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Review Article

Molecular Mechanisms of Persistence in Bacteria

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Abstract

A significant mortality rate from infectious diseases is largely mediated by the widespread and uncontrolled use of antibiotics, which has led to the emergence of drug-resistant strains of bacteria. The rapid evolution of 272 bacterial resistance to antimicrobials is a serious challenge for modern health care, mediates the need to create new antibiotic agents, as well as to intensify the study of molecular mechanisms underlying the formation of microorganism resistance. One of these mechanisms is bacterial persistence, manifested by the formation of persistent cells in the culture, which are a phenotypic variant of the isogenic population. The persistence of bacteria can occur spontaneously, regardless of exposure to antimicrobials or environmental reasons, such as lack of nutrients, oxidative stress or hypoxia. This small cell subpopulation is able to maintain viability even in the presence of antimicrobial agents at concentrations many times higher than therapeutic. The presence of persistent cells of pathogenic bacteria in the host organism reduces the effectiveness of antibiotic treatment, not due to the genotypic drug resistance of the microorganism, but due to the presence of phenotypic resistance of persister cells. The difference is fundamental, since cell-persisters are insensitive to any antibiotics and the development of fundamentally new antimicrobial strategies is necessary for their eradication. Persister cells are phenotypic variants of the maternal culture of bacteria that are present in all populations of microorganisms, and after the onset of favorable conditions, they are able to reclaim and form a new generation of vegetative bacteria. This review discusses modern concepts of the molecular genetic mechanisms of bacterial persistence with an emphasis on their clinical significance for the occurrence of persistent infections, and discusses innovative technologies for the eradication of resistant cell forms of microorganisms.

Keywords: *Bacterial Persistence; Persistent Infections; Molecular Mechanisms; Persister Cells; Resistance; Eradication; Modern Technologies*

Introduction

Soon after the discovery and triumphant use of antibiotics for the treatment of bacterial infections, which marked the beginning of a new historical era in medicine, the American microbiologist Gladys L. Hobby (1942) first drew attention to the mysterious phenomenon - the absence of complete sterilization by penicillin cultures of S. aureus [1]. A small surviving part of the cells continued to remain viable, and under favorable conditions gave rise to a new microbial population - the same sensitive to the antibiotic. This observation became convincing evidence that the new population of bacteria retained the biological properties of the mother culture and is not a genetically modified penicillin-resistant strain, and the surviving cells are its phenotypic variety. The revealed generation of cells under the influence of antibiotic therapy temporarily changed its biological properties, minimized metabolic and reproductive activity for the subsequent

revival of the dead population. The amazing resistance of these cells to antibiotics did not become an inherited trait, and the nature of this phenomenon has completely different mechanisms than the natural resistance of bacteria to antimicrobial drugs, mediated by genetic mutations.

2 years later J.W. Bigger (1944) obtained similar results and called the surviving subpopulation of bacteria persister cells ("non-dividing, inactive cells") [2]. However, in the wake of euphoria from the success of antibiotics in the 1940 - 1970s. works by G.L. Hobby and J.W. Bigger did not receive the attention they deserved, and further studies of cellular persistence were discontinued [3,4]. In addition, persistor cells were not cultured on conventional nutrient media and did not exhibit metabolic activity, which made it difficult to identify and study them by traditional microbiological methods [4,5] (Figure 1). At the turn of the XX and XXI centuries. the emergence of antimicrobial-resistant strains of pathogenic bacteria has become a serious threat to global health, and from the rostrum of the WHO they started talking about the coming decline of the era of antibiotics. In recent decades, against the background of an increase in infectious pathology, diseases have increasingly become protracted or chronic and associated with opportunistic microorganisms and hospital infection of patients [6] (Figure 1).

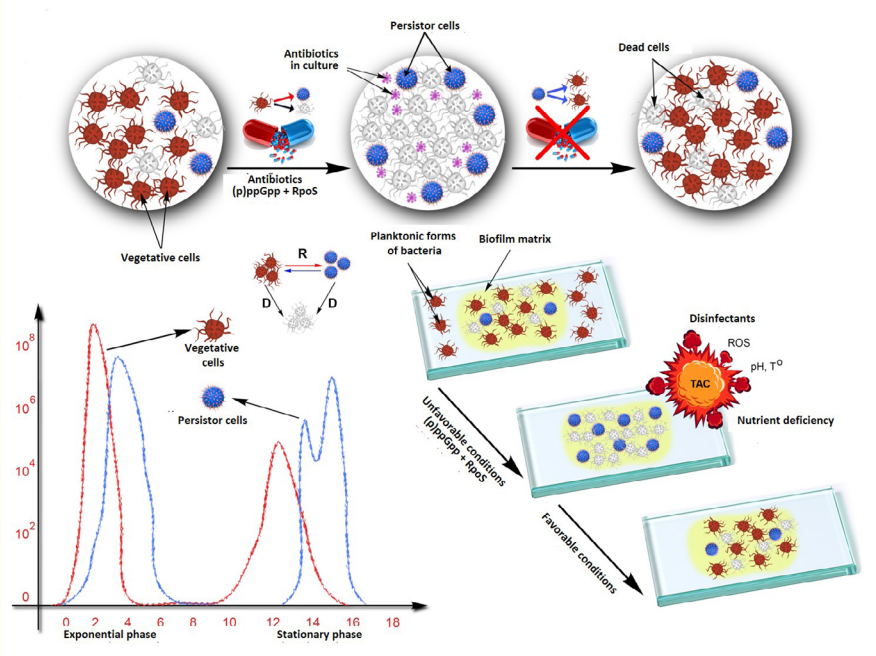

Figure 1: *Most bacterial cultures, being in the stationary phase of growth and favorable conditions, have a small (1–3%) non-dividing phenotypic subpopulation of persisters cells, the biological function of which is to preserve the population in the event of sudden changes in environmental conditions.*

In the current conditions, the search for and creation of new antibiotics is becoming more and more difficult, less and less promising and economically untenable process. And effectively combating bacterial resistance requires the development of innovative antimicrobial strategies, new targets and solutions. One of the promising approaches is the study of the persistence of bacteria. The growing scientific interest in this biological phenomenon became especially noticeable against the background of the emergence of new information about the molecular genetic mechanisms underlying their stability [3,5,6]. This review examines the modern concepts of the molecular genetic mechanisms of bacterial persistence with an emphasis on their clinical significance for the occurrence of persistent infections, discusses innovative technologies for the eradication of resistant cellular forms of microorganisms.

Bacterial persistence

One of the key signs of the viability of any prokaryotic cell is the coordinated reproduction of intracellular structures and the synthesis of macromolecules. However, the discovery in the middle of the twentieth century. the phenomenon of persistence of bacterial cells and its subsequent made it possible to expand the understanding of the adaptation strategies of microorganisms and the mechanisms of their preservation of the pathogenic potential [7-10]. Subsequently, it was shown that the majority of bacterial cultures, being in the stationary growth phase and favorable conditions, have a small (1 - 3%) non-dividing phenotypic subpopulation of persistors, the biological function of which is to preserve the population in the event of sudden changes in environmental conditions habitat [5,6,11]. In addition to the constant presence of this cell subpopulation in any culture of prokaryotes, an increase in their number occurs in response to the emerging threat of any unfavorable environmental factors. In addition to the already discussed effect of antimicrobial agents, it can be a deficiency (absence) of nutrients, oxidative stress, hypoxia, and temperature changes [6,9,10].

Depending on the conditions of existence, growth phase and environmental conditions of the microbial population, there is a constant transformation of vegetative forms into the generation of persistor cells and vice versa [3,4]. At the same time, the number of the inactive population can vary significantly, and under conditions of deficiency (absence) of nutrients, it can reach an almost total transition of the entire population to a state of persistence [12]. The biological significance of the presence and formation of these anabiotic cell generations lies in the preservation of populations after prolonged and extreme in magnitude exposure to unfavorable factors [7,8,13]. In the process of vital activity, vegetative (active) bacterial cells of populations can transform into a persistent phenotype and vice versa, while the rate of these reversions is focused on the growth phases and environmental conditions [3,7,8]. On the basis of the data obtained, it was concluded that the biological function of persistent cells is broader and that the induction of the generation of this bacterial subpopulation is related not only to the effect of antibiotics. For example, the phenomenon of bacterial persistence in recent years is increasingly considered as a universal adaptive ("defensive") strategy of microorganisms in response to competitive stress, which is the result of interspecific interactions and is of great importance for the preservation of the population in a community with dominant species [14-16].

Over the decades of studying the phenomenon of bacterial persistence, it has been established that persistent cells, in contrast to vegetative cell forms, are in a state of metabolic and reproductive dormancy. This allows them to evade the innate mechanisms of immune defines of the host organism and maintain viability after exposure to extreme environmental factors, including the influence of antibiotics, many of which are active in relation to only dividing cells [8,13,15]. The mechanisms of most of them are aimed at inhibiting key intracellular links of metabolism and reproduction: protein synthesis, cell wall and nucleic acid replication [17]. Of course, the presence in the body of inactive resistant cellular forms of pathogenic bacteria significantly contributes to the occurrence of persistent infections and their possible consequences: an increase in morbidity and mortality from the infection itself, as well as an increased risk of its spread [13,14,18]. The presence of this inactive generation of bacteria in the body reduces the effectiveness of antibiotic treatment, which is mediated not by the genetic resistance of the microorganism, but by the resistance of the phenotypic variant of the isogenic population - persistor cells. Thus, in contrast to genotypic resistance of microorganisms, resistance during persistence of bacteria is temporary and reversible. The difference is fundamental, since persistors are not sensitive to any antibiotics, and their eradication requires the development of fundamentally new antimicrobial strategies. Therefore, the treatment of persistent infections is difficult and, as a rule, is associated with the need for prolonged or repeated courses of antimicrobial therapy [3,4,17,18].

In recent decades, it has been established that persistence is a universal phenomenon. It was found not only in bacteria and archaea, but also in viruses [19], fungi [20], unicellular algae [21], cancer cells [22], plant seeds [23], which suggests the presence of common patterns and general biological significance this phenomenon for the survival of systems under various stress conditions [5,7,24]. The formation of persistor cell generation in a bacterial culture is well explained by the innovative paradigm of phenotypic heterogeneity of populations, which is most often a consequence of the spontaneous mechanism of molecular stochastic phenotype switching during the exponential or stationary growth phase of microorganisms [5,11,12]. However, the formation of persistors can also be mediated by

adaptive mechanisms in response to extreme environmental conditions, as well as at the beginning of the stationary phase with nutrient depletion [5,13,15]. This leads to the appearance of phenotypic heterogeneity, which increases the viability of the bacterial population [14,25-27].

The phenomenon of bacterial persistence is consistent with the hypothesis of a continuum of cell dormancy. According to this hypothesis, the formation of persistor cells along with the formation of viable but non-culturable (VBNC) cell forms of bacteria is considered as one of the most common ways for bacteria to survive in adverse conditions [28-30].

The similarity of the circumstances of formation and morphological characters indicates a rather close relationship of these resistant cellular forms, however, there is experimental data showing that VBNC is a form of deeper dormancy. To restore the growth parameters of these viable, but uncultivated bacteria after the termination of the stressor action, more time is required (up to a day or more), while the persistent cells are reclaimed after the completion of antibiotic therapy on solid nutrient media for several hours [28, 30,31]. This circumstance allowed some authors to suggest that persistor cells are a transitional form of transformation into the VBNC state [12]. Some authors [28-30] put an equal sign between persistor cells and VBNC, combining them on the basis of similarities in the circumstances of formation, morphological, physiological characteristics, as well as molecular genetic mechanisms of occurrence.

Molecular genetic mechanisms of bacterial persistence

Back in the 1950s. researchers wondered about the functioning of individual cells of microorganisms. This interest was based on the intuitive understanding that the physiology of individual cells in isogenic bacterial populations may differ from most others. Despite the fact that bacterial persistence has been known for about 80 years, the generation of persistor cells has been little studied. Technical problems with obtaining a laboratory model of dormant cells and the impossibility of their cultivation on conventional nutrient media were supplemented by the lack of sensitive analytical tools for studying the persistence of bacteria [11,13,28]. Significant achievements in molecular biology and genetics at the beginning of the XXI century. expanded their understanding of the molecular genetic mechanisms underlying the persistence of bacteria. With the emergence and development of the concept of single-cell microbiology, new analytical tools and methods of single-cell isolation (Raman spectrometry, microfluidics, flow cytometry, compartmentalization) were developed [32-34]. It became possible to isolate and grow previously uncultivated single cells to assess the viability, as well as to monitor the physiology and functions of individual cells, which was impossible 10 - 15 years ago [35-39].

Depending on the goals and objectives of research, these methods are combined with RNA sequencing (scRNA-seq), as well as with genetic, macromolecular, spatial, proteomic profiling of single cells [35-37]. Using these methods, it has been established that various independent molecular mechanisms are involved in the regulation of the formation of persistent cells [40-43]. Each of these mechanisms leads to the formation of a small generation of dormant cells in the population. For example, reacting to various external stimuli, bacteria use sensory systems that transform external signals into modulation of the intracellular content of secondary messengers-inductors. Among them are intracellular nucleotide alarm molecules (p) ppGpp (guanosine pentaphosphate and guanosine tetraphosphate). They play a key role in the response of a bacterial cell to external irritants, serving as one of the main mediators of "strict control" and a regulator of metabolic activity [42-45]. In turn, the concentration of these molecules in the cell is regulated by ppGpp synthases, the activity of which depends on the content of amino acids, with the direct participation of the RelA/SpoT superfamily of hormones, synthesizing ppGpp [42,44,46].

These molecules mediate the formation of persistor cells by changing the activity of a number of enzymes, primarily DNA primases, lysine decarboxylase, RNA polymerase, etc., and also regulate the rate of bacterial replication and their metabolic activity as signaling molecules [44,47,48]. In addition, the secondary messenger (p) ppGpp was identified as a regulator of the activity of numerous genetic operons encoding toxin-antitoxin systems (TAS) of prokaryotes, discovered at the end of the 20th century [49]. Their study made it pos-

sible to establish the wide distribution of these important operons in bacteria [28,50-52]. The subsequent establishment of a connection between TAS and the formation of the phenotype of bacterial persistence has become a powerful stimulus for the study of these genetic loci, which largely mediate the resistance of prokaryotes to antibacterial drugs [42,43,48].

Over the past decades, it has been revealed that the genetic TAS operons consist of two promoters located in the neighborhood on chromosomes and plasmids. They regulate the synthesis of stable toxin and unstable antitoxin sensitive to cellular proteases. Under normal conditions, these systems represent a non-toxic complex in which the antitoxin blocks the complementary toxin by direct binding of mRNA [42,48]. An increase in the level of (p) ppGpp causes a decrease in the activity of exopolyphosphatases and an increase in the intracellular content of polyphosphate. These high molecular weight polymers with the participation of Lon protease mediate the degradation of antitoxin, depolarization of cell membranes, as well as significant inhibition of metabolic activity and inhibition of reproduction [46,47,51] (Figure 2).

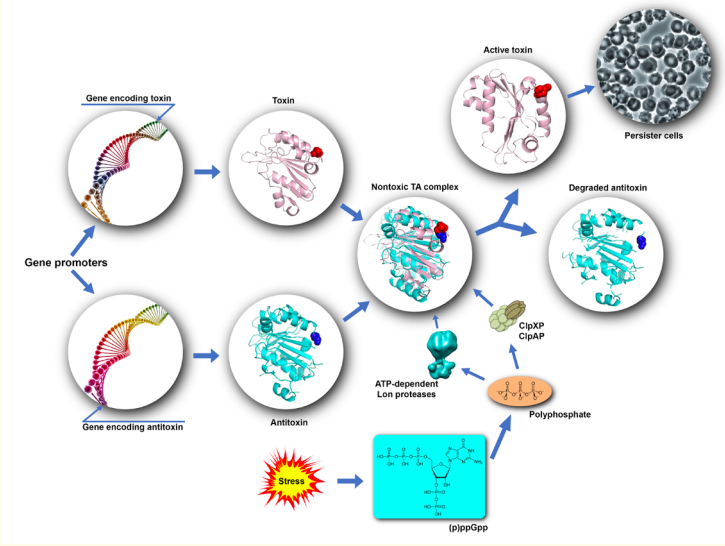

Figure 2: *TAS genetic operons consist of two adjacent promoters located adjacent to chromosomes and plasmids. Under normal conditions, these systems represent a non-toxic complex; under stress, an increase in the level of (p) ppGpp mediates the degradation of antitoxin, as well as a significant inhibition of metabolic activity and inhibition of bacterial reproduction.*

With the onset of extreme living conditions, the antitoxin is degraded by the homo-oligomeric ATP-dependent Lon (La) -protease and the two-component protease systems ClpXP and ClpAP, as a result of which the toxin is released and activated. The cellular target of the active toxin is intracellular enzymes, the inhibition of which mediates a significant slowdown in the rate of protein synthesis and the cell wall, inhibition of the metabolism and replication of bacterial DNA, which leads to their partial or complete resistance to etiotropic antibacterial therapy [43, 50]. Thus, activation of the RelE toxin (RelEB locus), according to [51], increases the resistance of *E. coli* to vancomycin by 10 thousand times, and the release of YafQ toxin (TA DinJYafQ locus) reduces the sensitivity of the same bacterium to cephalosporin the first generation antibiotic, cefazolin, 2400 times. It is important that TAS genes located on plasmids are capable of being transferred horizontally in biofilms to other pathological microorganisms, imparting antibiotic resistance to them [27,48,51].

To date, the existence of 6 types of TAS modules has been established, which differ in the structure of antitoxins and the nature of their interaction with complementary toxins [42,43,48]. Initially, the leading role in the formation of cell persistence was assigned to the most

studied type II TAC module and its hipBA locus, which contains the first bacterial resistance gene hipA discovered in *E. coli*. In this type of TAS, the toxin and antitoxin are proteins [42,46,51]. However, later on, the participation of other types of TAS in the formation of bacterial resistance was shown [27,52-54].

Different types of TAS use different mechanisms of toxin activation, which ultimately leads to its release, slowing down of metabolism and stopping reproductive activity, and the formation of persistor cells. Subsequent studies have shown that other types of TAS modules cause overexpression of toxins in dormant cells of the wild strain of *E. coli* (loci tisBistR, hokBsokB, etc.) [43,46,47]. Excessive activation of toxins leads to the destruction and death of cells, and this mechanism is proposed as one of the promising strategies aimed at combating the persistence of bacteria and their phenotypic resistance to antibiotics.

Promising strategies to combat bacterial persistence

The increasing clinical significance of bacterial persistence makes it increasingly important to search for fundamentally different strategies aimed at combating dormant forms of pathogenic microorganisms and alternative cellular targets (Table 1).

Target	Mechanism	Model	Links
Persister cells	DNA cross-linking agents penetrate and kill persister cells (cisplatin and mitomycin C)	*E. coli*; *Pseudomonas aeruginosa; S. aureus*	58
Block synthesis (p) ppGpp	The binding of the catalytic domains RelA / SpoT synthetic analog Relacin	*M. tuberculosis*	48
Lytic protease ClpP	Activation (modulation of activity) of the lytic protease ClpP	*Staphylococcus aureus*	59
Type II toxin antitoxin modules	Inhibition of the formation of the TAS complex, direct activation of the toxin after the introduction of biomolecules for antitoxin binding	*E. coli*	55
	Activation of mazE toxin by nucleic acid peptide oligomers	*Neisseria meningitidis*	56
	Pharmaceutical inhibition of antisense RNA antitoxin translation	*E. coli*	54
	Artificial TAC activation by antisense peptide nucleic acid oligomers	*E. coli*	60

Table 1: *Modern strategies aimed at inhibiting bacterial persistence.*

Taking into account the resistance of persistor cells to traditional antibiotics for their eradication, it was proposed to use approved anti-cancer drugs cisplatin and mitomycin C (form intrachain DNA cross-links). The use of these drugs against resistant cells of clinical strains of *E. coli* O157: H7 (EHEC), Pseudomonas aeruginosa and Staphylococcus aureus has shown high efficiency and promise for the treatment of chronic infections [55]. The discovery of the key role of toxin-antitoxin modules in the physiology of bacteria, as well as (p) ppGpp in the formation of the generation of antibiotic-resistant cell forms naturally linked one of the directions of the scientific search for the mechanisms of inhibition of the formation of persistent cells with these alarms. systems [43,47,48]. For example, K. Syal and colleagues [47] showed that blocking the synthesis of (p) ppGpp is mediated by the binding of the activity of the catalytic domains of synthetases/hydrolases RelA/SpoT of this messenger in Mycobacterium tuberculosis by the synthetic analogue Relacin.

B.P. Conlon*., et al.* [56] concluded that the acyldepsipeptide antibiotic (ADEP4), modulating the activity of the ClpP protease, kills persistent cells by degrading more than 400 intracellular proteins. In recent years, the use of TAS as antibacterial intracellular targets has been proposed as a promising innovation strategy [52,53]. Scientific interest in TASs as antibacterial strategies is due, on the one hand, to

their wide distribution among bacterial genomes, and, on the other hand, to their absence in eukaryotic cells, in particular, TASs have no analogues in humans. The main mechanisms of TAS use are associated with blocking the formation (degradation) of antitoxin, which leads to the destruction of the daughter cell by the complementary toxin ("post-segregation killing"). Another group of strategies is associated with artificial activation of the toxin or inhibition of TAS formation [46,57].

Bacterial persistence - hidden threat in fear of antibiotic resistance

Modern advances in medical science in the development of antibacterial drugs and their widespread and uncontrolled use have led to the formation of resistance by microorganisms, which reduces the effectiveness of the fight against bacterial infections. However, in fear of growing antibiotic resistance, the world is forgetting about another threat - the persistence of bacterial cells. These cells do not need mutations associated with horizontal transfer of antibiotic resistance genes, since all the protective potential is already encoded in its DNA. This phenomenon is called bacterial persistence [32,39,58].

For example, the mechanism of the anti-tuberculosis action of kanamycin is associated with the inhibition of the small 30S-ribosome subunit and the associated impairment of protein synthesis, which leads to the death of M. tuberculosis. Certain nucleotide mutations in 16S rRNA, which is part of the 30S subunit of ribosomes, modifies the main target of kanamycin and allows bacteria to grow even at high antibiotic concentrations [58,59]. The presence of the aph resistance gene in M. tuberculosis mediates the pathological agent to synthesize the protein aminoglycoside phosphotransferase. This enzyme phosphorylates the antibiotic kanamycin, chemically altering it and converting it to an inactive form [59].

Another example. The widespread use of penicillins, carbapenems and cephalosporin antibiotics in the treatment of infectious processes caused by gram-negative microorganisms has led to the development of genetically determined resistance due to the production of β-lactamase [5,11,30,61].

However (as shown above), unlike resistant bacteria, a bacterial cell exhibiting the property of persistence does not have specialized genes or mutations to protect against a particular antibiotic. Instead, it triggers its own internal defense mechanisms: before exposure to the antibiotic, the cell falls into a state of metabolic slowdown, which prevents damage to the cellular target of the antibiotic. This mechanism allows the bacterial cell to survive the action of the antibiotic, and after the end of antibiotic treatment, they can restore active metabolism, multiply, and after a long time again lead to the resumption of infection [29,30]. Thus, a small fraction of persistent cells underlying phenotypic heterogeneity will ensure the survival of the bacterial population under changing conditions (including antibiotic treatment). This mechanism appears to be largely responsible for the recent decline in M. tuberculosis susceptibility to isoniazid and rifampicin, which have been effective against tuberculosis for decades [58,60].

Both phenomena, resistance and persistence, are different strategies for bacterial resistance to antibiotics and do not occur simultaneously in the same strain. Unlike antibiotic resistance, bacterial persistence is difficult to measure, is therefore often overlooked, and can lead to treatment failure. However, in the evolution of the species as a whole, these strategies can complement each other [7,30]. So, persister cells experience the evolutionary pressure of an antibiotic, but do not die, but leave offspring. With each new generation, random mutations accumulate in surviving cells, and some of them can provide antibiotic resistance. Persistence makes it possible to enumerate such mutations even in the presence of an antibiotic, and not just before exposure to it, and in some cases can be converted into resistance [8,11,29].

The clinical significance of bacterial persistence is due to the fact that this phenomenon does not allow to cope with the disease completely: in some cases, the same infection recurs after some time even after successful initial antibiotic treatment and the retreat of the acute phase of infection [29,30]. Let me illustrate this process using the example of a tuberculosis infection. Tuberculosis can occur in an active form, when mycobacteria actively multiply in the patient's body, causing symptoms of the disease, but also in a latent form, when the bacteria persist in the lungs of the carrier without causing symptoms of the disease. During the treatment of tuberculosis, the

actively proliferating bacterial population is eliminated during the intensive care phase. But after that, a multi-month continuation phase of treatment is always required, the goal of which is to eliminate latent tuberculosis infection with a large number of persisters to prevent relapses [47,59]. If the strain is not resistant, then the antibiotics currently used will be relatively effective in the first phase of treatment. They will suppress the expansive growth of bacteria and cope with the manifestations of the disease. However, in the subsequent phase, traditional antibiotics are ineffective against resting persistent cells, which leads to a high proportion of recurrences of the infection [47,59,60].

Today, persistence is one of the main reasons for the difficulty in treating many chronic bacterial infections: tuberculosis, recurrent urinary tract infections, typhoid fever, staphylococcal infections, and many other infectious diseases [6,55,56]. In addition, bacteria that survive antibiotic exposure over again through persistence have the potential to acquire resistance mutations [55,56].

In hospital settings, the choice and use of empiric antimicrobial therapy should be justified as much as possible in order to increase the effectiveness of the treatment of patients with chronic infections. The solution to this problem depends, on the one hand, on timely routine microbiological determination of the etiological infectious agent and its biological properties, and on the other hand, on the performance of phenotypic screening of antibiotic resistance of microorganisms and the establishment of molecular genetic mechanisms in them for the formation of antibiotic resistance. In modern conditions, it has become possible to study resistance to antibacterial drugs in clinical strains of bacteria by the polymerase chain reaction (PCR) method [58,61].

In addition to the spread of resistance, the inappropriate prescription of antibiotics is also associated with other problems associated with the risk of side effects. Despite the fact that a large proportion of them are associated with allergic reactions [58], it should not be forgotten that the standard course of antibiotic therapy can lead to immunodeficiency, as well as gastroenterological, neurological or mental disorders [58].

In this concept, it is relevant to conduct an in-depth microbiological, molecular genetic study of the species composition of pathogens using modern analytical technologies and diagnostic platforms (including time-of-flight mass spectrometry with laser desorption/ionization, MALDI-TOF) and/or whole genome sequencing), which are increasingly used to reduce pathogen identification time [59,61].

There are certain difficulties in the strategy of detecting antibiotic resistance and bacterial persistence in the clinical setting. At the same time, routine microbiological procedures, including disk diffusion analysis and E-test with the determination of the minimum inhibitory concentration of antibiotics, are sufficient to identify resistant bacterial strains. However, methods for detecting the presence of bacterial persisters and their use in the clinic have not been sufficiently studied. In fact, there is still no rapid and accurate protocol for testing bacterial persistence, and levels of persistence observed and assessed *In vitro* may not correspond to levels of *In vivo* persistence in a patient [62].

In this sense, the "Seeding Replica Resistance Isolation System" (REPTIS) proposed by Matsuo., *et al.* (2019), which can be adapted for use in clinical microbiological laboratories [64]. In addition, the ColTapp method [65] is of interest, which can be used to detect and measure the heterogeneity of colony growth in a population with the subsequent use of additional technologies from the arsenal of the modern Single cell microbiology paradigm. Despite their limitations, these methods provide additional information that can help clinicians make therapeutic decisions. Despite their limitations, these methods provide additional information that can help clinicians make therapeutic decisions [64,65].

Conclusion

In recent decades, the growth rate of resistance has been increasing, and the commercial attractiveness of the development of new antibiotics has been declining. It appears that the antibiotic health crisis will only grow as long as medical biotechnologies ignore the phe-

notypic mechanisms of antibiotic resistance formation-persistence and biofilm formation. These mechanisms allow pathogenic bacteria not only to remain viable, but also to evolve in the presence of antibiotics.

Today, it is too early to talk about the final disclosure of the molecular mechanisms of bacterial persistence. Their study is in an active stage, and it is possible that new antimicrobial strategies aimed at the eradication of persistent cells will soon appear, which will expand and complement the existing regimens of traditional antibacterial therapy. However, it does not seem to be worth counting on an easy fight against bacterial persistence. Persistence mechanisms are too redundant and specific for each type of microorganisms, and a single and universal method for the eradication of resistant cells is not yet visible.

Approaches to the treatment of chronic bacterial infections require a paradigm shift and the need to recognize bacterial persistence as one of the mechanisms of antibiotic resistance as a clinically significant problem. To date, the most successful results have been obtained with the use of various metabolites (eg, mannitol, glucose, cardiolipin, etc.) for the recultivation of persister cells followed by aminoglycoside-mediated eradication [39,40,43,63]. The relevance of the development of methods for combating bacterial persistence is substantiated from the standpoint of the scientific and practical significance of the problem and economic feasibility. Treatment of persistent infections such as tuberculosis requires long courses of high doses of traditional antibiotics. The creation of combined treatment regimens aimed at eradicating an infectious agent and cell subpopulations resistant to it will optimize treatment regimens, reduce its cost and increase efficiency. Such approaches will preserve the wealth of antibacterial drugs that we currently have and prevent the emergence of resistance to new ones. drugs that will appear in the future.

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Conflicts of Interest

The authors declare no conflict of interest.

Bibliography

1. Hobby GL., *et al*. “Observations on the mechanism of action of penicillin”. *Proceedings of the Society for Experimental Biology* 50.2 (1942): 281-285.

2. Bigger JW. “Treatment of staphylococcal infections with penicillin by intermittent sterilization”. *Lancet* 244.6320 (1944): 497-500.

3. Van Den Bergh B., *et al*. “Should we develop screens for multi-drug antibiotic tolerance?” *Expert Review of Anti-infective Therapy* 14.7 (2016): 613-616.

4. Rehab Mahmoud abd El-Baky. “The future challenges facing antimicrobial therapy: resistance and persistence”. *American Journal of Microbiological Research* 4.1 (2016): 1-15.

5. Balaban NQ., *et al*. “Bacterial persistence as a phenotypic switch”. *Science* 305.5690 (2004): 1622-1625.

6. Lewis K. “Persister cells”. *Annual Review of Microbiology* 64 (2010): 357-372.

7. Van Teeseling M.C.F., *et al*. “Determinants of bacterial morphology: from fundamentals to possibilities for an-timicrobial targeting”. *Frontiers in Microbiology* 8 (2017): 1264.

8. Kysela DT., *et al*. "Diversity takes shape: understanding the mechanistic and adaptive basis of bacterial morphology". *PLOS Biology* 14.10 (2016): e1002565.

9. Kawai Y., *et al*. "Bacterial cell morphogene-sis does not require a preexisting template structure". *Current Biology* 24.8 (2014): 863-867.

10. Harms A., *et al*. "Mechanisms of bacterial persistence during stress and antibiotic exposure". *Science* 354.6318 (2016): aaf4268.

11. Maisonneuve E and Gerdes K. "Molecular mechanisms underlying bacterial persisters". *Cell* 157.3 (2014): 539-548.

12. Orman MA and Brynildsen MP. "Inhibition of stationary phase respiration impairs persister formation in E. coli". *Nature Communications* 6 (2015): 7983.

13. Randich AM and Brun YV. "Molecular mechanisms for the evolution of bacterial morphologies and growth modes". *Frontiers in Microbiology* 6 (2015): 580.

14. Stubbendieck RM and Straight PD. "Multifaceted interfaces of bacterial competition". *Journal of Bacteriology* 198.16 (2016): 2145-2155.

15. Gaivão M., *et al*. "Transmission fitness in co-co lonization and the persistence of bacterial pathogens". *The Bulletin of Mathematical Biology* 79.9 (2017): 2068-2087.

16. Dorosky RJ., *et al*. "Pseudomonas chlororaphis produces multiple R-Tailocin particles that broaden the killing spectrum and contribute to persistence in rhizosphere communities". *Applied and Environmental Microbiology* 84.18 (2018): e01230-e01218.

17. Fisher RA., *et al*. "Persistent bacterial infections and persister cells". *Nature Reviews Microbiology* 15.8 (2017): 453-464.

18. Grant SS and Hung DT. "Persistent bacterial infections, antibiotic tolerance, and the oxidative stress response". *Virulence* 4.4 (2013): 273-283.

19. Randall RE and Griffin DE. "Within host RNA virus persistence: mechanisms and consequences". *Current Opinion in Virology* 23 (2017): 35-42.

20. Böhm L., *et al*. "The yeast form of the fungus Candida albicans promotes persistence in the gut of gnotobiotic mice". *PLOS Pathogens* 13.10 (2017): e1006699.

21. Codony F., *et al*. "Persistence and proliferation of some unicellular algae in drinking water systems as result of their heterotrophic metabolism: short communication". *Water SA* 29.1 (2003): 113-116.

22. Pearl Mizrahi S., *et al*. "Persistence to anti-cancer treatments in the stationary to proliferating transition". *Cell Cycle* 15.24 (2016): 3442-3453.

23. Long RL., *et al*. "The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise". *Biological reviews of the Cambridge Philosophical Society* 90.1 (2015): 31-59.

24. Yafremava LS., *et al*. "A general framework of persistence strategies for biological systems helps explain domains of life". *Frontiers in Genetics* 4 (2013): 16.

25. Van Boxtel C., *et al*. "Taking chances and making mistakes: non-genetic phenotypic heterogeneity and its consequences for surviving in dynamic environments". *Journal of the Royal Society Interface* 14.132 (2017): 20170141.

26. Smith SE. "Organisms as persisters". *Theory, and Practice in Biology* 9.14 (2017).

27. Pu Y., *et al*. "Active efflux in dormant bacterial cells - new insights into antibiotic persistence". *Drug Resistance* 30 (2017): 7-14.

28. Kim JS and Wood TK. "Tolerant, growing cells from nutrient shifts are not persister cells". *mBio* 8.2 (2017): e00354-e00317.

29. Ayrapetyan M., *et al*. "Viable but non-culturable and persister cells coexist stochastically and are induced by human serum". *Infection and Immunity* 83.11 (2015): 4194-4103.

30. Ayrapetyan M., *et al*. "Relationship between the viable but nonculturable state and antibiotic persister cells". *Journal of Bacteriology* 200.20 (2018): e00249-e00218.

31. Amato SM., *et al*. "The role of metabolism in bacterial per-sistence". *Frontiers in Microbiology* 5 (2014): 70.

32. Ishii S., *et al*. "Single-cell analysis and isolation for microbiology and biotechnology: Methods and applications". *Applied Microbiology and Biotechnology* 86.5 (2010): 1281-1292.

33. Li M., *et al*. "Single cell Raman spectroscopy for cell sorting and imaging". *Current Opinion in Biotechnology* 23.1 (2012): 56-63.

34. Mazutis L., *et al*. "Single-cell analysis and sorting using drop-let-based microfluidics". *Nature Protocols* 8.5 (2013): 870-891.

35. Stuart T and Satija R. "Integrative single-cell analysis". *Nature Reviews Genetics 20*.5 (2019): 257-272.

36. Peterson VM., *et al*. "Multiplexed quantification of proteins and transcripts in single cells". *Nature Biotechnology* 35.10 (2017): 936-939.

37. Ramani V., *et al*. "Massively multiplex single-cell Hi-C". *Nature Methods* 14.3 (2017): 263-266.

38. Tóth EN., *et al*. "Single-cell nanobiopsy reveals compartmentalization of mRNAs within neuronal cells". *Journal of Biological Chemistry* 293.13 (2018): 4940-4951.

39. Hong-Geller E and Micheva-Viteva SN. Targeting bacterial persistence to develop therapeutics against infectious dis-ease (2020).

40. Lin JM. "Microfluidics for SingleCell Analysis". Beijing, China: Springer Singapore (2019).

41. Michiels JE., *et al*. "Molecular mechanisms and clinical implications of bacterial persistence". *Drug Resistance Updates* 29 (2016): 76-89.

42. Tian C., *et al*. "Synchronized switching of mul-tiple toxin-antitoxin modules by (p)ppGpp fluctuation". *Nucleic Acids Research* 45.14 (2017): 8180-8189.

43. Svenningsen MS., *et al*. "Birth and resuscitation of (p)ppGpp induced antibiotic tolerant persister cells". *Scientific Reports* 9.1 (2019): 6056.

44. Wood TK. "Combatting bacterial persister cells". *Biotechnology and Bioengineering* 113.3 (2016): 476-483.

45. Maisonneuve E., *et al*. "(p)ppGpp con-trols bacterial persistence by stochastic induction of toxin-anti-toxin activity". *Cell* 154.5 (2013): 1140-1150.

46. Manav MC., *et al*. "Structural basis for (p)ppGpp synthesis by the Staphylococcus aureus small alarmone synthetase RelP". *Journal of Biological Chemistry* 293.9 (2018): 3254-3264.

47. Syal K., *et al*. "Synthetic (p)ppGpp analogue is an inhibi-tor of stringent response in mycobacteria". *Antimicrobial Agents and Chemotherapy* 61.6 (2017): e00443-e00417.

48. Hauryliuk V., *et al*. "Recent functional insights into the role of (p)ppGpp in bacterial physiology". *Nature Reviews Microbiology* 13.5 (2015): 298-209.

49. Ogura T and Hiraga S. "Mini-F plasmid genes that couples host cell division to plasmid proliferation". *Proceedings of the National Academy of Sciences of the United States of America* 80.15 (1983): 4784-4788.

50. Page R and Peti W. "Toxin-antitoxin systems in bacterial growth arrest and persistence". *Nature Chemical Biology* 12.4 (2016): 208-214.

51. Van Melderen L. "Toxin-antitoxin systems: why so many, what for?" *Current Opinion in Microbiology* 13.6 (2010): 781-785.

52. Lee KY and Lee BJ. "Structure, biology, and therapeutic applica-tion of toxin-antitoxin systems in pathogenic bacteria". *Toxins* 8.10 (2016): 305.

53. Maleki A., *et al*. "MazE antitoxin of toxin-antitoxin system and fbpA as reliable targets to eradication of Neisseria meningitidis". *Current Pharmaceutical Design* 24.11 (2018): 1204-1210.

54. Cui P., *et al*. "Molecular mechanisms of bacterial persistence and phenotypic antibiotic resistance". *Yi Chuan* 38.10 (2016): 859-871.

55. Chowdhury N., *et al*. "DNA-crosslinker cisplatin eradicates bacterial persister cells". *Biotechnology and Bioengineering* 113.9 (2016): 1984-1992.

56. Conlon BP., *et al*. "Activated ClpP kills persisters and eradicates a chronic biofilm infection". *Nature* 503.7476 (2013): 365-370.

57. Równicki M., *et al*. "Artificial activation of Escherichia coli mazEF and hipBA toxin-antitoxin systems by antisense peptide nu cleic acids as an antibacterial strategy". *Frontiers in Microbiology* 9 (2018): 2870.

58. Llor C and Bjerrum L. "Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem". *Therapeutic Advances in Drug Safety - SAGE Journals* 5.6 (2014): 229-241.

59. Simmons JD., *et al*. "Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection". *Nature Reviews Immunology* 18.9 (2018): 575-589.

60. Ehrt S., *et al*. "Metabolic principles of persistence and pathogenicity in Mycobacterium tuberculosis". *Nature Reviews Microbiology* 16.8 (2018): 496-507.

61. Barlow G. "Clinical challenges in antimicrobial resistance". *Nature Microbiology* 3 (2018): 258-260.

62. Zhang Y. "Persisters, persistent infections and the Yin-Yang model". *Emerging Microbes and Infections* 3.1 (2014): e3.

63. Huemer M., *et al*. "Antibiotic resistance and persistence-Implications for human health and treatment perspectives". *EMBO Reports* 21.12 (2020): e51034.

64. Matsuo M., *et al*. "Genetic and Transcriptomic Analyses of Ciprofloxacin-Tolerant Staphylococcus aureus Isolated by the Replica Plating Tolerance Isolation System (REPTIS)". *Antimicrobial Agents and Chemotherapy* 63.2 (2019): e02019-e02018.

65. Bär J., *et al*. "Efficient microbial colony growth dynamics quantification with ColTapp, an automated image analysis application". *Scientific Reports* 10.1 (2020): 16084.

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