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Eradication of Persister Bacteria Note

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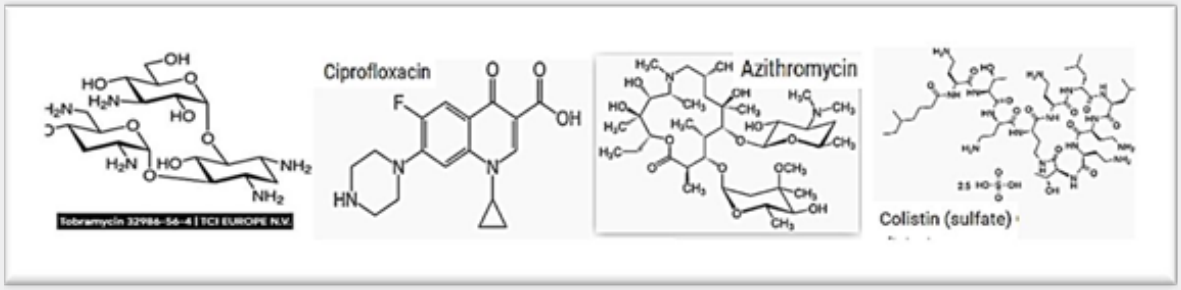
Several well-recognized puzzles in microbiology have remained unsolved for decades. These include latent bacterial infections, unculturable microorganisms, persister cells and biofilm multidrug tolerance. Accumulating evidence suggests that these seemingly disparate phenomena result from the ability of bacteria to enter into a dormant (non-dividing) state. The molecular mechanisms that underlie the formation of dormant persister cells are now being unravelled and are the focus of this Review.

It is difficult to "get rid of" highly resistant (HiP) pathogenic bacteria. Clinical records of treatment with antibiotics active against *Pseudomonas aeruginosa* are still conflicting. The 4 antibiotic classes leading to targeting P. aeruginosa prescribed to patients include aminoglycosides (top drug: tobramycin), fluoroquinolones (top drug: ciprofloxacin), macrolides (top drug: azithromycin), and polymyxins (top drug: colistin).



Colistin (polymyxin E) is a positively charged minute-peptide antibiotic agent. It disrupts the integrity of Gram-negative bacteria's outer membrane of the bacterial cell wall by binding to the fatty portion of lipopolysaccharides, resulting in cell death. The endotoxic activity of lipopolysaccharides is simultaneously inhibited. Colistin is increasingly being prescribed as a rescue treatment for multidrug-resistant bacterial infections. Nephrotoxicity and, to a lesser extent, neurotoxicity occur frequently during systemic colistin therapy, and have severely limited its application in the past. However, these side effects are largely reversible and can be managed with close monitoring. The former drug colistimethate sodium (CMS) is less toxic and is therefore the preferred formulation for parenteral administration. It is important to note that resistance to colistin appears to occur frequently, unless it is combined with other antibiotics, but more studies are needed on this phenomenon. The Pharmacokinetic and pharmacodynamic properties have received little attention, in part because of the physicochemical peculiarities of polymyxin antibiotics, especially their tendency to adhere to other molecules and surfaces. The relationship between the area under the curve of free colistin and the minimal inhibitory concentration (MIC) of the pathogen best predicts microbiological and clinical responses, but further studies are needed in this area. Also, there is a need for further standardization in the production and labeling of colistin formulations, and in the manner in which the sensitivity of bacteria to colistin isMore about this source textSource text required for additional translation information determined [1].

Despite intensive antibiotic therapy, often persists in the airways of cystic fibrosis (CF) patients for decades, and can do so without developing antibiotic resistance. Studies applying high-throughput screening assays of bacterial survival after high-dose ciprofloxacin treatment, we determined the prevalence of persisters in a large cohort of patients using 460 longitudinal isolates of P. aeruginosa from 39 CF patients.

Isolates were classified as high persistent variants (Hip) if they reappeared following antibiotic treatment in at least 75% of the experimental replicates. Strain genomic data, isolate phenotype, and patient care records were combined in a lineage-based analysis of persistence. The formation, and clinical impact. In total, 19% of the patients were classified as hip. Hip occurrence during lineage settlement time within the 22 Hip+ patients. Many hip+ lineages produced multiple hip isolates, but few hip+ lineages were dominated by hip.

Although we did not observe a strong signal of adaptive genetic convergence within the hip isolates, they generally appeared concurrently with or following the development of ciprofloxacin resistance and growth retardation. Transient lineages were the majority of Hip, while strains that persisted through a clinically

diagnosed 'elimination' period were the majority of Hip+. Patients received indistinguishable treatment regimens before hip onset, but hip+ patients overall were significantly more treated than hip patients, indicating recurrent treatment failure. When subjected to a similar dose of antibiotic *in vivo*, isolated femur survived better than femur in a structured biofilm environment. In conclusion, the femoral phenotype appears to contribute substantially to the Long-term recognition of a lineage in the CF lung environment. Results are against the existence of a single dominant molecular mechanism underlying bacterial antibiotic persistence. Instead, we show that multiple pathways, both phenotypic and genetic, are available for the 5persistent formation and resulting increase in stress capacity and treatment failure in CF airways.

The persistent phenotype, the ability of bacterial cells to survive antibiotic treatment without developing antibiotic resistance, is considered to be a majour reason to the failure of treatment in recurrent bacterial infections. Applying isolates of the bacterial pathogen *Pseudomonas aeruginosa*, collected over a decade from the airways of 39 young cystic fibrosis patients, were investigated the emergence, persistence, and contribution to fitness of the persistent phenotype in a clinical scenario with high levels of antibiotic therapy. Scientists see persistent variants in 56% of patients, but no signal of adaptive genetic convergence to support their appearance, and no difference in patients' treatment regimens before the appearance of the variant. However, bacterial lineages (distinct bacterial strains that infect the patient over time) that produce persistent high variants also produce patients with antibiotic resistance and/or just a slowed development rate. These lineages are also greatly and less likely to be transient and more likely to persist in the patient's health for long periods of time without detection in the clinic. In conclusion, we conclude that the persistent phenotype can appear in many adaptive ways, this offers significant fitness contributions in the complex *in vivo* environment of the cystic fibrosis airway.

Many pathogenic bacteria can infect and remain within their hosts for long periods of time. This can be due to host immunosuppression, immune-evasion by the pathogen microbes, and/or by ineffective killing of the antibiotics.

Many Bacteria can survive antibiotic treatment if they are resistant or tolerant to the drug. Persisters are a subpopulation of antibiotic-resistant bacterial cells that are often slow-growing or growth-arrested and are able to resume growth after lethal stress. The formation of persistent cells establishes phenotypic heterogeneity within a bacterial population, and is hypothesized to be important for increasing the chance of successful adaptation to environmental changes. The presence of persistent cells may result in resistance and recurrence of persistent bacterial infections, and is connected with the increased risk of developing antibiotic resistance during treatment. If the mechanisms of the formation and regrowth of antibiotic-tolerant cells were better understood, it could lead to the development of new approaches to eradicating persistent bacterial infections. Here scientists discuss recent developments in our understanding of persistent microbes and their potential implications for ongoing care. Infections [2].

Mechanism, by which some pathogenic bacteria are able to survive antibacterial treatment was revealed for the first time by researchers from the Hebrew University in Jerusalem. This work could pave the way for new ways to control such bacteria.

In addition to the well-known phenomenon where some bacteria become resistant to antibiotics through mutation, there are other types of bacteria, called "persistent bacteria", which are not resistant to antibiotics but simply continue to exist in a dormant or inactive state. exposed to antibacterial treatment. These bacteria "wake up" later when this treatment is over, resume their harmful tasks and present a dilemma of how to deal with them.

Until now, it was known that there is a connection between this type of bacteria and the naturally occurring toxin HipA in bacteria, but scientists did not know the cellular purpose of this toxin and how its activity induces the bacteria's dormancy [3].

During Mycobacterium tuberculosis infection, a bacterial population may become resistant to antibiotic killing in the absence of genotypic resistance, making treatment challenging. An *in vitro* model capable of generating an antibiotic-tolerant phenotypic subpopulation of cells, often called persisters, within populations of Mycobacterium smegmatis and M. tuberculosis. Researchers find that persisters differ from the larger antibiotic-susceptible population, as a small decrease in dissolved oxygen (DO) saturation (20%) enables their survival in the face of bactericidal antibiotics. Conversely, if high levels of DO are maintained, all cells succumb and the culture is sterile. With increasing evidence that bactericidal antibiotics induce cell death through the production of reactive oxygen species (ROS), we hypothesized that the decrease in DO reduces the concentration of ROS, thus facilitating sustained survival, and that maintaining high DO yields sufficient ROS to kill persistently. Consistent with this hypothesis, the hydroxyl radical compound thiourea, when added to M. smegmatis cultures maintained at high DO levels, rescues the persister population. In contrast, the antibiotic clopimin, which increases ROS through an NADH-dependent cycling pathway, successfully eradicates the persistent population. Recent studies suggest that environmentally induced antibiotic tolerance of bulk populations may be due to enhanced antioxidant capacities. We now show that the small persistent subpopulation within a larger antibiotic-sensitive population also shows differential susceptibility to hydroxyl radical addition. Furthermore, we show that stimulation of ROS production can eradicate persistent infections, thus providing a potential strategy for managing persistent infections [4].

In a phenomenon called persistence, a small number of bacterial cells survive even after exposure to antibiotics. Recently, bactericidal antibiotics have been shown to kill bacteria by increasing the levels of hydroxyl radicals within the cells. In the study, scientists report a direct correlation between the formation of intracellular hydroxyl radicals and bacterial persistence. By performing flow cytometric analysis in three-dimensional space, we resolved different bacterial populations in terms of intracellular hydroxyl radical levels, morphology and viability. We determined that after antibiotic treatment, a small subpopulation of Escherichia coli survivors does not overproduce hydroxyl radicals and maintains a normal morphology, while the majority of bacterial cells were killed by the accumulation of hydroxyl radicals and displayed a filamentous morphology. Our results indicate that bacterial persisters can be formed once they have transient defects in mediating reactions involved in the hydroxyl radical formation pathway. Thus, it is highly likely that the persisters do not share a common mechanism but that each persister cell responds to antibiotics in different ways, while all generally show reduced hydroxyl radical formation and increased antibiotic tolerance [5].

Selective Killing of Bacterial Persister by a Single Chemical Compound without Affecting Normal Antibiotic-Sensitive Cells.

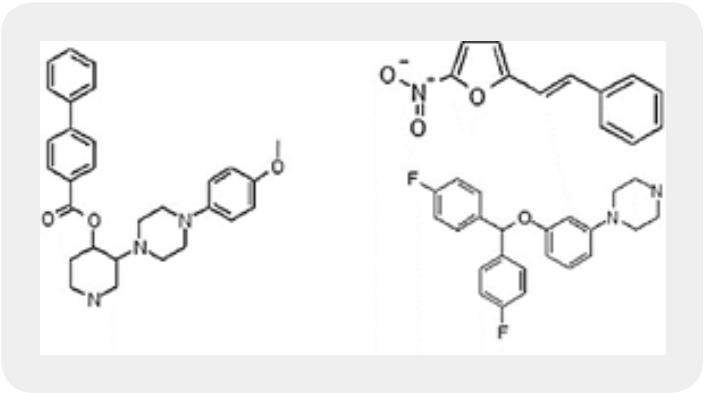


Figure: *Compounds effect of selected inhibitors on persister formation*

Researchers shaw that 3-[4-(4-methoxyphenyl)piperazin-1- yl] piperidin-4-yl biphenyl-4-carboxylate screened out of a chemical library, selectively kills bacterial persisterthat tolerate antibiotic treatment but does not affect normal antibiotic-sensitive cells. C10 led persister to antibiotic- induced cell death by causing reversion of persister to antibiotic-sensitive cells. This work is the first demonstration 1in wh1ich the eradication of bacterial persister is based on single-chemical supplementation. The chemical should be versatile in elucidating the mechanism of persistence [6].

The Disinfection of Bacteria Continues with Hydroxyl Radicals Formed in Antibiotics:

During Mycobacterium tuberculosis infection, a bacterial population may become resistant to antibiotic killing without genotypic resistance, making treatment challenging. Scientists describe an *in vitro* model capable of generating an antibiotic- tolerant phenotypic subpopulation of cells, often called persisters, within populations of Mycobacterium smegmatis and M. tuberculosis. We find that persisters differ from the larger antibiotic-sensitive population, as a slight decrease in dissolved oxygen saturation (20%) allows their survival in the face of bactericidal antibiotics. Conversely, if high levels of DO are maintained, all cells succumb and the culture is sterile.

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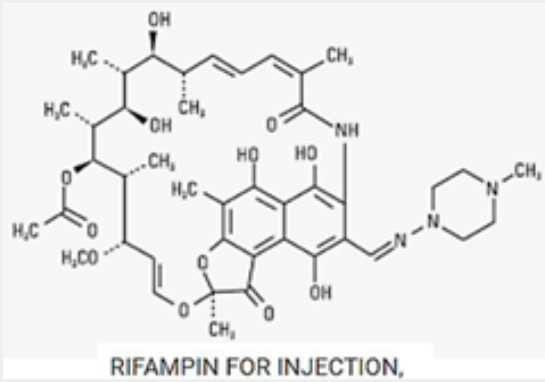
hydroxyl radicals. Furthermore, scientists show that stimulating ROS production can eradicate persisters, thus providing a potential strategy for managing persistent infections [7].

Medicines that Fight Infections caused by Persistent Bacteria:

Persisters are specialized survival cells that protect bacterial populations from being killed by antibiotics. Persistent are dormant phenotypic variants of normal cells rather than mutants. Antibiotics kill bacteria by subverting their targets to produce toxic products; Antibiotic tolerance occurs when targets are inactive. Transcriptome analysis of isolated pressors indicates toxin/antitoxin modules as a major component of constitutive formation. Continuous formation mechanisms are not necessary, which makes it difficult to 1eradi3cate these cells. In Escherichia coli, the toxins RelE and MazF induce dormancy by degrading mRNA; HipA inhibits translation by Ef-Tu phosphorylation; And TisB forms an anion channel in the membrane, leading to a decrease in pmf and ATP levels. Prolonged treatment of chronic infections with antibiotics selects for mutations in the hip that produce more persistent cells. Eradication of tolerant persistents is a serious challenge. Some of the existing antibiotics are able to kill persistently, and points to ways to develop drugs to treat chronic infections. Mitomycin is a prodrug that is converted to a reactive compound that forms adductswith DNA upon entering the cell. Prolonged treatment with aminoglycosides that cause mistranslation leading to folding peptides can sterilize a stationary culture of Pseudomonas aeruginosa, a pathogen responsible for chronic and highly tolerant infections in cystic fibrosis patients. Finally, one of the best bactericidal agents is rifampin, an RNA polymerase inhibitor, and we suggest that it "kills" by preventing ongoing resuscitation [8].



The emergence of bacterial resistance toward currently employed antibiotics has led to the reuse of 'abundant' antibiotics such as polymyxin B (PMB) toward multidrug resistance strains of P. aeruginosa, Enterobacter, Serratia and E. coli [9]. PMB is a naturally occurring cationic cyclic lipopeptide, isolated from Bacillus polymyxa [10], highly bactericidal to Gram-negative bacteria and considered one of the most efficient cell-permeabilizing compounds PMB is composed from a positively charged cyclic peptide ring and a fatty acid-containing tail (Fig. 1). The cyclic part is a seven- member amino acid ring containing four 2,4-aminobutyric acid (Dab) residues, one Thr residue and a hydrophobic segment of DPhe-Leu. The C-terminal carboxylic function of Thr9 forms an amide bond with the -amino group of Dab3 (Fig. 1).

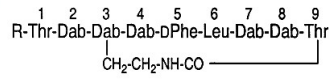
Table 1
Sequences of PMBN analogs

Peptide	Sequence	t_R (min)*
PMBN	TX*cyclo*[XXFLXXT]	19.6
[Ala_3]PMBN	AAATX*cyclo*[XXFLXXT]	20.5
[Ala_6]PMBN	AAAAAATX*cyclo*[XXFLXXT]	20.7
FmocPMBN	Fmoc-TX*cyclo*[XXFLXXT]	29.5
FmocDabPMBN	Fmoc-XTX*cyclo*[XXFLXXT]	29.6
PMB	6-methylheptanoyl/octanoyl-XTX*cyclo*[XXFLXXT]	25.7/26.9

D-amino acids are boldfaced. X, diaminobutyric acid (Dab).
*Retention time on C4 column using a linear gradient of 0–100% buffer B (see Materials and Methods) in 50 min.

Table 2
MIC values (μg/mL) of PMBN analogs

Peptide	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
PMBN	>500	>500	8
[Ala_3]PMBN	>500	>500	>500
[Ala_6]PMBN	>500	>500	>500
FmocDabPMBN	8	16	4
FmocPMBN	8	62	8
PMB	3	4	3



PMBN, R = H
PMB, R = 6-methylheptanoic/octanoic -Dab
FmocPMBN, R = 9-fluorenylmethoxycarbonyl
FmocDabPMBN, R = 9-fluorenylmethoxycarbonyl-Dab
[Ala_3]PMBN, R = Ala-Ala-Ala
[Ala_6]PMBN, R = Ala-Ala-Ala-Ala-Ala-Ala

Fig. 1. The chemical structure of PMBN and its analogs.

The linear N-terminal region is composed of two Dab and one Thr residues together with a 9 or 8 carbon fatty acid, i.e. 6- methyl octanoic [PMB1] and 6-methyl heptanoic acid [PMB2], respectively, forming a long hydrophobic tail. It was shown that both the positive charges and the acylated linear tail are crucial for antibacterial activity [11]. The unique structural features of the cationic peptide are suitable for specific binding to lipid A of gram-negative bacterial lipopolysaccharide (LPS).

Thereby allowing the acylated hydrophobic tail to penetrate through the Gram-negative bacterium's outer membrane [12]. PMB is unique among antibiotics in that it has potent anti- endotoxin activity in addition to its antibacterial activity. This property could be beneficial to patients who are experiencing Gram-negative bacterial sepsis and endotoxinmediated shock. PMB inhibits LPS-induced macrophage production of interferon-, tumor necrosis factor (TNF) and interleukin-1 and 16 (IL-61 and IL-6). *In vivo* PMB is able to act against most Gram- negative septicemia and prevents endotoxin lethality in several animal models [13]. The therapeutic applications of PMB are very limited, however, because of its relatively high toxicity and as a consequence it is used mainly for topical treatment.

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