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Persister cells and antibiotic resistance: an overview

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ABSTRACT

Persister cells are antibiotic-tolerant phenotypic variants that can survive exposure to high concentrations of antibiotics. Among all pathogens, a subset of the population usually exhibits this phenotypic attribute. Usually, when there is a decrease in antibiotic concentration, those cells that survive (persister) repopulate the population. There are diverse mechanisms by which these cells persist or withstand exposure to harsh environments. Interestingly, among all the major pathogens, persister cells have been found. Difficulty in treating most infections is usually due to certain cell populations not responding to antibiotic treatment. Thus, persister cells are implicated in antibiotic resistance, a major public health issue. They also have some association with biofilm formation and might be playing a central role in causing the difficulty in eradicating most chronic infectious diseases. Persistence could arise through different mechanisms, and resulting bacteria can withstand antibiotic treatment by developing resistance to drugs or exhibiting tolerance to them. The proportion of these persisters within a bacterial population tends to rise due to diverse stress factors, including exposure to antibiotics with bactericidal properties. So far, diverse models have been put forth to elucidate the mechanisms underlying the emergence of bacterial persistence. However, the exact mechanisms leading to persister formation remain elusive. This review delves into the recent advancements in understanding bacterial persisters and considers the implication of persister cell formation in treating bacterial infection. It also discusses the association of persistent cells with antibiotic resistance and diseases.

KEYWORDS

Persister cells; Antibiotic resistance; Antibiotics; Biofilm formation; Chronic infectious diseases

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Introduction

Persister cells are transiently antibiotic-resistant populations that arise from populations susceptible to antibiotics. They vary from resistant cells in that they survive antibiotic exposure due to dormant physiology rather than drug–target interactions being obstructed. Although persister cell production is stochastic, it is frequently driven by stress and exposure to certain environmental conditions [1]. Persister cells often arise due to several factors, and evidence from several quarters has shown that they are usually associated with several clinical outcomes [2]. Over the years, there have been many debates on the emergence of persister cells among microbial pathogens. However, it is important to emphasize that the production of persister cells is due to several molecular pathways. There are also growing shreds of evidence on the contribution of persister cells to antimicrobial resistance (AMR). There is a rapid increase in drug-resistant pathogens, and this issue is a global public health threat demanding urgent attention. Therefore, understanding how persister cells among bacterial pathogens influence AMR is very important. Herein, we discussed the role of persister cells in establishing antibiotic resistance and infectious diseases.

Persister Cell Biology: An Overview

The antibiotic-resistant issue is a burden on global health, complicating treatment efforts. Chronic and recurrent infections, on the other hand, are frequently linked to genetically sensitive bacteria that are resistant to even the most powerful and effective antibiotics [3]. This process is frequently associated with producing specialized "persister" cells. Tolerance of these cells has frequently been attributed to an alteration in the drug's active target site, allowing bacteria to survive [4,5]. The absence of a complete understanding of the biochemical or molecular mechanisms mediating drug resistance has been a significant obstacle to developing an effective treatment option for eliminating the drug-resistant persister population. Persisters can exist in biofilm, or they can be stress-induced. There are also stochastically-formed persisters (Figure 1).

Furthermore, the multifactorial nature of most persister and the inability to accurately predict their evolution are major factors hindering therapeutic efforts [1,6]. The evolutionary potential of most pathogens is usually anchored on their reproduction ability and the timeline for their

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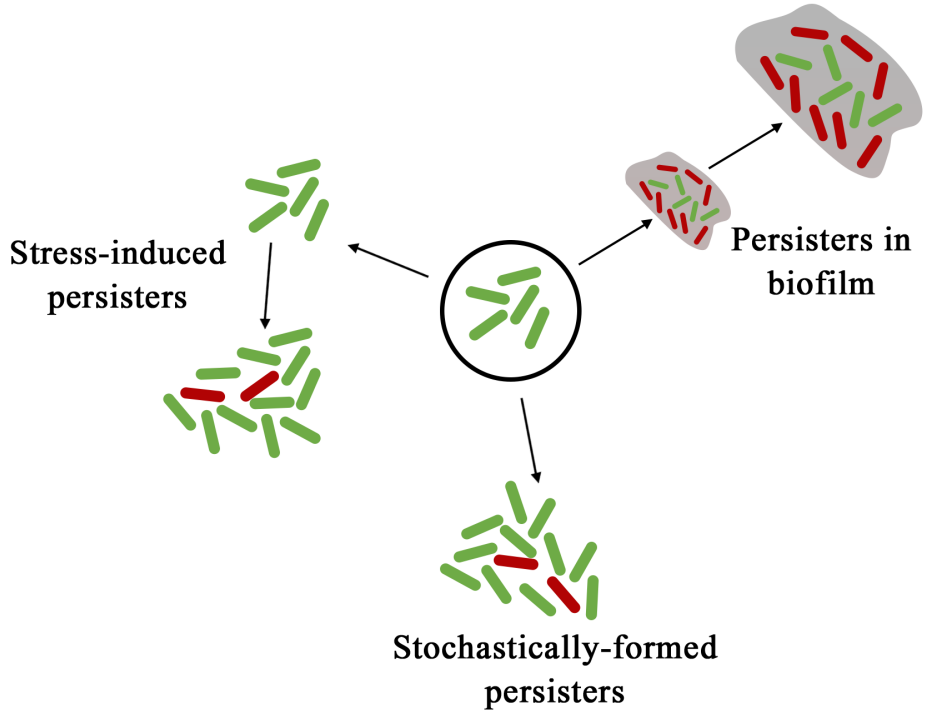

Figure 1. Stochastically-formed persisters.

reproduction. At first appearance, the formation of persister cells by clonal bacterial populations appears to be counter-intuitive, as they appear to be a waste of resources that may be better spent on population increase. This type of phenotypic heterogeneity has been observed in eukaryotes [7]. Persistent cells are usually selected during narrow antibiotic exposure in the laboratory [8].

In contrast to antibiotic resistance, the basic paradigm of bacterial persister development is that these cells achieve transient antibiotic tolerance by switching to a dormant state [1]. At this stage, the cells grow slowly or might not grow at all. In simpler terms, antibiotic resistance impairs the antibiotic's ability to reach its target site. However, persister cell development is due to alteration in the cell's physiology that is usually beneficial to the bacteria [9]. It has been established that bacterial growth rate upon antibiotic exposure is inversely proportional to their growth rate. Nonetheless, most of the cells that are not growing (usually in their stationary phase) are not tolerant to antibiotics [4,9]. This observation shows persisters "are not simply non-growing cells" but have extra physiologic alterations that underpin their survival and resuscitation [10].

Stochasticity and heterogeneity of persisters

As already mentioned, persister cells are a subpopulation of bacterial cells that exhibit temporary antibiotic tolerance, allowing them to survive antibiotic exposure even when most of the population is killed [11]. Stochasticity refers to the inherent randomness and unpredictability in the behavior of these persister cells [12]. This stochastic behavior arises from various molecular and physiological factors, such as gene expression, cell signaling, and environmental conditions [1]. Heterogeneity in persister cells refers to the diversity of phenotypes and behaviors within the persister subpopulation. This heterogeneity is driven by both genetic and non-genetic factors [13]. Genetic mutations, epigenetic modifications, and variations in gene expression levels can lead to different persister subpopulations with varying levels of antibiotic tolerance. Since the inception of single-cell investigations, it has become evident that clonal groups of cells showcase random fluctuations, also called noise, in gene expression [14]. This diversity can be found in any cell phenotype, but its impact on the growth rate is particularly noteworthy, closely tied to the persistence phenomenon. Growth rate variation is not only wide-ranging within a population but also strikingly discernible along cell lineages, even those of persister families. The outcomes presented by Hingley-Wilson and colleagues suggest that genes influencing epigenetic inheritance play a role in the formation of persister cells [15].

The stochastic and heterogeneous nature of persister cells has important implications for antibiotic resistance. Once antibiotic treatment is stopped, these persister cells can serve as a reservoir for reemergence of bacterial infections. The diverse persister subpopulations ensure that some cells are likely to survive even exposure to a range of antibiotics, contributing to the development of antibiotic-resistant strains over time [16,17].

AMR, tolerance, and persistence: different but complimentary terms

AMR, tolerance, and persistence are complementary terms in biology [18]. Generally, antibiotic resistance refers to bacteria's inherited ability to multiply in the presence of a medicine that would otherwise prohibit them from growing. The antibiotic's Minimum Inhibitory Concentration (MIC), inhibit bacterium multiplication, is the most widely used resistance indicator. Horizontal gene transfer and removal of drugs through some channels (efflux pumps) are some of the ways resistance develops. Other possible ways resistance develops include mutations in some critical genes [19,20]. This mutation could cause an alteration in drug targets,

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leading to the inability of the drug to reach its target. It can also lead to a decrease in the uptake of antibiotics. This is usually when the mutation affects genes playing critical roles in the cell membrane [21].

Conversely, "Tolerance" is the temporary ability of cells to resist killing when exposed to antibiotics. In this case, a mechanism essential for antibiotic activity is usually impeded. This frequently leads to markedly slowed growth, if not growth stoppage [22]. Other medications, on the other hand, may be able to kill strains that are resistant to these antibiotics [17].

Furthermore, in antibiotic persistence, a certain bacterial population survives when exposed to bactericidal antibiotic concentration. Usually, when the cells are subcultured in a fresh medium and exposed to the same antibiotic, the cells will exhibit the same susceptibility profile. Persistent cells re-cultured on the fresh medium will show the same susceptibility to the same antibiotic as the original culture, implying that only a subpopulation of the new culture will have the persistent phenotype [12]. Persisters cannot multiply as well as other cells in the presence of the medication. However, compared to the population they emanate from, they are killed at a lesser rate. Persistence is also different from heteroresistance. Heteroresistance occurs when a small subpopulation has a significantly (>8-fold) higher MIC for a short time [23]. In a time-kill experiment, a biphasic killing curve is obtained. This curve is a characteristic of antibiotic persistence. The slower killing phase represents the persisters. They usually surface after most of the bacterial population has been killed during rapid growth.

Antibiotic resistance and persistence allow bacteria to survive in the presence of antibiotics. The two terms may be used interchangeably in some qualitative investigations [24]. However, there are distinctions between perseverance and tolerance. A subpopulation of tolerant bacteria (usually 1%) that can tolerate drug doses far higher than the MIC (hence, the phenomenon may have been dubbed "heterotolerance") are the persisters. Lewis in their study found that mechanisms involved in tolerance are also involved in persistence. The heterogeneous killing exhibited in persistence distinguishes it from tolerance, i.e., there are diverse levels of the killing of the cells in a clonal culture [5]. As evidenced by the biphasic death curve, a subset of cells in their persistence state can withstand exposure to antibiotics more than the rest of the population.

The persistence of most cells when exposed to antibiotics is not limited to only one or two subpopulations. Most times, more than one persister subpopulation coexists. This can lead to a multimodal killing curve [1]. Two things usually stand out when analyzing persistence. The first is the mechanisms facilitating the survival of tolerance cells. The second is the mathematical concept leading to population heterogeneity, such as nonlinear mechanisms that cause bimodality by amplifying stochasticity [25,26].

Types of Persistent Bacteria

It's still up for debate whether the persistence phenotype is caused by a single general or numerous unique biological processes [8]. However, persisters can be created in the lab in different ways, allowing further studies. It's critical to distinguish between the different types of persistence since each one necessitates a distinct method for determining persistence levels.

Triggered persistence (type 1)

External factors, the most prevalent of which is malnutrition, cause antibiotic persistence in bacteria in most cases. Even after the pressure is released, some cells may remain inactive for long periods. These cells may finally end up in the survival fraction. When cells are regrown in a fresh culture, some may appear "exponentially growing,". However, few persisters cells activated nutrient deprivation might remain in a lag phase. As a result, the lag time distribution after exposure to stress is a critical component that might influence persistence [27]. Also, several stressors have been linked to induced persistence, including nutritional deprivation and oxidative and acid stress. Also, cell number and subinhibitory concentrations of drugs can induce persistence. Moreover, exposure to immunological protein and complement systems could also be associated with persistence [28].

A second complexity of the phenomena is linked to high doses of antibiotics, which cause growth arrest and, paradoxically, a decreased death rate and drug-induced persistence. Also, cell populations that trigger stress response as a survival tactic make bactericidal antibiotics become bacteriostatic when they sense an antibiotic in the environment. This type of response is independent of the culture's previous history of drug exposure and, hence could be attributable to spontaneous persistence. However, compared to other kinds, it may be more particular to the concentration and the antibiotic type used than other persistence forms [29].

Spontaneous persistence (type II)

In an exponentially growing culture, persistence may occur spontaneously. This type of persistence is not as common as Type I persistence. There have been no direct observations of spontaneous persistence in wild-type strains at the single-cell level. When all other parameters are kept in check, persistence may be observed even when no external inducer exists, especially when the cells are in the exponential growth phase. In this instance, spontaneous persistence may be noticed. Also, their fraction will remain constant as long as the exponential growth phase is maintained. Spontaneous persistence can also be referred to as type II persistence. However, this type of persistence is not as common as induced (triggered persistence) [30].

Genetic Basis of Persister Cells Formation

Since J. Bigger originally described persister cells in *Staphylococcus aureus* [31]. So far, investigations are available describing the mechanism underlying their emergence and survival when exposed to antibiotics. However, several experiments utilizing diverse approaches have shown that the process may be extremely repetitive at the population level. Thus, the process has been divided into two (specific and non-specific mechanisms). Some processes (non-specific mechanisms) produce antibiotic tolerance by altering bacterial physiology. Tolerance can also be by stochastic events. Specific mechanisms silence critical cellular activities (specialized mechanisms). Furthermore, many damage repair pathways aid persister cell survival [32,33].

Various damage repair processes also aid persister cells' survival. The shutdown of a certain physiological process usually favors damage repair events. However, the link

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between damage repair events and persister mechanisms remains blurred [10,33]. Furthermore, there is no precise persister regulator that could be controlling the molecular pathways. This is because these events are not purely stochastic but are incorporated into the regulons of different arms of stress signaling machinery. Moreover, starvation can trigger (p)ppGpp (second messenger). This protein could play a critical role in inducing persisters.

Diverse mechanisms and approaches are used to isolate and quantify persisters. Also, during experimental conditions, there might be an alteration or changes in persister cells because these cells are susceptible to change. This heterogenicity is a major issue in understanding the exact mechanisms of persistent cell formation [16]. Luidalepp et al. showed that the method of overnight culturing had a significant impact on the observation and penetrance of diverse phenotypic E. coli mutant phenotypes in the formation of persisters [16].

Furthermore, persister levels are linked to gene expression that protects against nutritional stress, DNA damage, heat shock, or oxidative stress. This emphasizes the significance of stress responses in persister cell formation [34,35]. However, elevated and decreased persister levels can be observed during some stress demands. Thus, this is evidence that more research is needed. For the past two decades, the knowledge of persistence has gradually grown. As a result, keeping track of genetic variables that influence persistence has become increasingly difficult. Genes mediating persister cell formation affect persistence in a certain state when mutated, deleted, or overexpressed [33,34,36].

Persister cells are a subset of bacterial cells that can tolerate antibiotics and other stresses, leading to treatment failure in various infections. The formation of persister cells is largely attributed to toxin-antitoxin (TA) systems within bacterial populations [11]. TA systems are genetic modules comprising a toxin gene and its cognate antitoxin gene, which act as a regulatory mechanism to control bacterial growth and survival under adverse conditions. TA systems play a crucial role in persister cell formation through a phenomenon known as "conditional cooperatively." In this mechanism, the antitoxin binds to the toxin and its gene promoter [37,38]. This dual binding prevents the expression of the toxin and antitoxin genes, maintaining a dormant state. However, certain stressors, such as nutrient deprivation or antibiotic exposure, can disrupt the equilibrium. The stressors either lead to the degradation of antitoxins or interfere with their binding to the promoter region, thereby allowing toxin expression [39,40].

The toxins produced by TA systems can target various cellular processes, including DNA replication, translation, and cell wall synthesis. By inducing a reversible growth arrest or dormancy, toxins protect a subpopulation of bacterial cells from the lethal effects of antibiotics. This temporary growth arrest enables persister cells to evade antibiotic treatments that primarily target actively growing bacteria [41]. Several types of TA systems have been identified in various bacterial species, highlighting their evolutionary significance. Some well-studied examples include the *Escherichia coli* mazEF system and the Staphylococcus aureus mazEF-like system [42]. These systems have been found to contribute to the formation of persister cells in response to different stress demands.

Overall, toxin-antitoxin pairs are key players in the formation of persister cells, allowing bacterial populations to survive under harsh conditions and potentially leading to recurrent infections. Understanding the molecular mechanisms underlying TA-mediated persister cell formation could offer new insights into developing more effective antimicrobial strategies [43].

Factors Enhancing Persister Formation

Nutrition stresses

For decades, scientists have proven that the bacterial growth rate is crucial in determining antibiotic effectiveness against the bacteria. The availability of carbon sources governs this [44] and hence, influence the antibiotic tolerance of an entire bacterial population. Furthermore, nutrient deficiency appears to be one of, if not the primary, causes of persister development. For example, nitrogen or amino acid starvation increases the number of microorganisms that survive antibiotic exposure. This has been demonstrated in *E. coli, P. aeruginosa*, and *S. mutans* [45,46]. It's also well known that as bacterial cultures progress into the stationary phase and beyond, persisters begin to accumulate. Persister cell proportions eventually reached values comparable to those found in biofilms. It has been demonstrated that biofilms with restricted nutrients and oxygen include a high fraction of persisters. Aside from the extreme stress that malnutrition causes, a change in carbon source affects the ecosystem and, hence, could influence persister cell formation.

Temporary starvation due to metabolic flux can also increase E. coli persisters [47]. A rise follows this shift in carbon sources in guanosine tetra- or pentaphosphate ((p)ppGpp). Thus, those persisters that arise from nutrient switching appear to sustain high ATP levels, allowing non-growth-related functions like membrane maintenance to continue [47].

Acidic, oxidative, and osmotic stressors

Oxidative, acidic, and osmotic stressors and dietary alterations have been implicated as causes of persister formation. A study by Vega et al. using E. coli showed that when the cells were exposed to hydrogen peroxide or salicylate, there was an increase in reactive oxygen species (ROS) generation, which led to an increase in persistent cell formation [28]. A similar investigation with similar results involving E. coli was reported by Wang et al. [48]. In E. coli, it was also discovered that indole promotes persister formation [28] The indole is an intermediate generated during tryptophan biosynthesis, and its production is boosted by oxidative stress.

Extracellular signalling

Exposure to extracellular chemicals produced in the environment by bacteria can also encourage the development of persister cells. CSP, a quorum-sensing peptide, stimulates competence (QS). S. mutans persister cell formation has been shown to be aided by a signalling molecule. This QS molecule is critical during the stress response. The creation of persisters is one of the signals passed by the molecule. A study that isolated bacteria from cystic fibrosis patients showed that quorum-sensing molecules from Pseudomonas influence persister cell formation. Similarly, several Pseudomonas QS compounds were reported to boost the persister cell fraction of Pseudomonas, Acinetobacter, and other bacterial species frequently isolated from cystic fibrosis patients combined.

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Non-Specific Mechanism in the Formation of Persister Cell

On several levels, antibiotic tolerance and persister development are intertwined with cellular metabolism. When comparing different bacterial mutations or growing settings, it's typical to find that persister cell development is negatively connected to metabolic activity and energy output [5]. The electron transport chain (ETC), a sequence of proteins found in the cytoplasm that distributes electrons from various sources such as NADH or succinate to receptors such as oxygen, is a key component of cellular energy metabolism. The energy released during the transfer of electrons helps in releasing protons from the cytoplasm, forming an electric field. Evidence has shown that ETC is linked to persister cell formation. However, how the formation and survival of persisters is linked to ETC remains largely elusive, as the link isn't always obvious [49]. As a result, by activating the ETC with appropriate metabolic stimuli, some persisters' intracellular drug concentrations can be greatly increased and killed [50]. Similarly, it's tempting to think that the ETC's role in ATP production influences persister development or survival. Different studies have found a relationship between persistent antibiotic tolerance and low levels of ATP. This could be induced by a planned shutdown or random ETC malfunction [51]. Drug tolerance is primarily viewed from this perspective as interfering with antibiotics' secondary killing effect [52].

Conversely, "PASH," or "Persistence As Stuff Happens," is one important alternative hypothesis used to explain persister cell formation and development. According to this theory, the numerous forms of different persister cells emerge likely by accident as a result of "various kinds of faults and defects" in reproduction and metabolism. It is widely accepted that PASH exists. Why persister development is so common in most tested organisms can be explained by PASH [51]. It has also been used to understand why all attempts to generate a mutant that does not form persisters have not been successful.

Persisters and Drug-Resistant Infectious Diseases

Antibiotic resistance is achieved through Genetic and biochemical or phenotypic tolerance provided by persister cells. While we have a good grasp of resistance mechanisms, we still have a lot to learn about tolerance, partially because the concept is only seen in a few cells. Nevertheless, our understanding of resistance mechanisms has greatly aided the treatment of acute illnesses. Furthermore, understanding the mechanisms behind the generation of persisters that can tolerate drugs might also be beneficial to treating chronic infections. Therefore, a clear understanding of the mechanisms behind persister creation and treatment options for eradicating these seemingly impenetrable cells is crucial.

Drug-resistant pathogens cause most infections. Worldwide, AMR is a critical issue complicating treatment efforts and increasing morbidity and mortality. Biofilm formation has been a critical phenotype that makes treating the most infections difficult. This is because cells in biofilm form an extracellular matrix, which protects the cells against antibiotic activity. Unfortunately, infections due to biofilms are very difficult to treat. Persister formation has some association with biofilm formation. Biofilms are not resistant but tolerant to antibiotic killing. The extracellular matrix of the biofilm also protects the cells against immune attack. Persister cells have temporary antibiotic-resistant phenotypes. They can be differentiated from permanent antibiotic resistance due to mutations or HGT. Inherenttoxin–antitoxin system has a way of affecting dormant cell state [4,51].

Furthermore, antibiotics do not kill persisters that survive to live another day. This can lead to relapsing of chronic infection. Persister cells can be protected from antimicrobial or other attacks by biofilm matrix [37]. Most chronic infection is usually due to protection from immunological attacks. This has been demonstrated in mycobacterium tuberculosis which can manoeuvre from immune proteins such as macrophages and granulomas. Also, *Helicobacter pylori* stomach has a way of protecting itself. In addition, *Neisseria meningitides* in the cerebrospinal fluid are also protected because of the limited presence of immunological proteins. Also, the recalcitrant nature of chronic infections is due to the presence of persisters [37].

Usually, immunocompromised patients are at higher risk of infection. This is usually due to the absence of a low immune response. Perhaps the most specific example of the resilience of a pathogenic cell during aggressive antibiotic therapy is that of cystic fibrosis (CF). The thick mucus layer formation in the lungs offers a favorable niche for pathogens that cannot be eliminated. CF is one of the major diseases in developed nations that is not treated by antibiotics. They can only be suppressed but not cured of the infection. Persisters are a possible explanation for the recalcitrance of chronic infections. Therefore, any agent that could target the resilient cells will be promising. However, understanding the relevance of persister cells in disease still demands more attention and investigation.

Following the Koch postulates, a link is established between persisters and disease. This can be demonstrated experimentally in the lab. First, the persister cells need to be isolated and inoculated into animals. Second, the ability of antibiotics to eliminate the microbial cell population will be measured while comparing it with regular strains serving as control. Unfortunately, the above-described approach is impractical. This is because lack of a good method or approach to isolate the persisters or even ensure they remain in that state. Even if it can be done, these supposed persisters might likely wake when introduced into animals.

Another approach could be to leverage the field of resistance. This can be done by creating a mutant with a high or low antibiotic tolerance. Then the activities of these mutants upon exposure to antibiotics can be measured and compared with the wild-type strains. Unfortunately, this might not work with a low-persister mutant since such does not exist yet. This type of research can indeed be carried out with a hip mutant if clinically relevant ones are available.

To date, the challenge of overcoming AMR seems to be constantly increasing. Most pathogens show MDR attributes. There is a need for new biomaterials targeting AMR pathogens and potentially killing persister cells and eliminating a chronic infection. Several studies are available explaining the formation of persisters cells and the development of resistance [29,30,52-54].

Anti-Persister Therapies: Potential and Prospects

Killing cells that evolve to survive sometimes is difficult. The inability of conventional antibiotics to kill persisters has made

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them prominent and gained global attention. The difference between stationary phase cells and a growing population has already been mentioned earlier. Killing at low population density (106 cells/ml) has been demonstrated in several studies. In this case, it is unlikely to see persisters, and antibiotics may show a total eradication of the pathogen. However, in a stationary population, this might not be observed. In contrast to other antibiotics, daptomycin acts against the membrane leading to persisters elimination, although not at safe concentrations. Antibiotic combinations have also been screened for their effect against persisters. Although some improvement in killing can be noticed with growing populations, they do not remove populations in their stationary phase. However, evidence has shown that adding gentamicin could help eradicate cells of a stationary culture. However, for several other pathogens, there is a need to find a solution. It is already known that antibiotics kill by disrupting the targets, which are inactive in persisters. An anti-persister biomolecule could provide more therapeutic value.

Furthermore, the resuscitation of persisters could be another strategy that could help to mitigate the increasing drug-resistant infectious disease crisis. Antibiotic efficiency when exposed to antibiotics could be increased when persisters are resuscitated [55]. So far, most of the treatments against persisters are not directed to inactivating persisters directly. In some quarters, attempts have been made to screen for chemicals that influence cell metabolism, resulting in persisters resuscitation and potentiating bactericidal antibiotics. For example, a chemical library screen identified a molecule that triggered resuscitation and eradicated ampicillin and norfloxacin persisters in *P. aeruginosa* and *E. coli* [49]. Although the molecular mechanisms modulating the resuscitation are unknown, inducing cell metabolism is a probable critical scenario.

Interference with and reduction of persisters formation is also another viable approach. Targeting global cellular processes necessary for persister development is also a viable approach. For example, (p)ppGpp enzymes involved in stringent response could also be targeted [56]. Moreover, targeting the SOS or oxidative stress response with inhibitors of the SOS or oxidative stress response is also another potent approach. For example, the stress response [57] could effectively treat chronic infections, QS, and Respiration [58].

Anti-persister formation therapy has also been explored using inhibitors [59]. It is, nonetheless, critical. It's important, however, to remember that persisters can arise very early on when the first pressures are encountered. As a result, these interventions would have to be preventative in nature. In this instance, vaccination is recommended and may be the most effective method in the long run.

Conclusions and Recommendations

Persisters are a subset of bacteria that can survive high concentrations of an antibiotic in a vulnerable population [60]. This precise and very narrow definition aims to energize the diverse field of bacterial research. A broader description may be necessary when evaluating various cell types that may adopt a roughly similar survival strategy. This is because heterogeneity may result in a diverse reaction to stress, with some of the population going into a growth halt to help them avoid a potentially lethal threat [18].

Conversely, the persister cells always seem highly undesirable for the host. This is more the case if the offspring emerge with new features relating to their pathogenicity and drug resistance. However, it is still subject to investigation if persistence might be positive. This can be considered in the case of commensals that could reconstitute the complex gut microbiota after antibiotic treatment. Even without antibiotic resistance, some infections can be hard to treat. Despite antibiotic therapy, non-proliferating or slow-growing bacteria have been found in persistently infected locations in infection models. The persister cells constitute a major threat to overcoming AMR. To adequately understand the relationship between persisters and antibiotic resistance, more robust techniques involving integrating next-generation sequencing techniques are critical. The introduction of cutting-edge techniques has tremendously aided this process in recent years to keep track of and study unusual non-growing microorganisms. Thus, the field is growing. However, more evidence and insight are needed to be able to properly define the emergence of persisters and their exact role in antibiotic resistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

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