Infection and Drug Resistance

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REVIEW

The Impact of Endogenous Hydrogen Sulfide on Bacterial Resistance

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Abstract: Infectious diseases, especially sepsis from bacterial infections, significantly threaten global health, with antimicrobial resistance (AMR) complicating treatment and increasing clinical burdens. Antibiotic overuse contributes to AMR by creating selective pressure, reducing the efficacy of traditional therapies, and necessitating new approaches. Endogenous hydrogen sulfide (H_2S), a gaseous signaling molecule produced by most bacteria through cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3MST), plays a crucial role in bacterial resistance. This review explores the biological functions of bacterial endogenous H_2S and its impact on AMR. H_2S enhances resistance by neutralizing antibiotic-induced reactive oxygen species (ROS), reducing oxidative stress and DNA damage, and promoting biofilm formation, which obstructs antibiotic penetration and facilitates resistance gene exchange. Furthermore, enhancing H_2S-based assays could significantly improve the diagnosis of AMR. Additionally, strategies such as targeting H_2S metabolism—through the use of H_2S synthase inhibitors or disrupting biofilms via H_2S clearance—or the combination of H_2S synthase inhibitors with antibiotics, may reverse resistance. A deeper understanding of the mechanisms by which H_2S mediates resistance is essential for the development of advanced diagnostic tools and innovative therapies to combat AMR. Its clinical translation may reverse AMR passivity, guide antibiotic sensitizer development, and optimize therapies, holding significant clinical and translational value.

Keywords: endogenous hydrogen sulfide, antibiotic resistance, infectious diseases

Introduction

Infectious diseases represent significant threats to human health, with sepsis being a clinical syndrome characterized by a dysregulated host response to infection, leading to organ dysfunction.[1] Infectious diseases pose substantial threats to human health, with sepsis identified as a clinical syndrome marked by an aberrant host response to infection, resulting in organ dysfunction. As a predominant condition within critical care medicine, sepsis exerts a considerable clinical burden. Globally, it accounts for over 48.9 million new cases and in excess of 11 million deaths each year, with a mortality rate of 19.7%, thereby imposing significant challenges on patients, healthcare systems, and society at large.[2] Bacterial infections constitute a primary precipitant of sepsis, wherein toxins released by pathogens, coupled with the host's exaggerated immune response, may culminate in organ failure. Clinical management strategies focus on the eradication of the infection source, judicious administration of antimicrobial agents, the application of immunoadjuvant therapies, and the rectification of pathophysiological disturbances.[3] Antibiotic therapy remains a cornerstone in the management of sepsis; however, the inappropriate use of antibiotics has contributed to the escalating issue of antibiotic resistance.[4,5] This resistance has emerged as a critical global public health challenge, primarily driven by selective pressure exerted through antibiotic use.[6] The escalating issue of antimicrobial resistance (AMR) has significantly diminished the effectiveness of conventional antibiotics, thereby heightening the risk of treatment failure and underscoring the critical necessity for the development of novel therapeutic strategies.[7] To address the clinical challenge of antibiotic resistance, strategies beyond the development of novel antibacterial agents are

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required. One promising approach involves targeting bacterial metabolic pathways, specifically the endogenous hydrogen sulfide (H_2S) metabolic pathway, which may offer a novel therapeutic avenue to combat antibiotic resistance.[8]

H_2S is a colorless gas characterized by a distinctive rotten-egg odor.[9] It is classified into exogenous and endogenous forms based on its biosynthetic origin. Exogenous H_2S is derived from external environmental sources or experimental interventions, primarily originating from: biogenic processes in anaerobic ecosystems, industrial chemical byproducts, and the decomposition of sulfur-containing organic matter during waste management in livestock and poultry farming.[10] In contrast, endogenous H_2S is synthesized through an organism's own metabolic processes and exhibits biological activity.[11] As a gaseous signal molecule, endogenous H_2S plays essential regulatory roles across various physiological systems, including the cardiovascular, nervous, digestive, endocrine, reproductive, and immune systems.[12–18] Disruptions in H_2S metabolism can lead to a range of pathological conditions, such as atherosclerosis, hypertension, diabetes, and neurodegenerative diseases. Despite substantial advancements have been achieved in researching the biological roles of H_2S in mammalian systems,[19] there is little research on the effect of H_2S on bacteria and its underlying mechanism, studies have shown that the majority of bacterial strains are capable of producing endogenous H_2S. H_2S is predominantly synthesized within the body through enzymatic catalysis involving cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3MST). This molecule exhibits intricate immunomodulatory and cytoprotective properties. In bacterial systems, H_2S is capable of activating antioxidant mechanisms, neutralizing reactive oxygen species (ROS) generated by antibiotic exposure, mitigating oxidative stress-induced damage, and preventing bacterial cell death resulting from such oxidative insults. Furthermore, H_2S facilitates the development of bacterial biofilms. These biofilms not only impede the physical penetration of antibiotics but also create a microenvironment conducive to the exchange of antibiotic resistance genes among bacteria. This process further augments the resistance of bacterial populations, thereby facilitating their survival and reproduction under antibiotic pressure.[20] H_2S serves as a multifunctional defense factor that is widely present in bacteria and is intricately linked to bacterial growth, bacterial antibiotic resistance, and virulence.

Biosynthetic Pathway of Endogenous H_2S in Bacteria

Specific enzyme systems (eg, cysteine desulfhydrases, sulfate reductases, etc.) are present in the bacterial cytoplasm. These enzyme systems can catalyze the sulfur-containing substrates (eg, sulfur-containing amino acids, sulfates) taken up by bacteria from the environment, facilitating metabolic reactions within the bacteria that ultimately produce H_2S. H_2S is released outside the bacteria through transmembrane diffusion. Endogenous H_2S in bacteria is synthesized through multiple pathways, which may function in a complementary manner. In certain bacterial species, H_2S production occurs via the metabolism of sulfur-containing amino acids. For instance, *Escherichia coli* (*E. coli*) generates H_2S through the catabolism of L-cysteine, facilitated by the enzyme cysteine desulfurase.[21] In 2011, Konstantin Shatalin's genomic analysis demonstrated that the majority of bacterial species possess homologues of mammalian CBS, CSE, or 3MST.[20] Notably, *Bacillus anthracisI*(*B. anthracis), Pseudomonas aeruginosa* (*P. aeruginosa*) *and Staphylococcus aureus* (*S. aureus*) contain homologues for CBS/CSE, yet they do not have homologues 3MST. Following the sequential inactivation of CSE and CBS using transposon insertion technology, the experimental results indicated that CSE serves as a crucial enzyme in the biosynthesis of bacterial endogenous H_2S, whereas CBS contributes a relatively minor role in this process.[20] Both CBS and CSE enzymes predominantly utilize cystathionine as a substrate, with pyridoxal phosphate and hemoglobin serving as cofactors. CBS synthesizes cystathionine from condensed cysteine and serine, while CSE catalyzes the conversion of cystathionine to L-cysteine, which is subsequently catalyzed by CSE/CBS to produce H_2S. CSE exhibits broad substrate specificity and facilitates H_2S production through various pathways. For instance, it catalyzes the conversion of cystathionine to L-cysteine, ammonia, and α-ketobutyric acid;[22] and it also mediates the decomposition of L-cysteine into pyruvate, ammonia, and thiocysteine, the latter of which further generates H_2S.[23] *E. coli* possesses orthologs for 3MST but lacks orthologs for CBS and CSE. The 3MST protein contains a rhodanese-like domain and exists in monomeric and dimeric forms. It is catalytically active in its monomeric state and becomes inactive upon transitioning to a dimeric structure at the conclusion of the reaction. Cysteine aminotransferase facilitates the conversion of cysteine and keto acids into 3-mercaptopyruvate, which is subsequently desulfurated by 3MST to generate H_2S.[24]

The gastrointestinal tract is the principal site for H_2S production, predominantly facilitated by the catabolism of cysteine by gut microbiota, including genera such as *Clostridium, Salmonella, Klebsiella*, and *Streptococcus* within the

intestinal lumen. A smaller fraction of H_2S is synthesized by sulfate-reducing bacteria (SRB). The gut environment is characterized by anaerobic conditions, which are conducive to the proliferation of SRB. These bacteria utilize organic or inorganic compounds as electron donors in their metabolic processes, reducing sulfate, which serves as an electron acceptor, to generate H_2S. The primary SRB involved in this process are species from the *Desulfovibrio* and *Vibrio* genera, which are capable of degrading and reducing sulfur-containing compounds such as sulfate, sulfite, and thiosulfate to produce H_2S.[25,26]

In natural environments, bacteria predominantly produce H_2S via two principal pathways. The first involves the reduction of inorganic sulfides; for example, in anaerobic settings such as the deep sea, sulfate-reducing bacteria synthesize H_2S by reducing sulfates, sulfites, and thiosulfates. The second pathway entails the decomposition of organic matter. Patricia Q. Tran et al conducted a screening of bacteria prevalent in natural lakes, identifying *Pseudomonas maltophilia, Bacillus bentonii*, and *Bacillus bifidus* as capable of degrading cysteine to produce H_2S under aerobic conditions.[27]

Importantly, the pathway for H_2S synthesis exhibits variability among different bacterial species, reflecting their specific species characteristics, environmental conditions, and metabolic needs.

Physiological Functions and Antibiotic Resistance of Endogenous H_2S in Bacteria

Antioxidant Stress Effect

Antioxidant and Free Radical Scavenging Activity of Endogenous H_2S

The Fenton reaction involves the generation of hydroxyl radicals (·OH) through the interaction of hydrogen peroxide (H_2O_2) with ferrous iron (Fe^{2+}). ·OH are produced when hydroxide ions lose an electron, resulting in highly oxidative species that can damage DNA, proteins, and lipids.[28] It has been observed that antibiotics can stimulate the production of ·OH in bacteria via the Fenton reaction. Shatalin demonstrated that bacteria can generate endogenous H_2S to mitigate oxidative stress, thereby enhancing resistance to antibiotics. The wild-type *E. coli* degraded H_2O_2 at a rate 1.5 times that of the 3MST-deficient strain, while the overexpressing strain exhibited an even higher degradation rate.[20,29] In a study, *E. coli* was treated with quinolone, β-lactam, and aminoglycoside antibiotics, all of which prompted the production of ·OH in the bacteria. The introduction of 2,2′-bipyridyl, an iron ion chelator, effectively inhibited the Fenton reaction, thereby reducing the production of ·OH and subsequently increasing bacterial survival. Furthermore, the treatment of *E. coli* with thiourea, a potent ·OH scavenger, effectively quenched the ·OH generated by the Fenton reaction, thereby enhancing bacterial survival. The addition of 2,2′-bipyridine or thiourea resulted in a bacterial survival rate that was three times higher than that of the control group. This observation underscores the role of ·OH in the bactericidal action of antibiotics. Additionally, it has been demonstrated that the introduction of H_2S donors markedly diminishes the bactericidal efficacy of antibiotics against *E. coli*. H_2S proved to be as effective as 2,2′-bipyridyl or thiourea in shielding *E. coli* from oxidative stress induced by gentamicin, thereby confirming that H_2S can scavenge ·OH and protect bacteria from oxidative damage (Figure 1).[30]

Effects of Endogenous H_2S on Bacterial Iron Metabolism

Antibiotics generate ·OH mainly through the reaction of Fe^{2+} with H_2O_2 in bacteria, and H_2S can directly scavenge ·OH, but also by regulating bacterial iron metabolism, which in turn reduces ·OH production. Iron-sulfur clusters (ISCs) are highly conserved protein cofactors consisting of proteins, iron, and sulfur, and knockdown of the ISCs gene significantly reduces the iron content in bacteria, and iron release from iron-sulfur clusters mainly relies on superoxide catalytic.[31] The introduction of antibiotics facilitates the conversion of NADH to NAD^+, resulting in the production of substantial quantities of superoxide, which subsequently exerts bactericidal effects.[32]

Ferric uptake regulator (*fur*) is the most important transcriptional regulator of iron metabolism in bacteria, using Fe^{2+} as a co-deterrent to inhibit the synthesis of iron carriers and repress the expression of iron uptake genes to maintain the dynamic balance of Fe^{2+} in bacteria.[33] *Fur* regulates the level of free iron, which in turn determines the antioxidant efficiency of H_2S. *ΔFur* (*fur*-deficient strain) leads to a surge in intracellular free iron and exacerbates oxidative damage. Exposure to H_2O_2 resulted in a 40-fold higher death rate in the *Δfur E. coli* mutant compared to the wild-type strain. In *Δfur* strains, the inhibitory effect of *fur* on iron uptake genes is abolished, resulting in a significant increase in intracellular free Fe^{2+} concentration.

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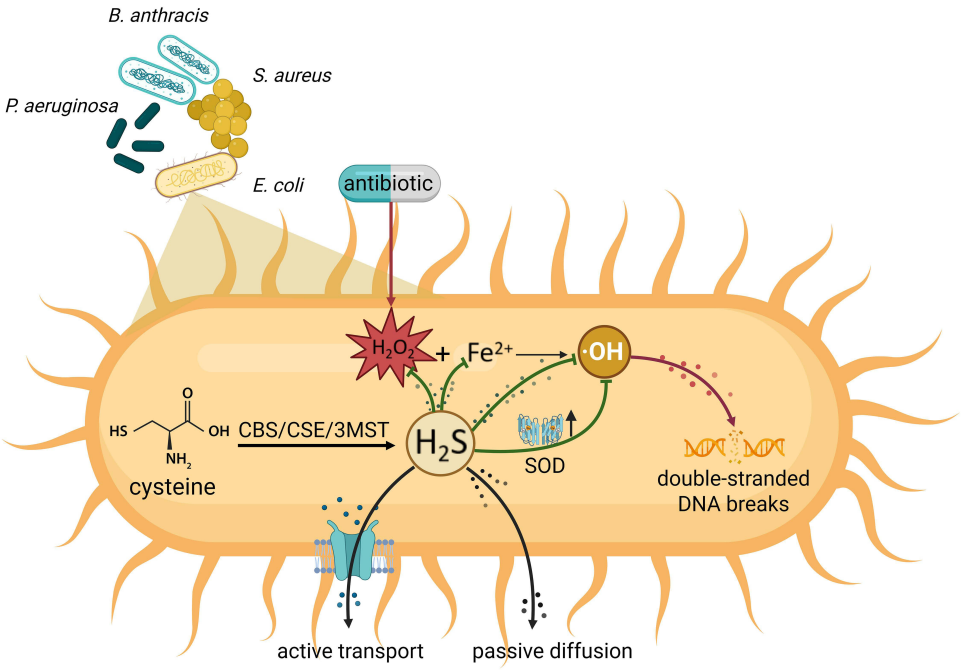

Figure 1 Endogenous hydrogen sulfide (H_2S) augments the bacterial antioxidant stress response and plays a role in the development of antibiotic resistance. Hydrogen peroxide (H_2O_2) facilitates the production of hydroxyl radicals (·OH) within bacteria via the Fenton reaction, which subsequently results in bacterial inhibition or death. In *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus anthracis* (*B. anthracis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and other bacterial species, cysteine metabolism leads to the production of H_2S via the enzymatic activity of cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3MST). H_2S can mitigate oxidative stress through several mechanisms: (1) it directly neutralizes ·OH; (2) it enhances the activity of antioxidant enzymes, such as superoxide dismutase (SOD), which subsequently reduces ·OH levels; and (3) it scavenges H_2O_2 or chelates Fe^{2+}, thereby diminishing ·OH production. ·OH are known to cause double-stranded DNA breaks. H_2S is exported from bacteria via passive diffusion and active transport mechanisms.

A large amount of Fe^{2+} participates in the Fenton reaction, generating more ·OH and drastically raising the risk of DNA damage and cell death. For *Δfur* strains, H_2S produced by 3MST can directly chelate excessive free Fe^{2+} and block the Fenton reaction, thereby restoring the cell's resistance to H_2O_2. This confirms that H_2S exerts a protective effect by chelating free iron. The reaction of L-cysteine with Fe^{3+} generates L-cysteine with Fe^{2+}, which in turn promotes the Fenton reaction. *E. coli* counteracts oxidative stress by chelating free iron using L-cysteine and H_2S.[20,30]

RyhB iron-responsive small regulatory non-coding RNA, first identified in *E. coli* induced by a low iron environment.[34,35] *RyhB* can post-transcriptionally regulate the target of encoded proteins in response to bacterial iron-starvation mRNA for iron storage and use; *ryhB* promotes iron carrier synthesis and uptake. In transcriptomics studies in *E. coli, ryhB* has been shown to directly or indirectly affect the transcription of many genes that are closely linked to Fe-S metabolism and ferritin maturation. *RyhB* is repressed by *fur*, and *Δfur* leads to constitutive iron import and hypersensitivity to oxidative DNA damage. Addition of $FeCl_3$ to *E. coli* resulted in a decrease in H_2S, suggesting that H_2S can directly chelate Fe^{3+}. Addition of H_2O_2 to non-H_2S-producing (*mstA* knockout) and *Δfur E. coli* resulted in lower survival, while *E. coli* overexpressing *mstA* and *Δfur* were almost completely immune to the killing effect of H_2O_2, as *mstA* expression is proportional to the H_2S-producing level, suggesting that endogenous H_2S can directly chelate iron to eliminate H_2O_2-mediated toxicity and iron overloading damage to bacteria.[30] *RyhB* indirectly affects the demand for H_2S by regulating iron metabolism. In *ΔryhB* strains, the intracellular free iron level is slightly higher than that in wild-type strains, and the demand for H_2S to chelate free iron increases accordingly. There is no significant difference in H_2O_2 sensitivity between the *Δfur ΔryhB* double mutant and the *Δfur* strain; however, overexpression of *mstA* restores the sensitivity of both strains. This confirms that the effect of *ryhB* on oxidative sensitivity is ultimately regulated by the chelation of free iron by H_2S. H_2S does not directly regulate the expression of *ryhB*. QRT-PCR detection showed that deletion or overexpression of *mstA* had no significant effect on the transcriptional level of *ryhB*, which further confirms that there is no direct regulatory relationship between the two. Instead, they are indirectly linked through the common "fur-iron" pathway.[30]

Fur and *ryhB* play an important role in regulating adaptive responses during bacterial infection, making them important targets against bacteria. *Fur* acts as an upstream regulator: it regulates the expression of iron uptake genes such as *ryhB* by binding to Fe^{2+}, thereby maintaining intracellular free iron levels. *RyhB* functions as a midstream executor: when *fur* is inactivated, it modulates intracellular iron distribution by degrading the mRNA of iron-consuming genes. H_2S serves as a downstream protector: by chelating the free iron regulated by the *fur/ryhB* pathway, it blocks the Fenton reaction and defends against oxidative damage. By sequencing *E. coli* RNA, H_2S was found to upregulate iron uptake genes and increase iron storage via *YgaV*, which is critical for *E. coli* resistance to oxidative stress and resistance to antibiotics.[6] H_2S levels in *Vibrio cholerae* are positively correlated with iron uptake, which in turn increases iron stores to increase catalase activity and enhance anti-oxidative stress.[36] However, no study has yet demonstrated a direct relationship between H_2S and iron. For example, how H_2S chelates intra-bacterial iron and whether it blocks intra-bacterial metal active sites to reduce antibiotic damage to bacteria.

Cysteine and Endogenous H_2S

Cysteine, a sulfur-containing amino acid, serves as a crucial substrate for H_2S production. *CysB*, a member of the *LysR* family of prokaryotic transcriptional regulatory proteins, plays a significant role in regulating sulfur metabolism across diverse bacterial species. It enhances the expression of genes involved in sulfate metabolism and cysteine synthesis while also monitoring endogenous L-cysteine levels.[37,38] Elevated concentrations of L-cysteine can be toxic to bacteria and facilitate the Fenton reaction. Reduced cysteine levels have been shown to increase antibiotic resistance in *E. coli* by modulating intracellular ROS levels. This modulation occurs through the influence of endogenous cysteine on the Fenton reaction, thereby impacting bacterial resistance.

Endogenous H_2S Enables Enzymes Activity

Antioxidant enzymes (such as superoxide dismutase (SOD), catalase, and glutathione peroxidase) can scavenge ROS induced by antibiotics, alleviate oxidative damage, and protect bacteria from oxidative stress mediated by antibiotics—thereby maintaining the integrity of bacterial physiological functions. This process indirectly enhances the survival ability of bacteria under antibiotic pressure, ultimately manifesting as increased bacterial resistance.[39] Endogenous H_2S has the capability to scavenge ·OH by augmenting the activities of catalase and SOD. Research indicates that H_2S can directly inhibit heme-containing catalase and enhance the bactericidal effect of H_2O_2. However, this inhibition is both transient and immediate. In contrast, H_2S functions as a long-term signaling molecule, promoting the expression of systems involved in the scavenging and repair of H_2O_2, thereby protecting bacteria from oxidative stress.[40] In wild-type *E. coli* stimulated by H_2O_2, the expression of SOD is more than 1.5 times greater compared to 3MST type *E. coli*.

The overexpression of 3MST in *E. coli* results in elevated SOD activity, with a direct proportionality observed between SOD activity and 3MST expression, thereby demonstrating that endogenous H_2S can enhance antioxidant enzyme activity.[20] In *Vibrio cholerae*, H_2S production is primarily catalyzed by CBS-mediated conversion of L-cysteine. This process reduces free iron through the activation of iron uptake and storage mechanisms and enhances peroxidase activity at the post-translational level, thereby augmenting the organism's resistance to oxidative stress.[36] Antioxidant enzymes can help bacteria resist the bactericidal mechanism of antibiotics through multi-level synergistic effects, including scavenging ROS, maintaining redox balance, and promoting damage repair, ultimately serving as a crucial system that supports bacterial antibiotic resistance.

Anti-DNA Damage Effects of Endogenous H_2S

Antibiotics facilitate the Fenton reaction, resulting in the formation of peroxides that induce double-stranded DNA breaks in bacteria. This DNA damage activates the SOS response, a repair mechanism triggered by bacterial DNA damage. The SOS response primarily involves homologous recombination, nucleotide excision repair, and transdamage synthesis. Initially, it was believed that the SOS response was primarily activated by abnormal single-stranded DNA; however, subsequent research has demonstrated that double-stranded DNA breaks also elicit this response (Figure 1).[41]

Among the mechanisms involved, *recA* expression plays a pivotal role in the repair of DNA double-strand breaks, primarily through the process of homologous recombination repair, which is facilitated by catalytic strand exchange and

invasion of homologous double-stranded DNA.[42] The introduction of antibiotics or H_2O_2 induces DNA double-strand breaks in bacteria. In contrast, the overexpression of 3MST or the administration of H_2S donors has been shown to inhibit DNA breaks in *E. coli*.[20]

Effect of Endogenous H_2S on Bacterial Growth

Persisters constitute subpopulations of dormant bacteria that endure exposure to lethal concentrations of antibiotics.[43] These cells exhibit slow growth or stagnation and demonstrate a transient yet high level of antibiotic resistance. Upon the removal of antibiotic stress, persisters revert to their normal bacterial phenotype, resuming growth and proliferation. They play a crucial role in the persistence of bacterial infections and significantly contribute to the development and regeneration of antibiotic-resistant bacteria during infection treatment.[44]

In 2021, Shatalin and his research team found that the sole condition of CSE deletion is sufficient to render *S. aureus* and *P. aeruginosa* sensitive to low doses of antibiotics from different classes, including gentamicin (an aminoglycoside), norfloxacin (a quinolone), and ampicillin (a β-lactam). The survival rate of CSE-deficient *S. aureus* and *P. aeruginosa* was significantly diminished following antibiotic treatment compared to wild-type strains.[45] *P. aeruginosa* exhibited a notably lower survival rate of persister cells post-antibiotic administration relative to wild-type strains, indicating that endogenous H_2S promotes the formation of persister bacterial populations. The surviving persister cells produce more H_2S than typical colonies, and elevated H_2S levels inhibit the tricarboxylic acid cycle, thereby reducing bacterial metabolism and enhancing antibiotic efficacy. Persistent bacteria are not antibiotic-resistant bacteria themselves, but their "phenotypic tolerance" trait serves as a key catalyst for the emergence of antibiotic-resistant bacteria. By providing a survival window for antibiotic-resistant mutant strains and surviving synergistically with antibiotic-resistant bacteria, persistent bacteria indirectly promote the development and spread of antibiotic resistance.

Effect of Endogenous H_2S on Bacterial Biofilm Formation

Biofilms constitute complex communities of microorganisms that adhere to either biotic or abiotic surfaces, exhibiting significantly enhanced resistance to antibiotics relative to their planktonic counterparts.[46] Notably, Gram-negative bacteria demonstrate a pronounced propensity for biofilm formation. For instance, *P. aeruginosa* is known to colonize both the human body and medical devices by forming a biofilm characterized by an asymmetric bilayer composed of phospholipids and lipopolysaccharides. This bilayer functions as a selective barrier, effectively impeding the penetration of antibiotics. The bacterial membrane is characterized by the presence of β-barrel protein channels, with *OprF* serving as the predominant pore protein that facilitates the non-specific uptake of ions and sugars, thereby impeding antibiotic penetration. Additionally, the membrane contains other specialized pore proteins, including *OprD*, which is specific for basic amino acids, *OprB*, which is specific for carbohydrates, and *OprP*, which is specific for phosphates. The formation of biofilms further complicates antibiotic treatment, resulting in infections that are challenging to manage and may lead to recurrent episodes.[47]

H_2S is integral to biofilm formation, as it facilitates the synthesis and stabilization of the biofilm matrix, thereby enhancing bacterial viability. In *P. aeruginosa*, the CSE leads to a reduction in biofilm formation, with a concomitant down-regulation of biofilm-associated genes, particularly those involved in the biosynthesis of alginate and other exopolysaccharides, as revealed by transcriptomic analyses.[45] Moreover, endogenous H_2S exerts a positive influence on the establishment of microbiota biofilms. In rodent models of colitis, the administration of H_2S donors has been shown to mitigate inflammation and restore microbiota biofilms.[48]

Effect of Endogenous H_2S on Antibiotic

CBS, CSE, and 3MST are critical enzymes involved in the biosynthesis of H_2S in bacteria. Inhibition of these enzymes has been shown to increase bacterial susceptibility to antibiotics in species such as *B. anthracis, S. aureus, P. aeruginosa*, and *E. coli*. Notably, *E. coli* deficient in 3MST, as well as *P. aeruginosa* and *S. aureus* deficient in CBS/CSE, did not exhibit significant growth defects. However, these H_2S enzyme-deficient strains demonstrated heightened antibiotic sensitivity compared to their wild-type counterparts. Furthermore, the introduction of H_2S donors mitigated the bactericidal effects of antibiotics on the enzyme-deficient strains, indicating that endogenous H_2S plays a role in enhancing antibiotic resistance to antibiotics.[20]

Additionally, studies on *Mycobacterium tuberculosis* have reported enhanced hypoxic survival in recombinant bacteria through increased H_2S production. In this context, *AlaE*, a cysteine efflux pump, has demonstrated significant cytoprotective effects.[49]

Methods for the Detection of H_2S in Bacteria

The detection of specific enzymes or metabolites in bacteria represents a viable approach for evaluating antibiotic resistance. Within the antibiotic metabolic pathways of bacteria, H_2S emerges as a notable antibiotic metabolite resulting from biodegradation. Consequently, methodologies for detecting H_2S-associated antibiotic-resistant bacteria are continually advancing. Additionally, novel detection methodologies, such as the fluorescent probe technique, employ fluorescent probes with specific affinities for H_2S or antibiotic-resistant bacteria, allowing for the rapid and sensitive detection of H_2S-associated antibiotic-resistant bacteria. This approach yields results in a relatively short timeframe and permits in situ detection, thereby offering a more convenient method for clinical diagnosis. H_2S, an emerging metabolic marker for β-lactam antibiotics, can be utilized for the screening of antibiotic resistance.[50]

Nanoprobes that selectively monitor fluctuations in H_2S concentrations for the imaging and screening of H_2S-associated antibiotic resistance represent a promising diagnostic approach for differentiating between β-lactam antibiotic-resistant *S. aureus* and non-resistant strains. This technique also facilitates the exploration of novel diagnostic strategies aimed at identifying H_2S-associated resistance pathways.[51] Furthermore, advancements in biosensor technology are increasingly being utilized for the detection of these bacteria. By integrating biometric elements with signal transduction components, these biosensors enable real-time and rapid detection of bacteria and their associated markers.

Detection of Antibiotic-Resistant Bacteria Associated with H_2S

Detection of specific enzymes or metabolites in bacteria can be one of the methods to assess antibiotic resistance, and H_2S is one of the antibiotic metabolites produced by biodegradation in the antibiotic metabolism pathway of bacteria, and the detection methods of antibiotic-resistant bacteria associated with H_2S are constantly evolving. The traditional culture method determines antibiotic resistance by culturing the bacteria on a specific medium, observing their growth and H_2S production characteristics, and combining it with antibiotic susceptibility testing, but the method is time-consuming and usually takes 2–3 days. Molecular biology-based detection methods, such as PCR technology, can rapidly detect genes related to H_2S production and antibiotic resistance in bacteria with high sensitivity and specificity. For example, by designing specific primers, fragments of genes related to H_2S synthase genes or antibiotic resistance genes can be amplified, so as to determine whether the bacteria are antibiotic-resistant bacteria associated with H_2S. Some new detection techniques such as fluorescent probe method, using fluorescent probes with specific recognition of H_2S or antibiotic-resistant bacteria, can realize rapid and sensitive detection of antibiotic-resistant bacteria associated with H_2S, and the results can be obtained in a shorter period of time and can be detected in situ, which provides a more convenient means of clinical diagnosis. Gholap S P proposed that H_2S, an emerging metabolic marker associated with β-lactam antibiotics, can serve as a tool to screen for bacterial antibiotic resistance.[50] Nanoprobes selectively monitoring changes in H_2S concentration for imaging and screening of antibiotic resistance can be used as a specific diagnostic technique for screening β-lactam antibiotic-resistant *S. aureus* and non-resistant *S. aureus*, and exploring new diagnostic strategies for the identification of H_2S-associated resistance pathways.[51] In addition, biosensor technology is gradually being applied to the detection of such bacteria, which allows real-time and rapid detection of bacteria and related markers by combining biometric elements with signal conversion elements.

Although the current research on the application of H_2S markers in the diagnosis of bacterial antibiotic is relatively limited, this H_2S-based diagnostic method has certain advantages, such as relatively simple detection, and can directly reflect the antibiotic resistance to specific antibiotics. With further research, it is expected to further expand the application of H_2S markers in the diagnosis of more types of bacterial antibiotic and improve the accuracy and efficiency of diagnosis.

Potential Applications of H_2S in the Treatment of Antibiotic Resistance

Development of H_2S Synthase Inhibitors

H_2S exhibits potential applications in the treatment of bacterial antibiotic resistance. In particular, the inhibition of H_2S production or activity may serve as a therapeutic strategy in instances where endogenous H_2S production contributes to antibiotic resistance against antibiotics. This approach involves the development of inhibitors targeting H_2S-producing enzymes, thereby compromising the antibiotic resistance mechanisms and enhancing antibiotic efficacy. In 2021, Konstantin Shatalin employed a virtual screening method to identify compounds capable of specifically binding to *S. aureus* cystathionine-γ-lyase (SaCSE), utilizing the X-ray crystallographic structure of SaCSE as a basis for this identification. Subsequent to this, both in *vitro* and in *vivo* assays were conducted to evaluate various functionalities, ultimately leading to the identification of three compounds capable of inhibiting H_2S-producing enzymes. These compounds, when combined with antibiotic-enzyme inhibitors, have the potential to reduce treatment failure in acute infections, decrease colonization, prevent conversion to chronicity and relapse, shorten the duration of treatment, and mitigate the risk of antibiotic resistance emergence or spread.[45] In contrast, for bacteria that do not naturally produce H_2S, such as *Acinetobacter baumannii*, the external supplementation of H_2S can impart resistance to a wide range of antibiotics. This observation indicates a potential novel strategy for managing infections caused by these bacterial strains.[52]

Clearance of Endogenous H_2S to Disrupt Bacterial Biofilm Formation

In contrast to the inhibition of H_2S synthase at its source, direct scavenging of H_2S has been explored through the development of various materials aimed at reducing H_2S levels within bacteria. Wei Zhang, following chemical and structural optimization, designed compound 7b based on nitrobenzofurane scaffolds, which can accurately identify and scavenge H_2S. This compound enhances the bactericidal capabilities of macrophages and neutrophils, thereby inhibiting and eradicating bacterial biofilm formation. Furthermore, it significantly amplifies the antimicrobial efficacy of gentamicin in models of *P. aeruginosa* induced pneumonia and skin wounds.[53] Metal-Organic Frameworks, a class of porous materials composed of inorganic metal ions or clusters as central nodes and organic ligands, form a crystalline network through coordination bonds. These materials possess advantageous physicochemical properties, such as high specific surface area, adjustable pore size, high porosity, and excellent biocompatibility, which facilitate efficient drug loading, targeted delivery, and controlled release, making them highly promising for biomedical applications.[54]

The metal-organic framework zirconium (IV) terephthalate (UiO-66-MA), subsequently loaded with gentamicin to form UiO-66-MA@Gm, serves as a low-toxicity, structurally stable, and high-quality drug delivery carrier. It effectively removes H_2S produced by bacteria and enhances the sensitivity of antibiotics. This methodology offers innovative perspectives for the design of novel antibacterial materials. Weizhong Yang developed an "enzyme-mimicking bioheterojunction", primarily composed of $CuFe_2S_3$ and lactate oxidase. $CuFe_2S_3$ facilitates the production of potent bactericidal ·OH from H_2O_2 and depletes antioxidant substances within bacteria, such as glutathione, thereby disrupting bacterial metabolism. Bacterial infections and lactic acid accumulation create a localized acidic environment in wounds, prompting the material to release H_2S. As a reducing gas, H_2S can reduce metal ions from a high valence state (eg Fe^{3+}, Cu^{2+}) to a low valence state (eg Fe^{2+}, Cu^{+}), thereby continuously generating ROS and accelerating the valence cycle of metal ions. This cycling enables the material to persistently produce ROS, such as ·OH, thereby enhancing its antibacterial efficacy.[55]

Conclusions and Future Prospects

H_2S, produced by bacteria through enzymes like CBS, CSE, and 3MST, functions as a defense mechanism that significantly enhances bacterial antibiotic resistance. Antibiotics induce bacteria to produce ·OH, which kill bacteria through oxidative stress. Endogenously produced H_2S in bacteria counteracts this oxidative stress by scavenging ·OH, chelating Fe^{2+}, and enhancing antioxidant enzyme activity. Moreover, H_2S can attenuate antibiotic-induced DNA damage in bacteria, facilitating the formation of persister bacterial populations. It also promotes biofilm formation, enhances biofilm stability, and contributes to bacterial colonization.

H_2S presents significant potential for advancements in bacterial antibiotic research. In the realm of diagnostics, it is anticipated that assays based on H_2S will undergo further optimization and expansion. This includes enhancing current H_2

S detection technologies to improve their sensitivity and specificity, thereby enabling more accurate diagnosis of bacterial antibiotic resistance. Concurrently, research is being conducted to explore the feasibility of utilizing H_2S as a biomarker for a broader range of bacterial antibiotics, with the aim of extending its application in clinical diagnostic settings.[50] From a therapeutic perspective, comprehensive research into the optimal combination of H_2S and antibiotics, including the determination of the appropriate H_2S dosage, timing, and modality of combination, may yield more effective therapeutic outcomes. Furthermore, elucidating the mechanisms by which H_2S modulates the expression of bacterial drug-resistance genes could facilitate the development of novel therapeutic strategies centered on H_2S regulation, such as reversing bacterial antibiotic resistance through the modulation of H_2S-related signaling pathways. Concurrently, exploring the impact of H_2S on bacterial biofilms presents a promising avenue for developing innovative approaches to disrupt biofilms and enhance the efficacy of antibiotics against antibiotic-resistant bacteria within these biofilms.

Abbreviations

H_2S, hydrogen sulfide; AMR, Antimicrobial Resistance; CSE, cystathionine-γ-lyase; CBS, cystathionine-β-synthase; 3MST, 3-mercaptopyruvate sulfurtransferase; ROS, reactive oxygen species; *E. coli, Escherichia coli; B. anthraci, Bacillus anthracis; P. aeruginosa, Pseudomonas aeruginosa; S. aureus, Staphylococcus aureus*; SRB, sulphate-reducing bacteria; DSV, *Desulfovibrio bacteria*; OH, hydroxyl radicals; H_2O_2, hydrogen peroxide; Fe^{2+}, ferrous iron; ISCs, iron-sulfur clusters; *fur*, ferric uptake regulator; SaCSE, *S. aureus* CSE; UiO-66-MA, metal-organic framework zirconium (IV) terephthalate.

Author Contributions

All authors made a significant contribution to the work reported, including conception, drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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