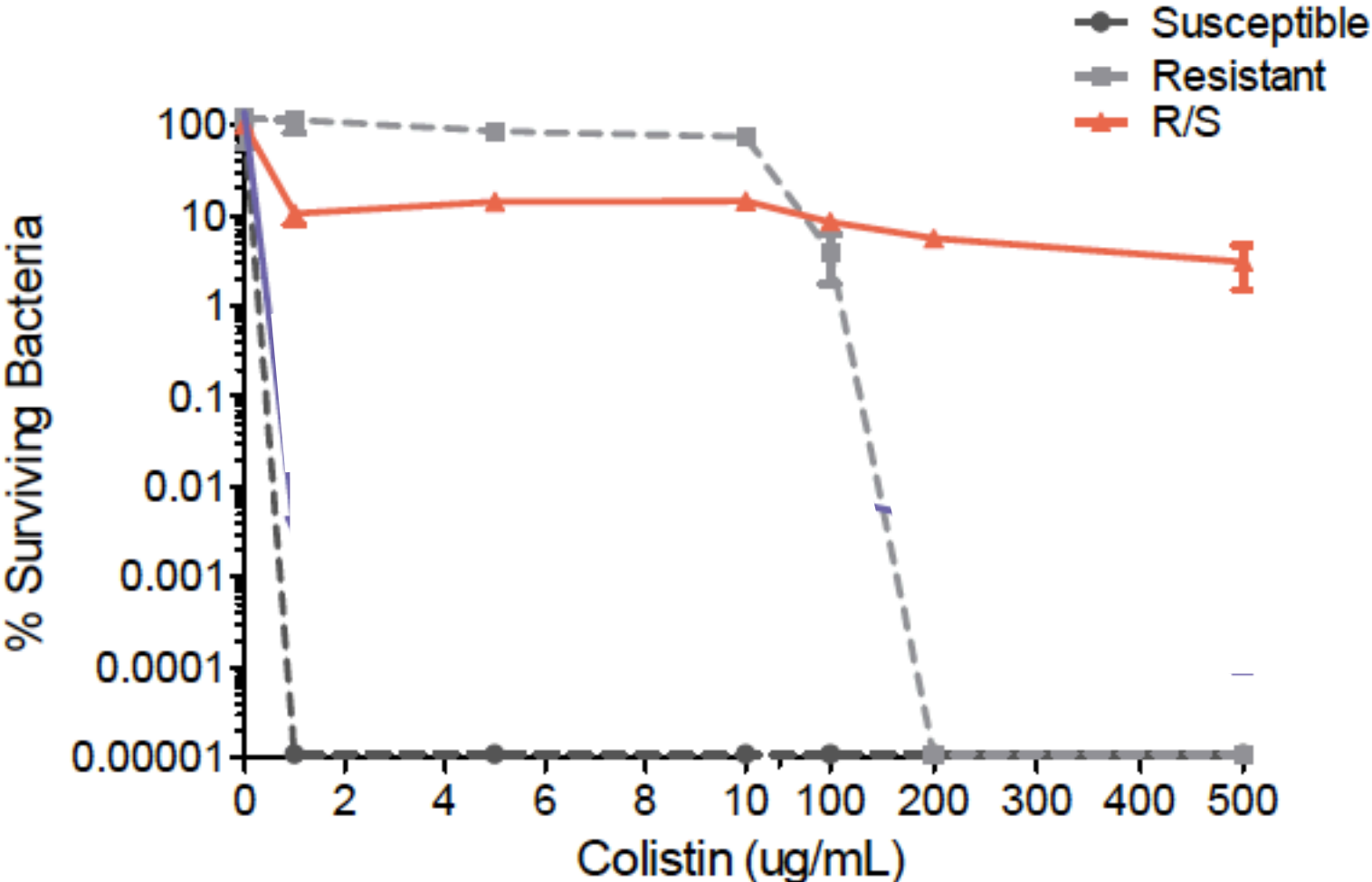


Conclusions

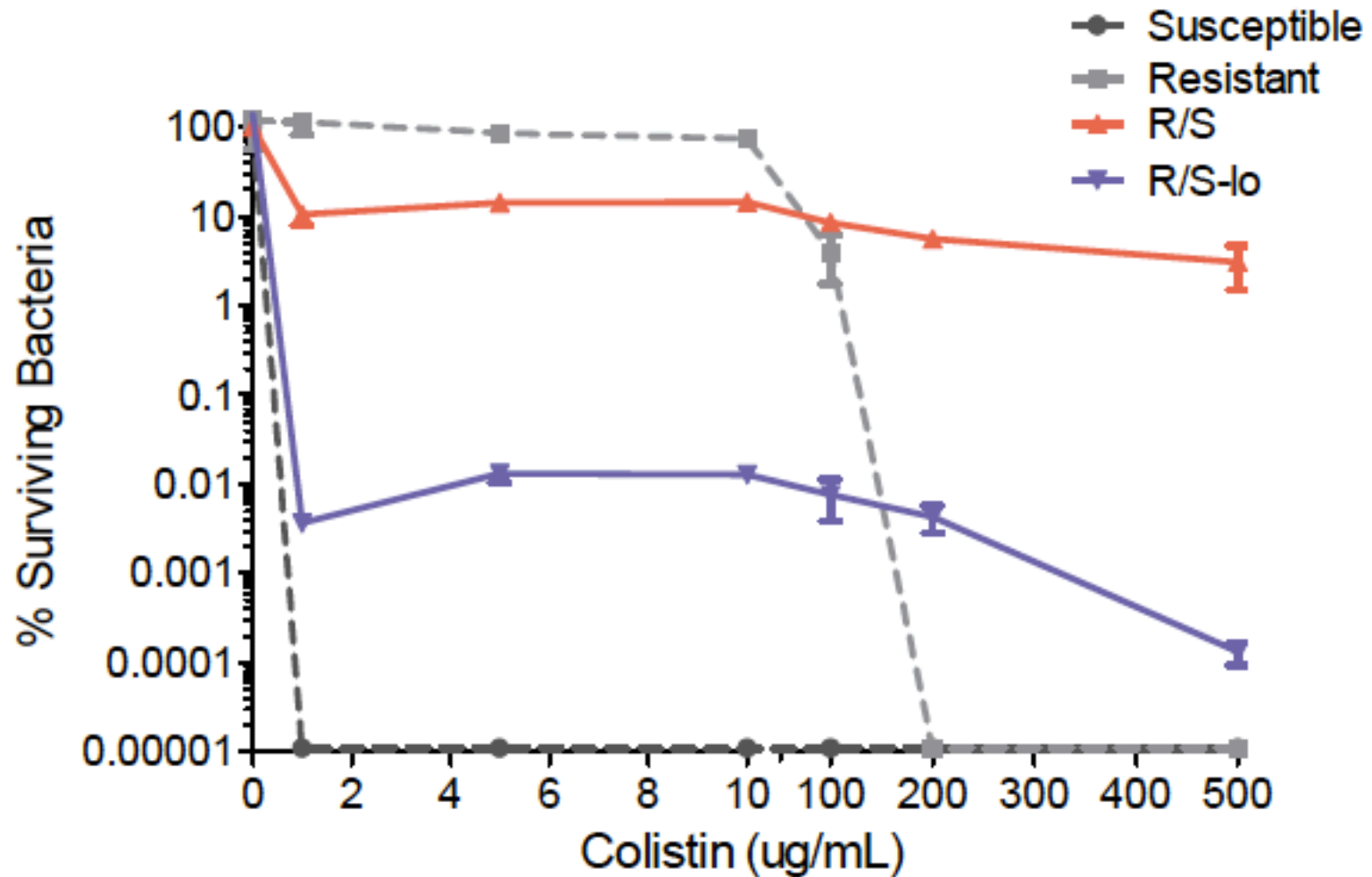
PhoQ is critical for colistin heteroresistance

Absence of PhoQ sensitizes the bacteria to colistin therapy

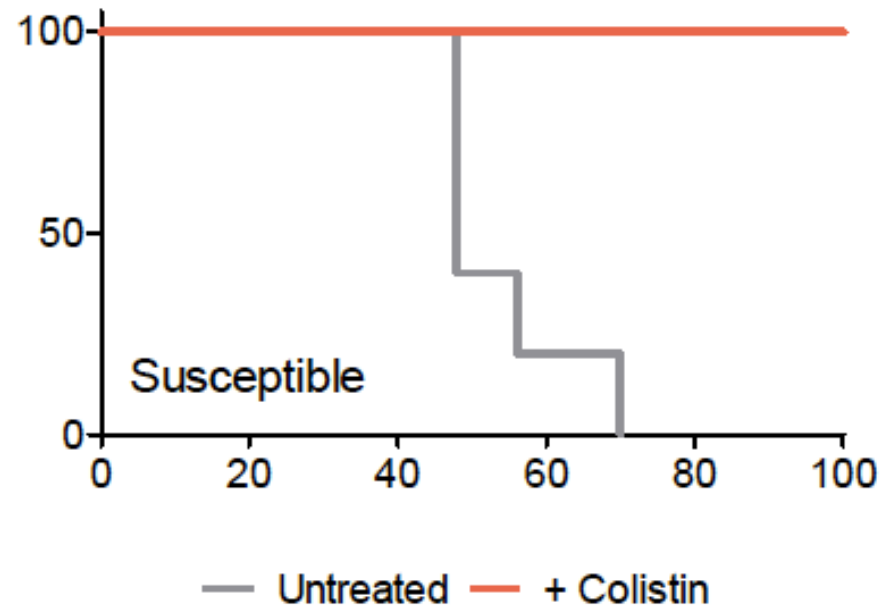
Highly resistant subpopulation



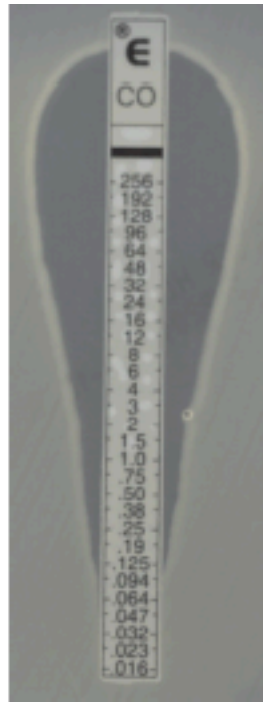
Lower frequency colistin heteroresistant isolate



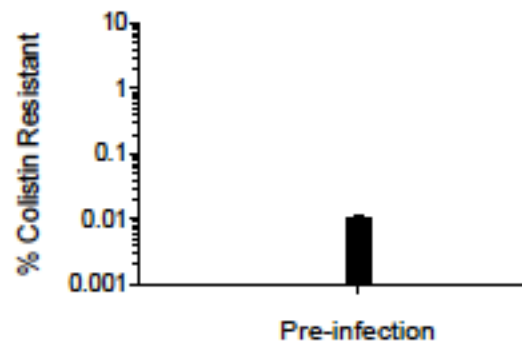
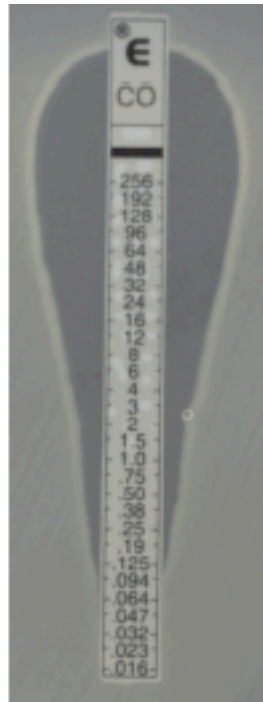
Resistant subpopulation mediates treatment failure



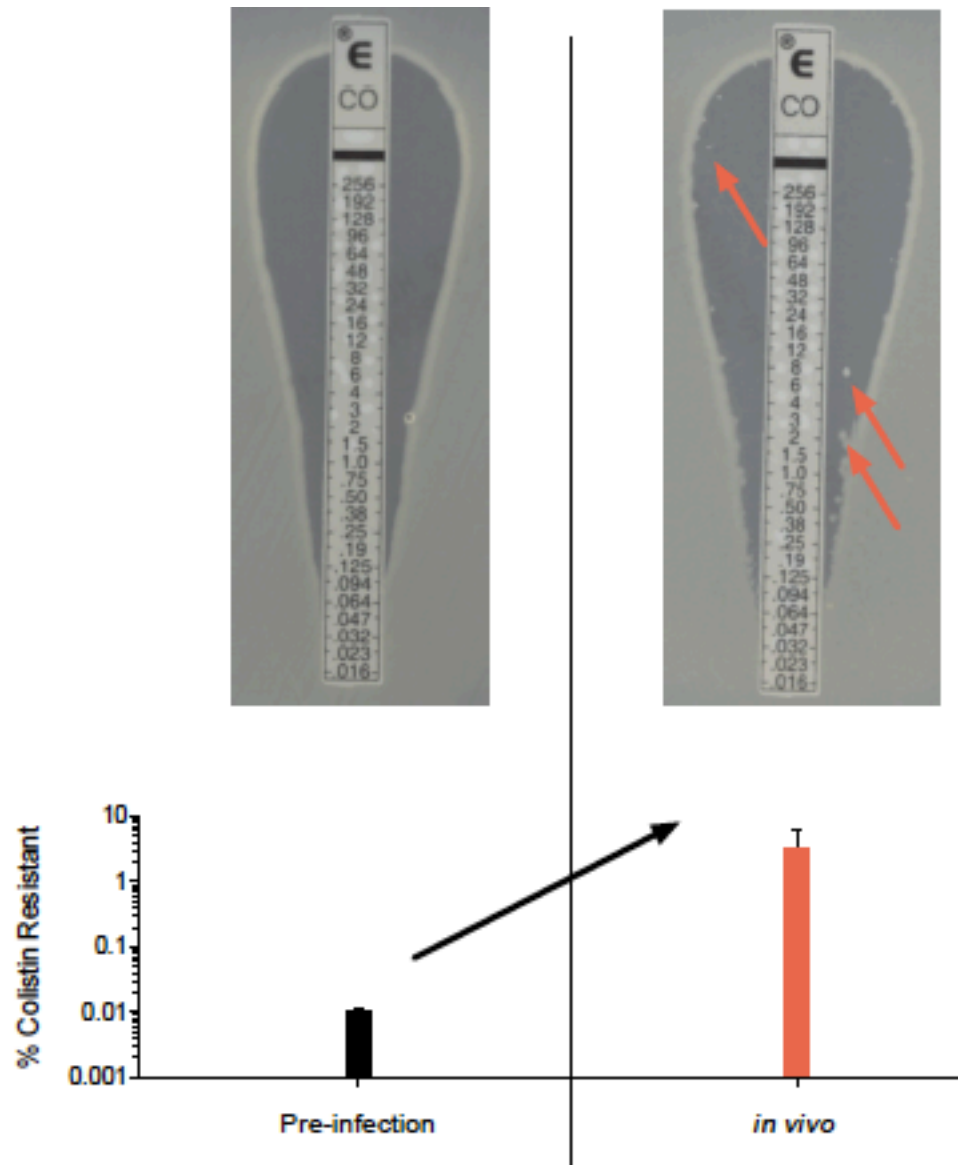
Undetected resistant subpopulation increases in frequency during *in vivo* infection



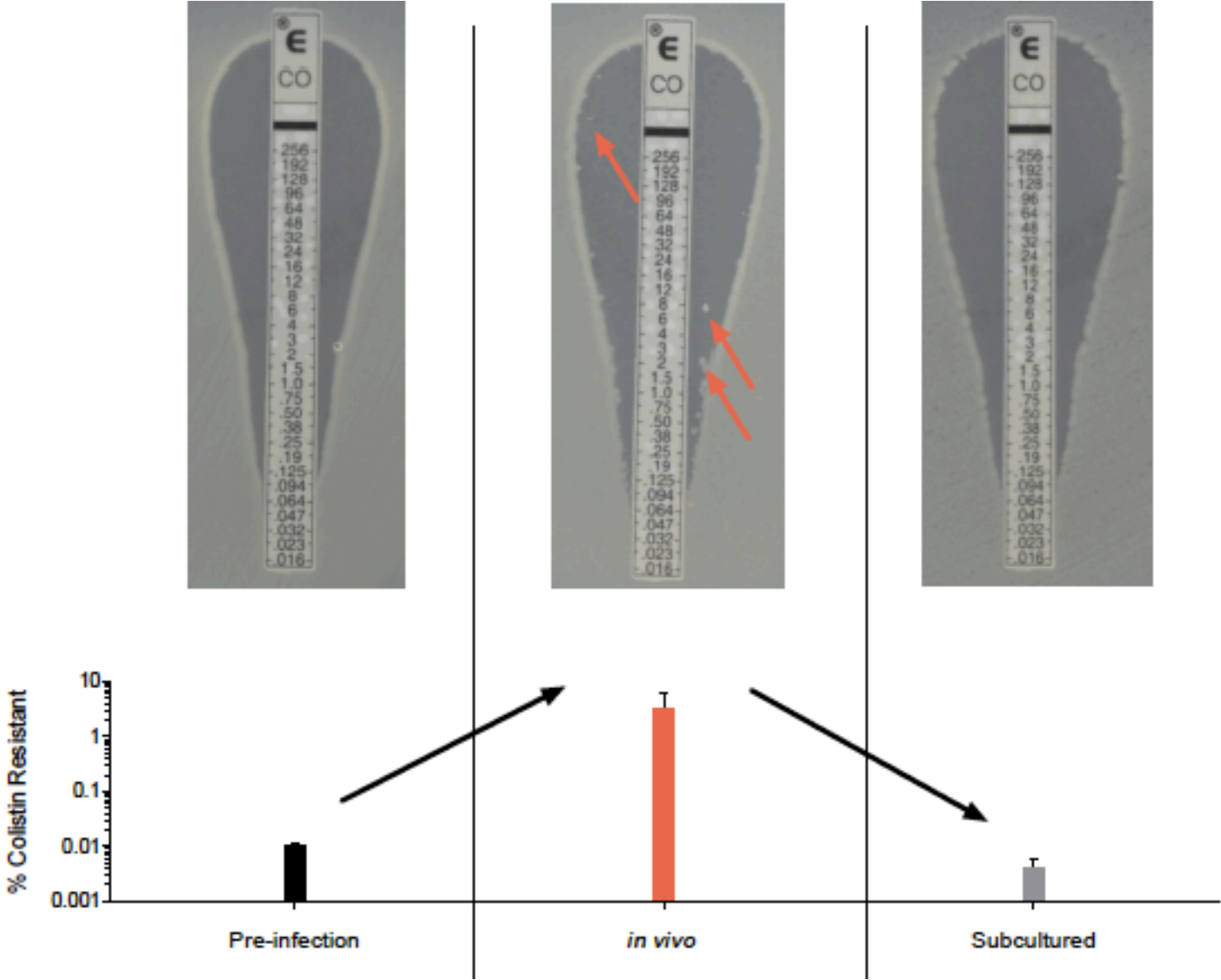
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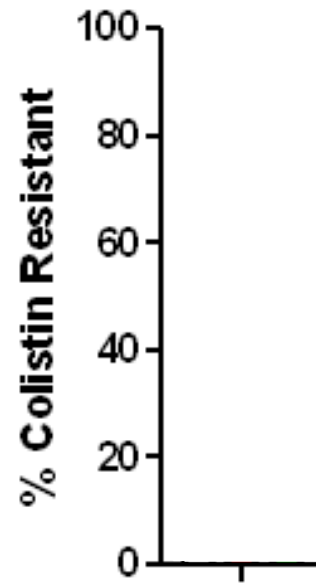
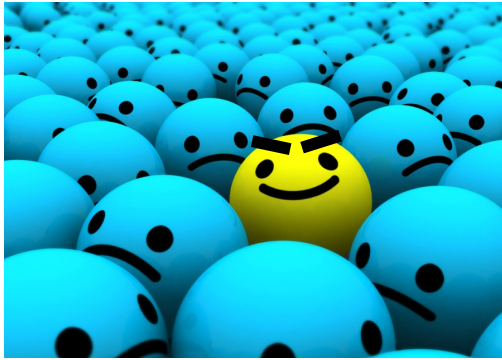
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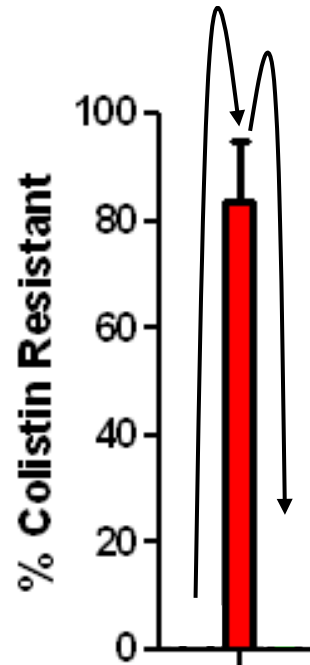
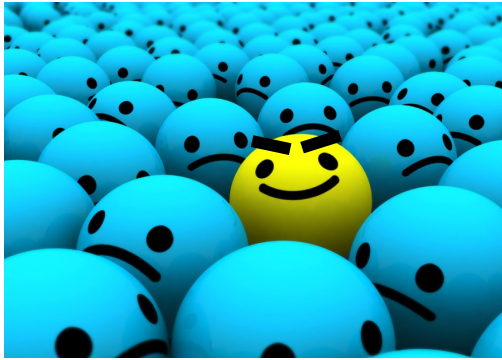
Undetected resistant subpopulation increases in frequency during *in vivo* infection



Model



Model



Low level heteroresistance is undetected in the clinical lab and may cause unexplained treatment failures

Acknowledgements

Weiss Lab

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Steve Bosinger (Yerkes)

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VA

Bill Shafer

Phil Rather



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