Bacterial Persisters - a Thorny Problem in Clinical Antibiotic Treatment

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Uropathogenic E. coli (UPEC)

- Acute Urinary Tract Infection, **20%-30%** will have a recurrent infection within 3-4 months.

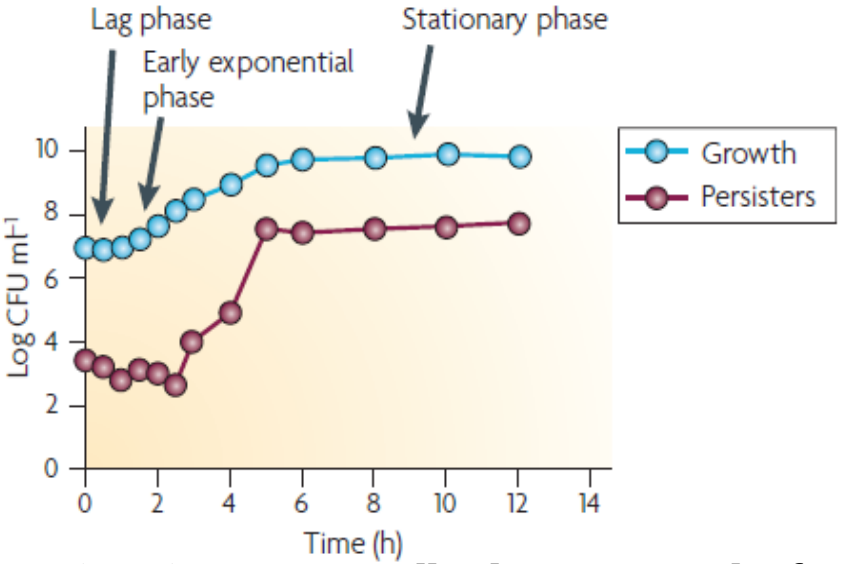
  Foxman, B. (2002). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *The American journal of medicine*, *113*(1), 5-13.

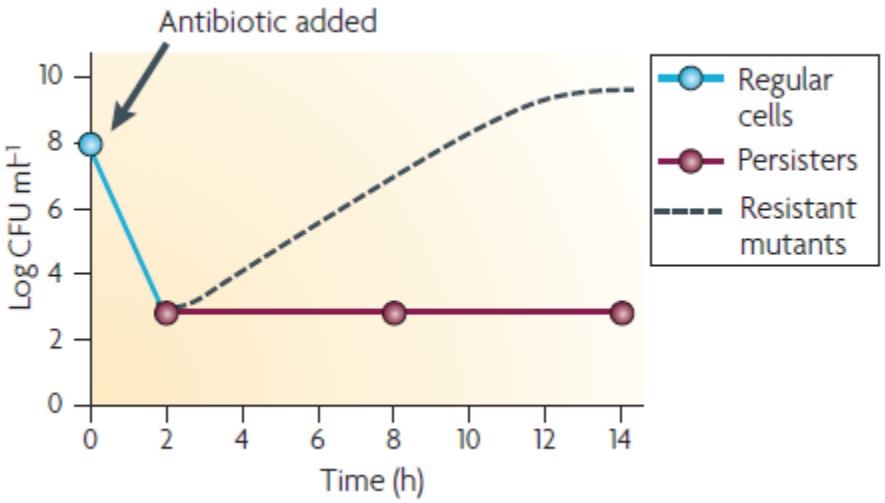
- Clinical chronic infection.

http://newpaper.dahe.cn/dhjkb/html/2013-03/26/content_868584.htm?div=-1

Bacterial Persisters- main reason of chronic disease

- **Bacterial Persisters**: a transiently multidrug-tolerant subpopulation of bacteria.





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Bacterial Persisters- Clinical chronic infections

	Pathogen	Persistent disease	Biologic mechanisms
Asymptomatic persistent infections	*Mycobacterium tuberculosis*	Latent tuberculosis	Intracellular growth, persisters
	Helicobacter pylori	Gastritis, gastric cancer	Intracellular growth
	Salmonella Typhi	Chronic carrier, gall bladder carcinoma	Intracellular growth, biofilm formation
	Treponema pallidum	Latent syphilis	Intracellular growth
Symptomatic persistent infections	*Pseudomonas aeruginosa*	Bronchiectasis/pneumonia in CF patients	Biofilms, small colony variants, persisters
	Escherichia coli	Recurrent urinary tract infections	Intracellular growth, biofilms
	Staphylococcus aureus	Broncicctasis/pneumonia in CF patients; device-associated infections	Biofilms, small colony variants
	Hemophilus influenza	Recurrent otitis media	Biofilms
	Mycobacterium leprae	Leprosy	Intracellular growth

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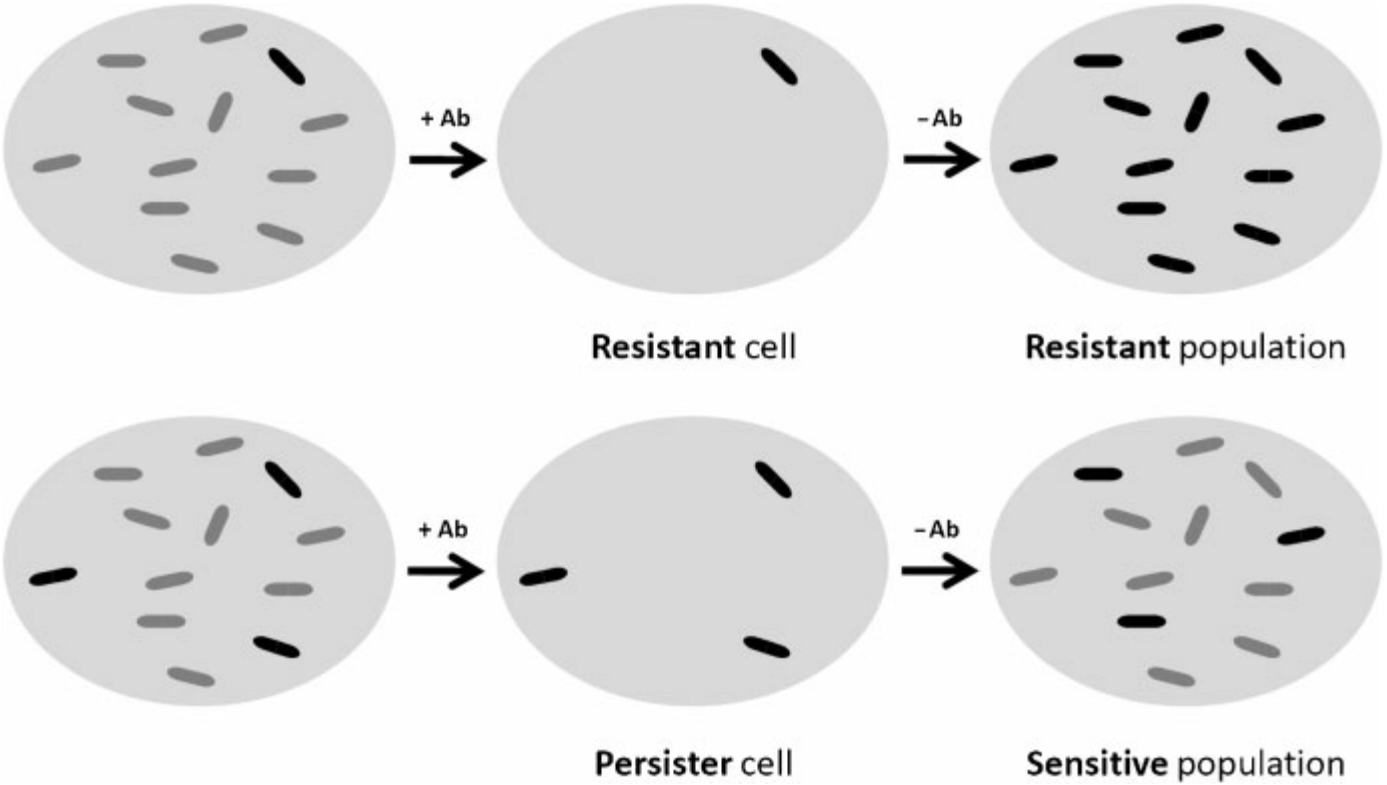
Bacterial Persisters- multidrug tolerance



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Bacterial Persisters- Characteristics

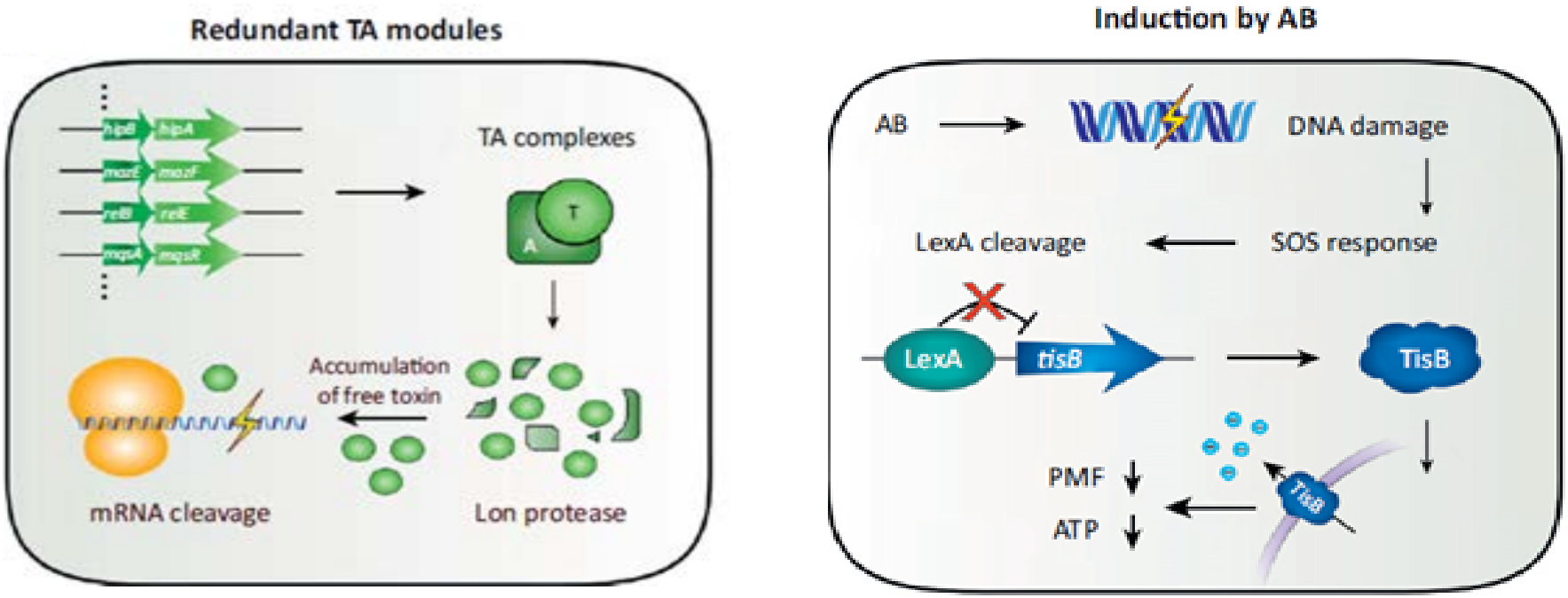
- A minor part of a bacterial population (from10^{-6} to 10^{-1}).
- Multidrug Tolerance.
- Non-heritable phenotypic variation.
- Transient switch.

Bacterial Persisters- Formation

TA (Toxin- Antitoxin) modules

- Main model for the formation of persisters.
- Toxin: non-secreted, inhibits essential cellular functions.
- Antitoxin: normal circumstance, neutralizes the toxin, so that bacterial cell growth is unaffected.

Bacterial Persisters- Formation



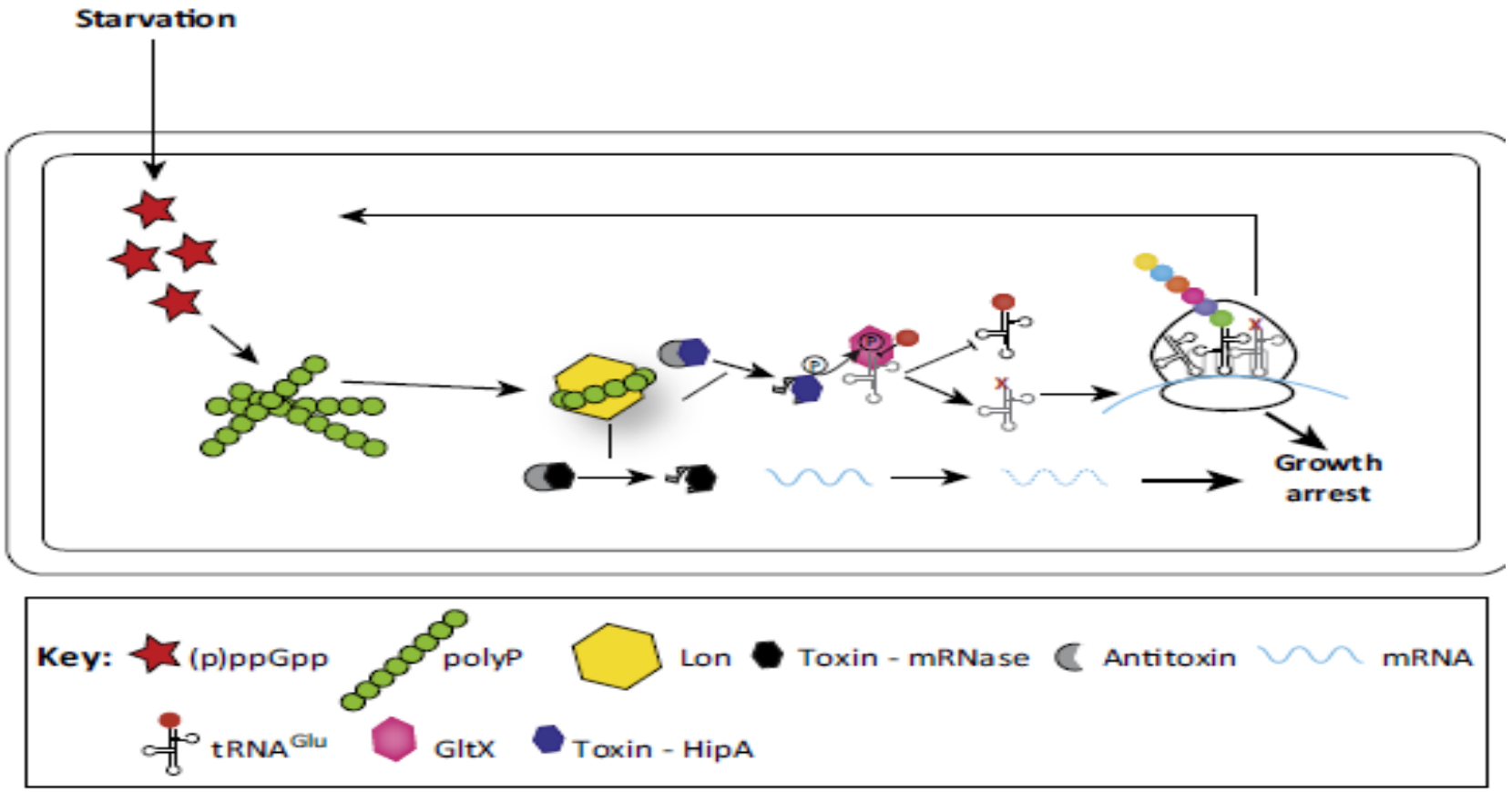
Kint, C. I., Verstraeten, N., Fauvart, M., & Michiels, J. (2012). New-found fundamentals of bacterial persistence. *Trends in microbiology*, *20*(12), 577-585.

Bacterial Persisters- To be or not to be

- **Triggers**: a variety of environmental conditions.

- Starvation, host environment, indole, quorum sensing, SOS response and antibiotics, etc.

Bacterial Persisters- To be or not to be



Helaine, S., & Kugelberg, E. (2014). Bacterial persisters: formation, eradication, and experimental systems. *Trends in microbiology*.

Bacterial Persisters- To be or not to be

Inherent variability underlying persistence	Trigger mechanism	Mechanism for noise amplification	Organism
Growth arrest	Starvation	Unknown	*E. coli*
	Starvation	TA	*E. coli*
	Starvation	TA threshold	*E. coli*
	Prolonged starvation	Unknown	*M. smegmatis*
	Prolonged starvation	Unknown	*E. coli*
	Quorum sensing	Unknown	*P. aeruginosa*
	Biofilm formation	TA, threshold?	*E. coli*
	Intracellular residence	Unknown	*S. enterica*
Stringent response induction	Amino-acid starvation	Bi-stability	*M. smegmatis*
SOS response induction	Ciproflaxin	TA, threshold?	*E. coli*
Efflux pumps activation	Intracellular residence	Unknown	*M. tuberculosis*, *M. marinum*
Down regulation of virulence factors	Intracellular residence	Unknown	*S. aureus*
Chromatin state in cancer cells	Unknown	Unknown	Mammalian cells

Balaban, N. Q. (2011). Persistence: mechanisms for triggering and enhancing phenotypic variability. ***Current opinion in genetics & development, 21*****(6), 768-775.**

Bacterial Persisters- Experimental systems

- Persisters: focus on pathogenic bacteria, and preferably those cause recalcitrant infections.

- Persisters: pay attention to the disparity between in vitro and in vivo experiments.

Internalization of *Salmonella* by Macrophages Induces Formation of Nonreplicating Persisters

Sophie Helaine,* Angela M. Cheverton,† Kathryn G. Watson,† Laura M. Faure, Sophie A. Matthews, David W. Holden*

Salmonella infected Macrophages

Many bacterial pathogens cause persistent infections despite repeated antibiotic exposure. Bacterial persisters are antibiotic-tolerant cells, but little is known about their growth status and the signals and pathways leading to their formation in infected tissues. We used fluorescent single-cell analysis to identify *Salmonella* persisters during infection. These were part of a nonreplicating population formed immediately after uptake by macrophages and were induced by vacuolar acidification and nutritional deprivation, conditions that also induce *Salmonella* virulence gene expression. The majority of 14 toxin-antitoxin modules contributed to intracellular persister formation. Some persisters resumed intracellular growth after phagocytosis by naïve macrophages. Thus, the vacuolar environment induces phenotypic heterogeneity, leading to either bacterial replication or the formation of nonreplicating persisters that could provide a reservoir for relapsing infection.

Many studies, mostly focusing on bacteria grown in laboratory media, have shown that persisters are an unstable, nongrowing, multidrug-tolerant subpopulation that result from phenotype switching (*1*–*3*). In contrast to antibiotic-resistant bacteria arising from heritable mutations, the progeny of persisters are mainly antibiotic-sensitive cells. Most studies on persistent infections are based on the assumption that a proportion of the bacterial population is nonreplicating (*4*); however, this consensus was challenged recently in studies that showed replicating antibiotic-

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Activated ClpP kills persisters and eradicates a chronic biofilm infection

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Deep-seated mouse thigh infection model.

Chronic infections are difficult to treat with antibiotics but are caused primarily by drug-sensitive pathogens. Dormant persister cells that are tolerant to killing by antibiotics are responsible for this apparent paradox. Persisters are phenotypic variants of normal cells and pathways leading to dormancy are redundant, making it challenging to develop anti-persister compounds. Biofilms shield persisters from the immune system, suggesting that an antibiotic for treating a chronic infection should be able to eradicate the infection on its own. We reasoned that a compound capable of corrupting a target in dormant cells will kill persisters. The acyldepsipeptide antibiotic (ADEP4) has been shown to activate the ClpP protease, resulting in death of growing cells. Here we show that ADEP4-activated ClpP becomes a fairly nonspecific protease and kills persisters by degrading over 400 proteins, forcing cells to self-digest. Null mutants of *clpP* arise with high probability, but combining ADEP4 with rifampicin produced complete eradication of *Staphylococcus aureus* biofilms *in vitro* and in a mouse model of a chronic infection. Our findings indicate a general principle for killing dormant cells—activation and corruption of a target, rather than conventional inhibition. Eradication of a biofilm in an animal model by activating a protease suggests a realistic path towards developing therapies to treat chronic infections.

Bacterial Persisters- Experimental systems

Table 1. Experimental models to study bacterial persisters during infection of their host

Experimental model	Bacterial species	Tools to analyse persisters[a]
Cultured host macrophages	*Mycobacterium tuberculosis*, *Salmonella* Typhimurium	CFU, microscopy, and FD
Zebrafish larvae	*Mycobacterium marinum*	CFU and microscopy
Isoniazid-treated mice	*M. tuberculosis*	STM and CFU
Mouse urinary tract-inserted catheters	*Escherichia coli*	CFU
Murine subcutaneous biofilm	*Pseudomonas aeruginosa*	CFU
Mouse thigh infection	*Staphylococcus aureus*	CFU

[a]Abbreviations: CFU, colony-forming unit; FD, fluorescence dilution; STM, signature-tagged mutagenesis.

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Bacterial Persisters- Eradication

- **Mathematical modeling**:

1. Periodic dosing of antibiotics.

2. Prolong treatment with current antibiotics.

Bacterial Persisters- Eradication

- **Sugars**: led to increased uptake of aminoglycosides.
- **Weak electrochemical currents** and **quorum sensing inhibitors**.
- **Oxygen**: Reactive Oxygen Species (ROS) .
- **Lytic phages**: lyse bacteria when growth is resumed.

Bacterial Persisters- Eradication

- **Small molecule compounds**: **ADEP4** activates and corrupts ClpP, results in over 400 various functional types of proteins degradation.

Bacterial Persisters- Conclusion

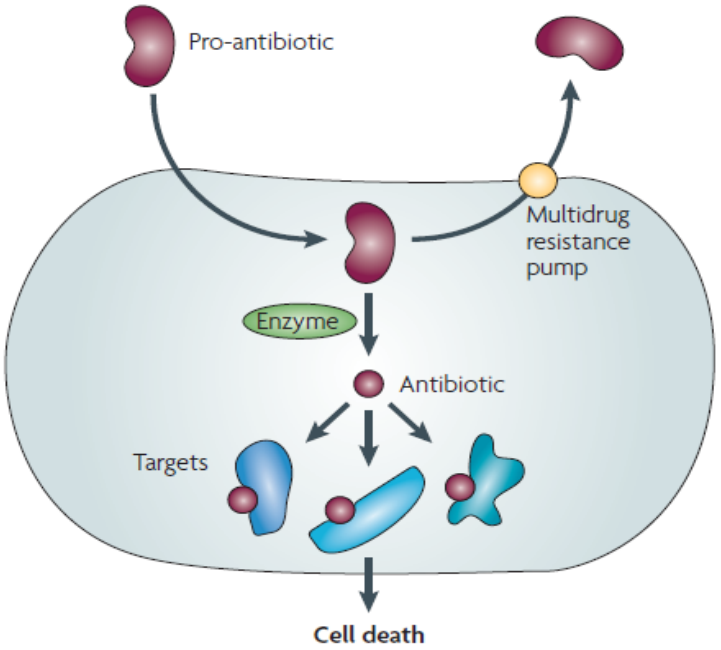
- Persisters may result in the **enrichment of multidrug resistance bacteria**.
- Persisters formation is very **complex and under multigenic control**.
- Persisters formation include **bacterial** species, **host** environment, and **treatment** regimens.

Bacterial Persisters- Futher Diretion

- Design successful drugs.

1. Metabolite-enabled eradication of bacterial persisters: awake or inhibit.

2. pro-antibiotic: nonspecifically, affect all bacterial cells.



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Thank you!