International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.2, pp 204-212, 2015

Bacterial persistence: molecular mechanisms, biofilm, pathogenicity and eradication

Moumita Roy Chowdhury

Department of Biotechnology, School of Chemical & Biotechnology, SASTRA University, Thirumalaisamudram, Thanjavur 613401, India.

Abstract: Relapse of tuberculosis, typhoid and various other infectious diseases after remotion of antibiotics is attributed to the presence of 'persisters' in the bacterial population. This phenomenon is referred to as 'persistence'; often in conjunction with multicellular like biofilm complicates the treatment of infectious diseases. Persistence is a non-heritable trait as the progeny cells are as sensitive to antimicrobial agents as their ancestors. In the recent past several breakthroughs have shown that several factors induction heterogeneity of a bacterial population. Of this Toxin Antitoxin Systems (TAS) are better understood to mediate metabolic reduction and induce non dividing state of a small fraction of bacterial population. These cells, referred to as persisters, are tolerant to even high concentrations of several antibiotics. The factors involved in the regulation of TAS are thought to be stochastically activated which in turn activate different TAS to induce slow growing state. Biomolecules like ppGpp, inorganic polyphosphate and various proteases like Lon are involved in TA system mediated bacterial persistence. In this review, recent developments are summarized with a focus on possible targets to counter the persistence phenomenon.
Key words: persisters, Toxin-Antitoxin systems, Lon protease, ppGpp.

Introduction

When rapidly growing bacterial population is exposed to antibiotic or stress conditions like nutrient depletion, amino acid and phosphate starvation or any other extreme environmental condition, most of the bacteria get killed in the process. But few slow growing or non growing bacteria survive through the stressed condition and become dormant. After removal of the stressed condition these bacteria stochastically switch back to rapidly growing cells[1]. This phenomenon is called bacterial persistence and the bacterial cells which survived through the stresses are called "persisters"[2]. When the persister cells are sub cultured it is found that the daughter cells are sensitive to antibiotic or other stressed conditions[3]. Hence bacterial persistence is a phenotypic trait.

Bacterial persistency is an epigenetic phenotypic property which is drug independent although it is debatable as few studies have shown that persister cell formation is induced after the antibiotic exposure[4]. Many pathogens escape antibiotic killing by forming persisters. Experiment on animal model has shown that rapidly growing bacterial cells are easily killed by antibiotics at an early infection stage. But persister pathogenic cells escape from the killing and become tolerable to antibiotics at late stage of infection which leads to the relapsing of the diseases like cystic fibrosis and urinary tract infection. Biofilm enriched with persister cells are responsible for chronic infections of various implanted medical devices. Role of TA system in biofilm production has long been debated. But information is accumulating favoring the relationship among TA system, persistency and biofilm production in pathogens like *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*.

Discovery of bacterial persistence

In the early 1940s Joseph Bigger found that rapidly growing culture of *Staphylococcus aureus* is not efficiently killed by penicillin. Some of the bacteria survive through the process. Penicillin interrupts bacterial cell wall synthesis and kills rapidly growing cells efficiently. But penicillin is unable to kill slow or non-growing bacteria hence unable to sterilize the culture of *Staphylococcus aureus*. He called this survival process as "persistence" and coined the term "persisters" for the surviving cells. The stochastic mechanism behind this phenomenon helps the rapidly growing cells to switch to the non growing dormant cells in response to antibiotics or other stressful condition. After surviving through the antibiotics or other environmental assaults the persisters switch back to the rapidly dividing cells causing chronic infection. Very slow or non growing physiological state makes the persisters tolerant to the antibiotics as most antibiotics affect the cell wall synthesis thus effectively kill the rapidly growing cells. Another view of this phenomenon suggests the presence of high persistence (hip) mutants which exhibit multidrug tolerance. It has also been proved that persistence is dependent on the toxin-antitoxin (TA) loci which can be present on the plasmid or in the bacterial chromosome of *Escherichia coli*. Type II *hipBA* toxin-antitoxin locus encodes HipA protein responsible mediating persistence. Along with *hipBA* other toxin antitoxin families like *relBE, mazEF*, *vapBC* [5] and *mqsRA* [6] gene families are also found to be responsible for persistence in *Escherichia coli*. In Toxin-Antitoxin systems toxin is responsible for the cell dormancy by interfering with various cellular mechanisms such as cell division, replication, protein translation and cell wall synthesis [7]. Antitoxin is an antidote of toxin and inhibits or represses the action of toxin by forming tight toxin-antitoxin complex or by repressing the transcription of Toxin-Antitoxin systems.

Molecular mechanisms of persistence

Toxin-Antitoxin systems

Toxin-Antitoxin (TA) system were first discovered and characterized on plasmid. Later several chromosomal TA systems were discovered on most of the fully sequenced free living bacteria and archae. Plasmidic TA systems are implicated in aiding the maintenance of plasmid within a population by a phenomenon called post segregational killing (PSK)[8]. However, the physiological significance of chromosomal TA systems are implicated in stress survival, biofilm formation, persistence and programmed cell death (PCD) [9]. TA system is a bicistronic autoregulatory operon in which antitoxin mostly present upstream of the toxin gene. Toxins are in general stable proteins which inhibit various cellular responses like cell division, DNA replication, protein translation, and cell wall synthesis[10].Antitoxin regulates the expression of toxin. Antitoxins are loosely folded hence proteolytically unstable than toxins and present in abundance. There are 5 types of TA systems till now discovered. In type I TA system toxins are hydrophobic proteins whose translation is suppressed by the antitoxin which are antisense RNAs. Antitoxins form tight complexes with toxins by inactivating them in type II TA systems and both toxins and antitoxins are proteins in nature. In type III TA system antitoxin RNA binds to toxin protein by forming a tight protein-RNA complex and finally repressing the function of toxin protein. Type IV and V TA systems have been discovered recently and they also act by forming tight complexes where antitoxin binds to either toxin or the target of toxin protein[11]. Antitoxins can be degraded by Lon (Long form filament phenotype) or Clp proteases under various stressed condition. Degradation of antitoxins by Lon protease leads to the activation of mRNA endonucleases which in turn increases persistency in bacteria by inhibiting the global cellular translation[9]. To date more than thirty TA systems are discovered in *E. coli* and more than eighty TA systems in *Mycobacterium tuberculosis* [5].

Type I TA system

In type I TA system toxins are small hydrophobic proteins which damage inner cell membrane thus conferring toxicity to the cells. Antitoxins are antisense RNAs which forms base pairing with the toxin's mRNA and inhibits translation of the toxin protein. Afterwards Toxin-Antitoxin duplex is degraded by RNaseIII [7]. Hok-Sok is the well known type I TA system which includes a third component named Mok [5] where Sok antisense RNA forms base pair with *hok* mRNA hence inhibiting the translation of Hok toxin [11].Other well understood chromosomal type I TA families are SymE-SymR which induced as a SOS response. Other TA families of this group are fst-RNAII, FlmA-FlmB, TisB-IstR, LdrD-RdlD etcetera.

Type II TA system

Toxin and antitoxin both are proteinaceous in nature in type II TA system where the antitoxin forms a tight complex with the toxin hence inhibiting toxin to act on its targets. Sometimes antitoxins are DNA binding

proteins and it inhibits transcription of the toxin gene by binding to the operator region. Type II antitoxins are susceptible to ubiquitous Lon or Clp proteases and degradation of antitoxins leads to the overproduction of toxins hence increasing the persistence and programmed cell death. Well understood type II gene families are *hipBA, mazEF, relBE* and *vapBC*. Other type II TA systems include Phd-Doc, CcdA-CcdB, HicA-HicB etcetera.

Type III Toxin-Antitoxin system

An RNA antitoxin regulates the transcription of toxin protein in type III TA system. ToxI-ToxN system is the best understood type III TA system till now which is found in plant pathogens like *Pectobacterium atrosepticum* and *Erwinia carotovora*. ToxN inhibits translation in a similar manner like MazF. ToxI RNA binds to three ToxN proteins and in turn inactivates the toxin. ToxN induced persistency by cleaving ToxI mRNA [12]. Type III TA system confers resistance to bacteriophage.

Type IV Toxin-Antitoxin system

Type IV TA system does not involve the formation of toxin-antitoxin complex. In this system antitoxin directly binds to the targets of toxin hence inhibiting the toxin activity. Toxin CbtA and antitoxin YeeU are the constituents of type IV TA system and well understood till now. CbtA inhibits polymerization of two cytoskeleton proteins FtsZ and MreB. YeeU antitoxin binds to FtsZ and MreB and neutralizes the activity of the toxin [13].

Type V Toxin-Antitoxin system

In this TA system antitoxin endoribonuclease cleaves the toxin mRNA which involves in membrane lysis and causes ghost cell formation. GhoT toxin either causes cell death or causes increment in persister cell formation. GhoST is the best understood type V TA system where the toxin GhoT (previously known as YjdO) is a small, hydrophobic protein. Activity of GhoT is regulated by a non labile antitoxin GhoS(previously known as YjdK) [14].

Lon protease plays an important role in regulation of Type II TA systems

Antitoxins are unstable in nature but produced in abundance compared to toxin proteins. Antitoxins are degraded by Lon protease and toxin proteins can freely bind to its target and inhibit translation machinery in the absence of antitoxins. In the absence of Lon protease persister cell formation decreased dramatically. Deletion of various Toxin-Antitoxin systems decreased the formation of persister cells by several folds although deletion of individual TA systems did not show much effect on persister cell formation. Lon overproduction cannot increase persister cell formation in the absence of 10 TA systems which proves that there is a strong relationship between Lon protease and TA systems [15].

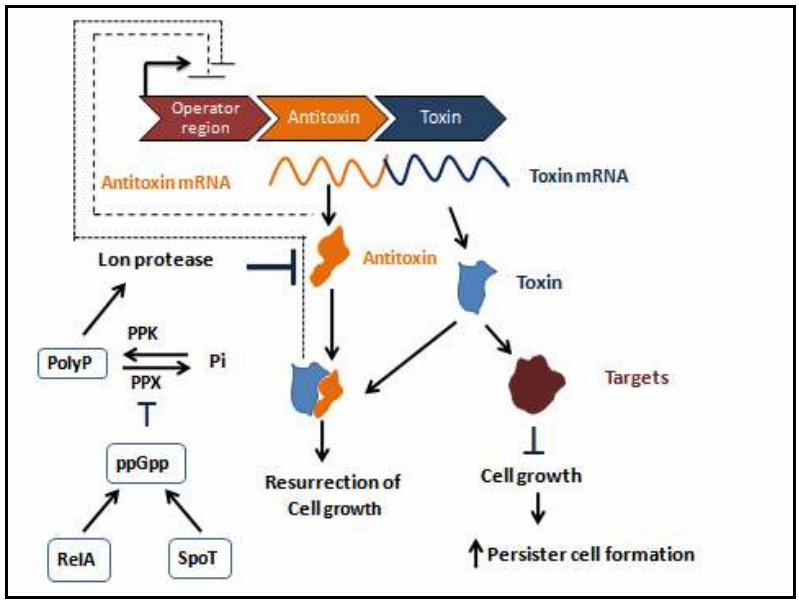

Fig: Type II TAS produces proteolytically stable toxin proteins and proteolytically unstable antitoxins. Type II toxins inhibit translation hence inhibit cell growth. In normal condition Antitoxin binds directly to Toxin or the operator region of TAS and resurrects cell growth. Toxin-Antitoxin complex also inhibit translation of Toxin by binding to the operator region of TAS. Under stressed condition RelA protein synthesized ppGpp alarmone which inhibits exopolyphosphatase (PPX). PolyP- a stretch of orthophosphates degraded by PPX and synthesized by polyphosphate kinase (PPK). PolyP induces Lon protease cleaves antitoxin and in turn induces persistence.

***hipBA* gene families**

HipA toxins inhibit cell growth and induce persistence. HipA toxin is inhibited by HipB antitoxin which is a helix-turn-helix protein. *hipBA* genes are specifically known for their role in bacterial persistency. HipA is a serine kinase which inhibits the translation via phosphorylating the elongation factor TU (EF-Tu) [16] thus increasing persistency. HipB dimers form a tight complex with the HipA toxin. HipB is degraded by Lon protease and HipA is overproduced and arrests the cell growth by increasing persistency.

***mazEF* gene families**

mazEF gene families which are found in the chromosome of *E. coli* and other pathogenic bacteria previously thought to be present in the bacteria as an extra chromosomal trait. Earlier idea was that *mazEF* is responsible for the plasmid stabilization hence regulating post-segregational killing (PSK) of plasmid free daughter cells. Involvement of (p)ppGpp in the bacterial programmed cell death (PCD) by MazF toxin was the earlier idea which is later ruled out by recent studies. MazF which is an endoribonuclease inhibits global translation by cleaving mRNAs at ACA or ACU sites in a ribosome independent manner which in turn induce PCD. MazE antitoxin which is located upstream of the toxin MazF, binds to the promoter region and inhibits the transcription of the toxin[17]. MazE is degraded by ClpAP in an ATP-dependent manner and the toxin inhibits the cell growth hence induce persistency.

***relBE* gene families**

relBE gene families consist toxin RelE whose expression is regulated by antitoxin RelB. RelE which is an endoribonuclease inhibits global cellular translation by cleaving mRNA at the ribosomal A site. RelE cleaves mRNA in the same manner as MazF but it requires the involvement of ribosome as purified RelE is unable to cleave mRNA in absence of ribosome [5]. Like other type II TA systems, dimer of the antitoxin RelB binds to the promoter region and inhibits the transcription of toxin gene[18]. Upon degradation with Lon protease antitoxins are no longer available for the repression of the toxin gene and the toxin is available for the cell growth inhibition in turn inducing bacterial persistence.

***vapBC* gene families**

VapBC TA systems were first discovered in *Dichelobacter nodosus*- a gram negative obligate anaerobe. *vapBC* gene families which are plentiful in both enteric and other pathogenic bacteria like *Mycobacterium tuberculosis, Leptospira interrogans, Shigella flexneri* produce VapC toxin and VapB antitoxin [19]. VapB antitoxin acts as a repressor of the toxin gene by binding to the promoter region. VapC when present in abundance induces persistency by destabilizing the TA complex and finally inhibits translation process.

Stringent response

Under amino acid starvation, heat shock, limitation of phosphate and other stress condition bacteria possess a stringent response which is controlled by guanosine 3',5'-bispyrophosphate (ppGpp) and guanosine 3'-diphosphate,5'- triphosphate (pppGpp)[20]. It has been proved since 1978 that *Escherichia coli* strains lacking *relA* and *spoT* genes are unable to accumulate (p)ppGpp[21]. RelA and SpoT are the proteins responsible for the synthesis of (p)ppGpp alarmone during amino acid starvation [22]. During amino acid starvation uncharged t-RNA occupy free ribosomal A site and translation is stalled which is sensed by RelA. RelA is a synthase enzyme which synthesize ppGpp from GDP and pppGpp from GTP with the help of ATP[23]. SpoT is a hydrolase enzyme although in certain condition SpoT can act as synthase too and it can sense the phosphate starvation and elevate the level of (p)ppGpp[24]. Relation between bacterial persistence and stringent response especially in various intracellular pathogens is being proved by the recent studies. Pathogens like *Mycobacterium*

tuberculosis, Streptococcus equisimilis and *Bacillus subtilis* possess *relA* homologue gene which helps these bacteria persist during stress condition. In *Streptococcus pyogenes* and *Staphylococcus aureus* homologue of *relA* gene rel_{Spy} and rel_{Sau} genes have proved to be involved in stringent response which may linked to persistence of these extracellular pathogens[25]. (p)ppGpp inhibits the activity of exopolyphosphatase (PPX) enzyme which is responsible for the degradation of inorganic polyphosphate (PolyP). PolyP is a polymer of several hundreds of orthophosphates which is synthesized by PPK (polyphosphate kinase). According to recent studies PolyP activates Lon protease which is responsible for the degradation of antitoxin hence increasing persistence. Deletion of *ppk* and *ppx* gene from the wild type strain has shown several fold of reduction in persister formation while exposing to bactericidal antibiotics[26].

Induction of persistence due to SOS response

Evidences are gradually accumulating in favor of the idea that bacterial persistence is not a stochastic mechanism and SOS gene networks play a major role in the formation of persister cells. Fluoroquinolones are the drugs which inhibit DNA gyrase or topoisomerase activity by altering the protein conformation hence resulting in double strand breaks in DNA [27]. This double strand breaks induce the SOS genes which are involved in the formation of persister cells. This observation has lead to the idea that multi drug tolerance may not be always a drug independent process and can occur in response to the antibiotic like ofloxacin. Strain lacking RecA and RecB has shown decrement in persister formation upon exposure to fluoroquinolone antibiotics [4].

Efflux pump

Tet(L) is a tetracycline efflux protein found in *Bacillus subtilis* and MdfA is a multidrug resistance (MDR) efflux protein found in *Escherichia coli*. Tet(L) and MdfA both the two efflux proteins found to be responsible for the bacterial persistence in recent studies[28].

ROS and antioxidants

Many bactericidal antibiotics including fluoroquinolone antibiotics kill the cells by producing reactive oxygen species (ROS) and hydroxyl radical[29] in the presence of high concentration of dissolved oxygen and causes cell death. Studies on *Mycobacterium smegmatis* and *M. tuberculosis* have proved that persister cells escaped killing by various bactericidal antibiotics by producing high level of antioxidant. Little drop in dissolved oxygen leads to the elevation of persister formation in the presence of bactericidal antibiotics. It has also been proved that in the maintenance of high concentration of dissolved oxygen is able to exterminate the persister population[30].

Role of quorum sensing in bacterial persistence

Pathogen like *Pseudomonas aeruginosa* causes chronic and persistent infection with the help of quorum sensing. Recent studies have proved that quorum sensing deficient mutants can be cleared faster from various implants than their wild type siblings. It has also been proved that application of inhibitors of quorum sensing have shown efficient clearing of biofilm from implants [31].

Persistence and biofilm formation

Biofilm is the cause of dental plaque, various urinary tract infections, catheter infection, cystic fibrosis, endocarditic diseases, coating on contact lenses and infections in indwelling devices like heart valves etc. Research has proved that biofilm is resistant to almost all antimicrobial agents while not possessing any well characterized resistance mechanism. This is mainly due to the presence of persisters or multi drug tolerant cells [32]. Persister cell formation in biofilm is stage dependent as more persister cells are formed during stationary phase than log phase[33]. Most of the cells in biofilm are susceptible to antibiotic killing except the persister cells. Immune systems of host cell have the ability to remove both normal pathogenic cells and persister cells but immune systems can not remove the persister cells present in the biofilm. After the removal of antibiotic persister cells present in the biofilm resume their growth and form the biofilm again which may cause chronic infection. *Candida albicans and Pseudomonas aeruginosa* are specially known for biofilm mediated chronic infections and both form persisters when exposed to antibiotics or any other antimicrobial agents. The idea of involvement of TA systems in biofilm formation was not accepted before as the homologues of MazF and RelE

proteins did not show any effect on biofilm formation. Later by other experiment involving MqsRA TA systems in biofilm formation was proved. Involvement of an uncharacterized protein YjgK in the formation of biofilm was proved by transcriptome analysis. This YjgK protein was reported to decrease the biofilm formation after 8 hours and increased after 24 hours[33-34]. Recent studies have also proved the involvement of type V TA systems in biofilm formation. Deletion of *ghoT* causes decrement in biofilm formation when deletion of *ghoS* causes increment in biofilm formation[14].

Role of persistence in pathogenicity

Helicobacter pylori, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenza, Salmonella enterica serovar typhi, Streptococcus pyogenes, Shigella flexneri, Proteus vulgaris* are well known bacteria which persist in human body for long time by evading the innate and adaptive immune system. These pathogens are responsible for various chronic infections in mammalian hosts like tuberculosis, typhoid fever, gastroenteritis, peptic ulcers even cancer[35].

These pathogenic bacteria colonize in reticuloendothelial system for long periods of time. Granulomas within the macrophages are found to be the favorite residence of these persistent bacteria although the proper site of infection during persistency is still unknown. These bacteria survive within the granulomas by either inhibiting the fusion of macrophages with lysosomes or by adapting the acidic environment of phagolysosome. The mechanism of persistence in the presence of well evolved immune system of host is still now in the research level. Recent studies on *Escherichia coli* have shown that persistency is linked to the Toxin-Antitoxin system of bacteria although the proper mechanism is unknown. Evidences are growing in the support of this idea for the pathogenic bacteria too. ω-ε-ζ are the three components of Toxin-Antitoxin system found in some gram positive pathogenic bacteria like *Streptococcus pyogenes and Enterococcus faecalis*. These components along with type I TA system are found to be responsible for the virulence of these pathogenic bacteria.

How to tackle persistence

Research is going on in order to kill the persister cells without affecting the host. Bactericidal antibiotics from aminoglycoside group like gentamicin, kanamycin or streptomycin which are potent inhibitor of translation can be used to kill persister cells with the help of various metabolites which potentiate the action of these aminoglycosides. Although less sensitive to aminoglycosides, persister cells are effectively killed in the presence of high concentration of various metabolites involved in glycolysis or pentose phosphate pathway like glucose, fructose or pyruvate[36]. Use of Trimethoprim-Sulfamethoxazole antibiotic for ten days has shown reduction in persister formation in acute urinary tract infection caused by uropathogenic *Escherichia coli* [37]. Various antibacterial agents like 3-[4-(4-methoxyphenyl)piperazin-1-yl]piperidin-4-yl biphenyl-4-carboxylate (C10)[38] and (*Z*)-4-bromo-5-(bromomethylene)-3-methylfuran-2(5*H*)-one(BF8)[39] are recently proved to inhibit persistence. Where C10 specifically targets the persister cells specifically without affecting the antibiotic sensitive cells BF8 restores the antibiotic sensitivity of the persister cells. Recent studies have proved that use of various antimicrobial peptides and membrane active antibiotics or other biomolecules which damage the bacterial cell membrane along with these chemotherapeutic agents can also prevent persister cell formation. Lipoglycopeptides like telavancin, oritavancin, dalbavancin etcetera and daptomycin which is a membrane active antibiotics can be used to remove persister cells from the bacterial population[40]. In presence of high dissolved oxygen concentration bactericidal antibiotics are also able to remove persister cells by generating various reactive oxygen species (ROS) and hydroxyl radicals. Use of clofazimine which induce the generation of ROS have the ability to eradicate persister cells of *Mycobacterium tuberculosis* from the host[41].

Possible drug targets for the eradication of persistence

As conventional antibiotics often fail to eradicate persistence new drugs must be developed in order to control persistence. Throughout this review various molecular mechanisms are discussed which may be the cause of bacterial persistence. According to recent research a complex network of proteins are involved in persistence and many of the proteins can be targets for various drugs. Many proteins in this network inhibit persistence directly or indirectly and various drugs can be used mimicking those proteins. Toxin-Antitoxin systems are the most promising drug targets as various studies have shown reduction in persistence in absence of Toxin-Antitoxin systems. Antitoxins inhibit toxin proteins by forming tight complex with Toxins or their

targets but Antitoxins are proteolytically unstable. Proteolytically stable drugs mimicking antitoxins can be used to treat bacterial persistence. Recent studies have proved that Antitoxins are degraded by Lon protease hence increase persistence. Drugs inhibiting Lon proteases can be used to eradicate persistence. Involvement of PolyP and ppGpp under stressed condition has also been proved. PolyP enhances activity of Lon protease and polyphosphate kinase (PPK) synthesizes PolyP from inorganic phosphate. Both PolyP and PPK can be used as drug targets in order to control persistence. ExopolyphosphaToxin-Antitoxin systemse (PPX) degrades PolyP hence excess amount of PPX or drugs mimicking PPX can be used to combat persistence. PPX is inhibited by (p)ppGpp under stringent condition which is regulated by RelA and SpoT proteins where RelA synthesizes (p)ppGpp and SpoT hydrolizes (p)ppGpp. RelA can be the potential drug targets for the eradication of bacterial persistence as in absence of RelA (p)ppGpp will not be produced and PPX will produce in large number which will hydrolyze PolyP. As Lon protease activity depends on PolyP, in absence of it Lon protease cannot degrade Antitoxins.

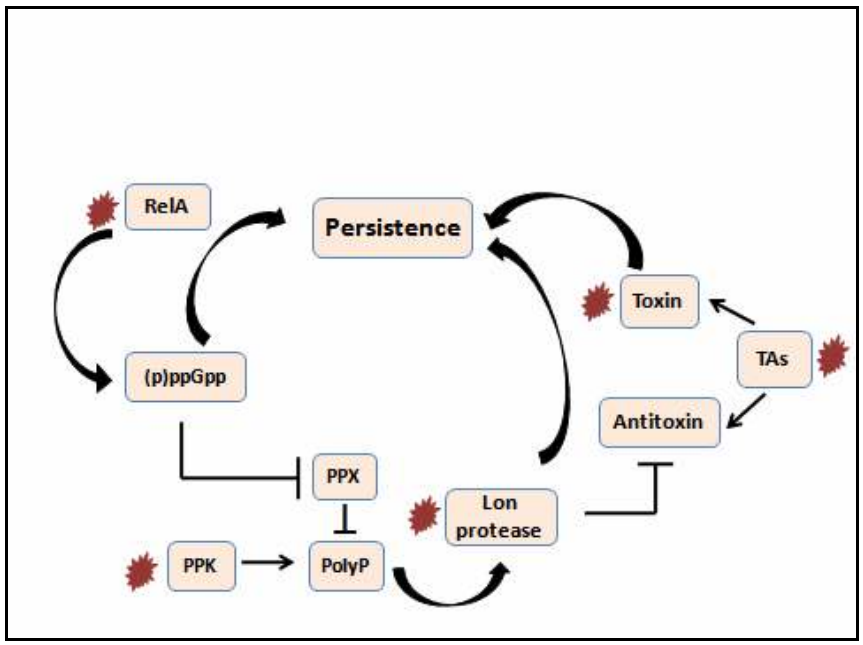

Fig: Possible drug targets to treat persistence. RelA, PPK, Lon protease, TAs (Toxin-Antitoxin systems) and toxin proteins coded by the TAs can be used as drug targets for the eradication of persistence.

Conclusion:

After the discovery of penicillin and other antibiotics it was thought that various serious infectious diseases can be cured readily. But due to the high mutation rate or phenomenon like persistence bacteria often escape killing by antibiotics or other antibacterial agents.

So far we have discussed about the history of bacterial persistence, finding of persistent genes, effects of various Toxin-Antitoxin systems on bacterial dormancy and other possible molecular mechanisms behind bacterial persistence. Escaping antibiotic killing by various pathogenic bacteria through the formation of persister cells is the overgrowing concern nowadays as most pathogenic bacteria become persistent in the mammalian host cells and causes various chronic infections. Information is steadily accumulating favoring strong relationship between TA system, bacterial persistence and biofilm formation. Deletions of various TA systems have shown significant decrement in persistence and biofilm formation. As the persisters are well known for the production of biofilm, it is a challenge to treat biofilm related diseases. Although recent studies regarding the use of various chemotherapeutic agents or combinatorial antibiotic treatment have shown eradication of persister cells lot more research is needed to be done. Until the correct mechanism of the phenotypic switching of persistence is discovered persister pathogenic bacteria possess enormous threat to the mankind.

Bacterial persistence can be theoretically related to the formation of cancerous cells and the anti cancer treatment. In case of non-small cell lung cancer persistence is observed. Chromatin remodeling may play a role in this kind of persistency which is also seen in some pathogens [42]. Bacterial persistence can be the role model for these malignant cells. More development in the field of bacterial persistence will lead to the development of new antibiotics which will be able to eradicate the persister cells completely without affecting other rapidly growing cells.

Reference

1. Kussell E, Kishony R, Balaban NQ, Leibler S. Bacterial persistence a model of survival in changing environments. Genetics 2005;169(4):1807-14.
2. Bigger J. Treatment of staphylococcal infections with penicillin by intermittent sterilisation. The Lancet 1944;244(6320):497-500.
3. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K. Persister cells and tolerance to antimicrobials. FEMS microbiology letters 2004;230(1):13-18.
4. Dörr T, Lewis K, Vulić M. SOS response induces persistence to fluoroquinolones in Escherichia coli. PLoS genetics 2009;5(12):e1000760.
5. Gerdes K, Maisonneuve E. Bacterial persistence and toxin-antitoxin loci. Annual review of microbiology 2012;66:103-23.
6. Hong SH, Wang X, O'Connor HF, Benedik MJ, Wood TK. Bacterial persistence increases as environmental fitness decreases. Microbial biotechnology 2012;5(4):509-22.
7. Wen Y, Behiels E, Devreese B. Toxin–Antitoxin systems: their role in persistence, biofilm formation, and pathogenicity. Pathogens and disease 2014;70(3):240-49.
8. Van Melderen L, De Bast MS. Bacterial toxin–antitoxin systems: more than selfish entities? PLoS genetics 2009;5(3):e1000437.
9. Hayes F. Toxins-antitoxins: plasmid maintenance, programmed cell death, and cell cycle arrest. Science 2003;301(5639):1496-99.
10. Sofos N, Xu K, Dedic E, Brodersen DE. Cut to the chase–Regulating translation through RNA cleavage. Biochimie 2015.
11. Faridani OR, Nikravesh A, Pandey DP, Gerdes K, Good L. Competitive inhibition of natural antisense Sok-RNA interactions activates Hok-mediated cell killing in Escherichia coli. Nucleic acids research 2006;34(20):5915-22.
12. Blower TR, Short FL, Rao F, Mizuguchi K, Pei XY, Fineran PC, et al. Identification and classification of bacterial Type III toxin–antitoxin systems encoded in chromosomal and plasmid genomes. Nucleic acids research 2012;40(13):6158-73.
13. Masuda H, Tan Q, Awano N, Wu KP, Inouye M. YeeU enhances the bundling of cytoskeletal polymers of MreB and FtsZ, antagonizing the CbtA (YeeV) toxicity in Escherichia coli. Molecular microbiology 2012;84(5):979-89.
14. Wang X, Lord DM, Cheng H-Y, Osbourne DO, Hong SH, Sanchez-Torres V, et al. A new type V toxin-antitoxin system where mRNA for toxin GhoT is cleaved by antitoxin GhoS. Nature chemical biology 2012;8(10):855-61.
15. Maisonneuve E, Shakespeare LJ, Jørgensen MG, Gerdes K. Bacterial persistence by RNA endonucleases. Proceedings of the National Academy of Sciences 2011;108(32):13206-11.
16. Schumacher MA, Piro KM, Xu W, Hansen S, Lewis K, Brennan RG. Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. Science 2009;323(5912):396-401.
17. Mittenhuber G. Occurence of mazEF-like antitoxin/toxin systems in bacteria. Journal of molecular microbiology and biotechnology 1999;1(2):295-302.
18. Gotfredsen M, Gerdes K. The Escherichia coli relBE genes belong to a new toxin–antitoxin gene family. Molecular microbiology 1998;29(4):1065-76.
19. Zhang YX, Xiao Kui G, Chuan W, Bo B, Shuang Xi R, Chun Fu W, et al. Characterization of a novel toxin-antitoxin module, VapBC, encoded by Leptospira interrogans chromosome. Cell research 2004;14(3):208-16.
20. Haseltine WA, Block R. Synthesis of guanosine tetra-and pentaphosphate requires the presence of a codon-specific, uncharged transfer ribonucleic acid in the acceptor site of ribosomes. Proceedings of the National Academy of Sciences 1973;70(5):1564-68.

21. O'Farrell PH. The suppression of defective translation by ppGpp and its role in the stringent response. Cell 1978;14(3):545-57.
22. Primm TP, Andersen SJ, Mizrahi V, Avarbock D, Rubin H, Barry CE. The stringent response of Mycobacterium tuberculosis is required for long-term survival. Journal of Bacteriology 2000;182(17):4889-98.
23. Agirrezabala X, Fernández IS, Kelley AC, Cartón DG, Ramakrishnan V, Valle M. The ribosome triggers the stringent response by RelA via a highly distorted tRNA. EMBO reports 2013;14(9):811-16.
24. Cashel M. Regulation of bacterial ppGpp and pppGpp. Annual Reviews in Microbiology 1975;29(1):301-18.
25. Godfrey HP, Bugrysheva JV, Cabello FC. The role of the stringent response in the pathogenesis of bacterial infections. Trends in microbiology 2002;10(8):349-51.
26. Maisonneuve E, Castro-Camargo M, Gerdes K. (p) ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. Cell 2013;154(5):1140-50.
27. Drlica K. Mechanism of fluoroquinolone action. Current opinion in microbiology 1999;2(5):504-08.
28. Krulwich TA, Lewinson O, Padan E, Bibi E. Do physiological roles foster persistence of drug/multidrug-efflux transporters? A case study. Nature Reviews Microbiology 2005;3(7):566-72.
29. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. Cell 2007;130(5):797-810.
30. Grant SS, Kaufmann BB, Chand NS, Haseley N, Hung DT. Eradication of bacterial persisters with antibiotic-generated hydroxyl radicals. Proceedings of the National Academy of Sciences 2012;109(30):12147-52.
31. Christensen LD, Moser C, Jensen PØ, Rasmussen TB, Christophersen L, Kjelleberg S, et al. Impact of Pseudomonas aeruginosa quorum sensing on biofilm persistence in an in vivo intraperitoneal foreign-body infection model. Microbiology 2007;153(7):2312-20.
32. Lewis K. Persister cells and the riddle of biofilm survival. Biochemistry (Moscow) 2005;70(2):267-74.
33. Kim Y, Wang X, Ma Q, Zhang X-S, Wood TK. Toxin-antitoxin systems in Escherichia coli influence biofilm formation through YjgK (TabA) and fimbriae. Journal of Bacteriology 2009;191(4):1258-67.
34. Wang X, Wood TK. Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. Applied and environmental microbiology 2011;77(16):5577-83.
35. Monack DM, Mueller A, Falkow S. Persistent bacterial infections: the interface of the pathogen and the host immune system. Nature Reviews Microbiology 2004;2(9):747-65.
36. Allison KR, Brynildsen MP, Collins JJ. Metabolite-enabled eradication of bacterial persisters by aminoglycosides. Nature 2011;473(7346):216-20.
37. Schilling JD, Lorenz RG, Hultgren SJ. Effect of trimethoprim-sulfamethoxazole on recurrent bacteriuria and bacterial persistence in mice infected with uropathogenic Escherichia coli. Infection and immunity 2002;70(12):7042-49.
38. Kim J-S, Heo P, Yang T-J, Lee K-S, Cho D-H, Kim BT, et al. Selective killing of bacterial persisters by a single chemical compound without affecting normal antibiotic-sensitive cells. Antimicrobial agents and chemotherapy 2011;55(11):5380-83.
39. Pan J, Bahar AA, Syed H, Ren D. Reverting antibiotic tolerance of Pseudomonas aeruginosa PAO1 persister cells by (Z)-4-bromo-5-(bromomethylene)-3-methylfuran-2 (5H)-one. PLoS One 2012; 7(9):e45778.
40. Hurdle JG, O'Neill AJ, Chopra I, Lee RE. Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. Nature Reviews Microbiology 2010;9(1):62-75.
41. Marrakchi M, Liu X, Andreescu S. Oxidative Stress and Antibiotic Resistance in Bacterial Pathogens: State of the Art, Methodologies, and Future Trends. Advancements of Mass Spectrometry in Biomedical Research: Springer; 2014. p. 483-98.
42. Balaban N. Persistence: mechanisms for triggering and enhancing phenotypic variability. Current opinion in genetics & development 2011;21(6):768-75.

*****