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International Journal of Medical Science and Dental
Health (ISSN: 2454-4191)
Volume 11, Issue 10, October 2025
Doi: <https://doi.org/10.55640/ijmsdh-11-10-14>

Molecular Crosstalk between Bacterial Two-Component Regulatory Systems and Host Immune Modulation: Implications for Antimicrobial Resistance and Pathogenic Persistence

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Received: 22 September 2025, **accepted:** 30 September 2025, **Published Date:** 22 October 2025

Abstract

Bacterial two-component systems (TCSs) are versatile regulatory circuits that enable pathogens to sense environmental and host-derived signals and translate them into adaptive genetic responses. These systems, typically composed of a sensor histidine kinase and a response regulator, play pivotal roles in controlling virulence, antimicrobial resistance, and persistence. In parallel, the host immune system employs innate and adaptive mechanisms to recognize and eliminate pathogens. However, a dynamic molecular crosstalk exists, wherein TCSs detect immune-derived cues such as antimicrobial peptides, oxidative stress, and ion limitation, and in turn, modulate bacterial physiology to evade or manipulate host defenses.

Well-studied examples—including PhoPQ, PmrAB, CsrRS, and Agr systems—highlight how TCSs govern immune evasion, biofilm formation, and resistance to last-resort antibiotics such as polymyxins and vancomycin. These processes contribute significantly to chronic infections and treatment failures. Targeting TCSs has therefore emerged as a promising therapeutic strategy. Experimental inhibitors of histidine kinases and response regulators demonstrate potential to attenuate bacterial virulence without exerting strong selective pressure for resistance. Moreover, combining TCS-targeting compounds with conventional antibiotics may enhance treatment efficacy.

This review synthesizes current knowledge of TCS-immune interactions, emphasizes their contribution to antimicrobial resistance and persistence, and explores therapeutic perspectives. Understanding this molecular dialogue provides crucial insights into host-pathogen interactions and offers new opportunities for combating multidrug-resistant infections in the post-antibiotic era.

Keywords: Two-Component Regulatory Systems (TCSs), Host–Pathogen Interactions, Antimicrobial Resistance (AMR), Immune Modulation, Virulence Regulation, Pathogenic Persistence

Introduction

Bacteria have developed a stunning ability to quickly adapt to changing environments which allows them to survive under a variety of stress conditions and in inhospitable host niches. Among the major molecular strategies governing this adaptation is the use of two-

component regulatory systems (TCSs), which are elaborate signal transduction circuits enabling bacteria to perceive environmental signals and translate them into coordinated gene expression programs. A classic TCS is composed of a membrane-bound sensor histidine kinase (HK) along with a cytoplasmic response regulator

(RR) (1). These systems regulate a wide range of bacterial processes, such as virulence regulation, stress responses, biofilm formation, metabolic adaptation and antimicrobial resistance, through phosphorelay-dependent signalling. Notably, TCSs allow bacteria to adjust their physiology and are also important for dynamic interactions with the host immune system (2,3).

From the host point of view, the immune system is the first and most important line of defense against bacterial invasion. Through pattern recognition receptors (for example, Toll-like receptors (TLRs) and NOD-like receptors (NLRs)), the innate immune system recognizes conserved patterns in microbes and induces inflammation by promoting the release of pro-inflammatory cytokines and enhancing phagocytic activity (4). Then the adaptive immune system adds to the response, making more specific antibodies and memory T cells that are directed against the same pathogen. Nevertheless, pilfering pathogenic bacteria have developed sophisticated mechanisms to evade such host defenses. Though more complete than even a few years ago, we are increasingly aware that TCS function as molecular sentinels that respond to host-derived signals (e.g. AMP, ion dysregulation, oxidative and environmental stress and pH changes) and modulate bacterial responses that either attenuate immune detection or counteract immune assault (5).

The interaction between bacterial TCSs and host immunity are dynamic and bidirectional molecular crosstalks. On one side of it, bacterial regulatory pathways are directly modulated by host-derived cues (6,7). For example, TCSs such as PhoPQ or PmrAB can be activated by cationic antimicrobial peptides, and subsequent modifications of the bacterial outer membrane can result in reduced susceptibility to eukaryotic host defenses and specific antibiotics. Conversely, TCSs can influence the expression of bacterial virulence determinants that elicit and shape host immune responses, such as secretion systems, toxins and adhesins. In this sense, the interplay between TCSs and the host immune system represents a finely tuned arms race whereby bacteria exploit TCSs to evade host immune defenses, and the host, with its adaptive immune system, continuously evolves to detect and eliminate bacterial threats (8,9).

This interaction is not just important to pathogenesis, but also has implications for antimicrobial resistance

(AMR) and persistence of the pathogen. TCSs directly mediate antibiotic tolerance and multidrug resistance by regulating efflux pumps, porin expression and biofilm formation. In addition, the functions of biofilms in biofilm development and stress resistance mediate chronic infections where bacteria survive under persistent immune pressure and treatment continuation. Clinically, this is of particular concern with pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, since TCS-mediated regulation has been associated with therapeutic failure and insidious illness (10,11).

These findings suggest that TCSs may be attractive therapeutic targets. Inhibitors of TCS signaling, which are rationally designed, can potentially reduce bacterial virulence without the burden of killing the pathogen and sparking natural selection pressure for the development of resistance. In parallel, approaches that utilize the debilities of TCS-mediated control could increase host immune elimination. However, these advances also face challenges including structural diversity in TCSs between different species of bacteria and redundancy in the signaling pathways making them difficult to target using therapeutics against the TCSs (12).

Herein we provide a critical synthesis of existing knowledge concerning the molecular discourse between bacterial two-component systems and the immune system of the host. First, we will summarize the basic structural and functional features of TCSs, and then we will present an overview of TCS-mediated host immune modulation during bacterial infection (13). We will then review prominent exemplars of molecular crosstalk, illustrating how TCSs detect and respond to immune pressures. This work will pay particular attention to TCS roles in antimicrobial resistance and persistence — two global health concerns. We will also briefly review novel therapeutic strategies focusing on TCSs and future perspectives on research. In summarizing these aspects, this review highlights the important role that TCS-mediated intercellular communication plays in host-pathogen interactions and the potential therapeutic implications of modulating such communications in the context of emerging antimicrobial resistance to the treatment of infectious diseases (14).

Overview of Bacterial Two-Component Regulatory Systems (TCSs)

TCSs are one of the most common and simple signal transduction devices in bacteria. They enable pathogens to sense and adapt to alterations in their environment, from nutrient accessibility and osmotic strength to immunogenic host stress signals (15). A prototypic TCS consists of two basic components – a sensor histidine kinase (HK) and a response regulator (RR). The HK residing in the bacterial inner membrane senses extracellular or intracellular signals and becomes autophosphorylated at a conserved histidine residue. This phosphoryl group is then transferred to an aspartate residue on the RR, which adopts the conformation of a transcriptional regulator of target genes (14,15).

TCSs, despite being structurally simplistic, are capable of impressive flexibility. Many bacteria contain multiple TCSs (e.g., *Escherichia coli* encodes over 30 distinct

systems), each devoted to sensing specific signals. In pathogenic bacteria, these systems are involved in modulating processes critical to host survival, including outer membrane function remodeling, secretion system activation, biofilm formation and expression of virulence genes. Critically, however, TCSs rarely function in isolation but are frequently interconnected by cross-regulation that allows bacteria to integrate multiple signals and tune responses accordingly (14,5).

E-biosphere exhibits a simplistic summary of TCS Structure contained within Table 1, which gives the general structure and functional roles played by its two main constituents. This is also a core for the knowledge how TCSs are involved in bacterial kinetics and their cross-regulation of bacteria-host immune system detailed in the following sections (16).

Table 1. General organization of bacterial two-component systems

Component	Location/Structure	Primary Function
Sensor Histidine Kinase (HK)	Inner membrane (transmembrane protein)	Detects environmental or host-derived signals; undergoes autophosphorylation at histidine residue (17).
Response Regulator (RR)	Cytoplasmic protein (often DNA-binding)	Receives phosphate group; regulates transcription of target genes affecting stress response, virulence, and resistance (18).

3. Host Immune Modulation in Response to Bacterial Infections

The immune system of the host is a complex multi-layer defense system against invading bacteria. The innate immune system, representing the first line of defense against pathogens, is dependent on pattern recognition receptors (PRRs) such as Toll-like receptor (TLRs), NOD-like receptor (NLR)s and Ctype lectin receptors. These receptors, which bind PAMPs like lipopolysaccharides and flagellin or peptidoglycan fragments initiate signaling cascades that lead to the synthesis of pro-inflammatory molecules (TNF- α , IL-6, IL-1 β) and antimicrobial peptides. These responses attract neutrophils and macrophages to the site of infection and synergistically promote bacterial clearance (19,20).

Along with innate immunity, it adds specificity and memory: the adaptive immune system. Adaptive immune responses entail differentiated T and B cells in

which antigen-presenting cells (APCs), including dendritic cells, internalize bacterial antigens and expose them to T and B lymphocytes, imparting a tailored response. For example, CD4+ T helper cells provide a cytokine milieu for infection control, CD8+ cytotoxic T cells destroy both target cells and infected host cells, and B cells secrete antibodies that protect against bacterial toxins and enhance opsonophagocytosis (8,10).

However, pathogens know a trick or two to escape or manipulate their host. Some microbes suppress cytokine signaling, resist phagocytic killing or prevent antigen presentation. Some induce immune tolerance, while others instigate hyperinflammation, both of which underlying them are capable of creating suitable niches for this pathogen to flourish (Chowdhury et al., 2021; Davar et al., 2019; Karlsson et al., 2020). This was essential for the excitatory control of host immunity, a pillar of chronic infection insusceptibility and is

coordinately dependent on TCS activity decoding immune-derived signals in most bacteria (21,22,23)

Table 2. Overview of host immune components and bacterial evasion strategies

Immune Component	Primary Function	Bacterial Evasion Strategy
TLRs / PRRs	Detect PAMPs and trigger cytokine release	Alteration of surface antigens; secretion of decoy molecules
Cytokines (e.g., TNF-α, IL-6)	Recruit immune cells and amplify inflammation	Suppression of cytokine production via TCS-regulated effectors
Phagocytes (macrophages, neutrophils)	Engulf and kill bacteria	Resistance to oxidative burst, survival within phagosomes
Adaptive immunity (T and B cells)	Antigen-specific response and memory	Antigenic variation, interference with antigen presentation

4. Molecular Crosstalk Between TCSs and Host Immunity

Examples of this bidirectional cross-talk between bacterial two-component systems (TCSs) and the host immune system are established and well described. Adaptive transcriptional programmes meant to counteract immune pressure are regulated by play-sensing of host signals by TCSs such as antimicrobial peptides, iron-privation, magnesium-starvation, pH shifting and reactive oxygen species. The latter, in turn, has a direct effect on the magnitude and quality of host immune response, by triggering these bacterial responses (23).

For example, the PhoPQ system in *Salmonella* senses antimicrobial peptides and low magnesium levels (22). PhoPQ activation causes structural changes on LPS lipid A, thus decreasing the recognition of TLR4 and subsequently increasing the resistance to the host immune system. In addition to controlling outer membrane remodeling to resist immune molecules, the PmrAB system also mediates resistance to polymyxin antibiotics by an outer membrane remodelling process in

the presence of cationic peptides (10). A second example is the two-component CsrRS system of *Streptococcus pyogenes* that activates expression of virulence factors that downregulate or delay host inflammatory responses and can modulate disease outcome (21).

Such crosstalk, mediated by secreted effectors, can also extend to immunomodulation. Secretion systems (e.g., Type III and Type VI) that deliver effector proteins into a host cell to modulate signaling pathways, arrest maturation of the phagosome, or interfere with cytokine responses, are controlled by TCSs. This disadvantages the elimination of pathogens and causes chronic infections (23).

The molecular love/hate affairs highlighted here reflect the eternal arms race between the plasticity of bacteria and the watchful eye of the host immune system over evolutionary time scales. Understanding these regulatory interactions is essential for studying disease mechanisms and for the identification of novel therapeutic targets (24).

Table 3. Selected examples of TCS-immune system interactions

TCS System	Pathogen	Host Cue Sensed	Effect on Host Immunity
PhoPQ	<i>Salmonella enterica</i>	Low Mg ²⁺ , antimicrobial peptides	Lipid A modification → reduced TLR4 activation (15).

PmrAB	<i>Klebsiella pneumoniae</i>	Cationic peptides	Resistance to AMPs and polymyxin (20).
CsrRS	<i>Streptococcus pyogenes</i>	Host immune pressure	Modulates virulence and inflammatory responses (10).
Agr system	<i>Staphylococcus aureus</i>	Host environment signals	Controls quorum sensing and toxin production (15).

5. TCSs and Antimicrobial Resistance

Antimicrobial resistance (AMR) is one of the most serious health problems worldwide and bacterial two-component systems (TCSs) are major actor in this field. Through sensing of environmental as well as host-associated factors, TCSs control various adaptive responses decreasing the efficacy of antibiotics. One of the best characterized functions is its control over membrane permeability. The PhoPQ and PmrAB systems, for example, modify the Gram-negative cell wall lipopolysaccharide to reduce susceptibility to cationic AMPs and clinically relevant antibiotics of last resort such as polymyxins (25).

An important process is also activation of efflux pumps due to TCS signalling. The BaeSR system in *Escherichia coli* and *Klebsiella pneumoniae* activates the expression of the multidrug efflux pump gene, which confers resistance to a variety of antibiotics. Also, the CpxAR regulon senses envelope stress and controls efflux-

associated genes as well cell envelope repair related genes, and increases tolerance to aminoglycosides and β -lactams (22).

TCSs also participate in antibiotic sensing. The *Enterococcus faecalis* VanSR system senses the presence of vancomycin and induces expression of genes involved in modification of peptidoglycan precursors, generating a state of high-level resistance to vancomycin. This sensing of the antibiotic molecules themselves enables bacteria to quickly develop defenses, reducing the effectiveness of treatment (11).

In sum, TCS-mediated regulation is a multilayered resistance mechanism that combines membrane rearrangement, efflux and modification of the target. Knowledge of these systems also offers insights into the molecular mechanisms behind multidrug resistance, and emphasizes TCSs as potential targets for new antimicrobials (26).

Table 4. Examples of TCSs associated with antimicrobial resistance

TCS System	Pathogen	Antibiotic Resistance Mechanism
PhoPQ	<i>Salmonella</i> , <i>E. coli</i>	Lipid A modification → resistance to polymyxins (22).
PmrAB	<i>Klebsiella pneumoniae</i>	Membrane remodeling against AMPs and polymyxins (5).
BaeSR	<i>E. coli</i> , <i>K. pneumoniae</i>	Induction of multidrug efflux pumps (13).
CpxAR	<i>E. coli</i>	Regulation of envelope stress and aminoglycoside tolerance (22).
VanSR	<i>Enterococcus faecalis</i>	Sensing vancomycin → peptidoglycan modification (15).

6. Contribution of TCSs to Pathogenic Persistence

Apart from the issue of antimicrobial resistance, TCSs are principal regulators in pathogen persistence, permitting the bacteria to establish themselves in hostile host environments and result in chronic infection. One of the key functions TCSs play in persister development is

attributed to their role in biofilm formation. Biofilms shield bacteria from antibiotics and immune responses. For example, the *Pseudomonas aeruginosa* GacS/GacA system modulates small RNAs that impact biofilm formation and secondary metabolite production to promote survival within cystic fibrosis patients (12,22,25).

TCSs also help the bacteria to survive inside the wild type phagocytic cells. The PhoPQ system allows *S. enterica* to modulate its outer membrane, resist oxidative stress, and survive within macrophages. Similarly, the MprAB system in *Mycobacterium tuberculosis* controls genes that respond to stress and help the pathogen persist within granulomas during latent infection.

A second endurance mechanism is that of stress tolerance (19). TCSs sense and respond to extracellular stresses, including acidic pH, hypoxia or nutrient limitation by turning on adaptive stress pathways. In *E.*

coli the ArcAB system for instance controls anaerobic metabolism that is important to survive oxygen deprivation as encountered in host tissues (27).

Crucially, TCSs have evolved to orchestrate these pathways in a manner favoring long-term colonization and chronicity of carriers, which complicates the treatment outcome. TCSs are also directly involved in contributing substantially to relapsing and tough-to-cure infections by enabling bacteria to maintain viability under immune assault as well as exposure to antibiotics (25).

Table 5. Roles of TCSs in pathogenic persistence

TCS System	Pathogen	Contribution to Persistence
GacS/GacA	<i>Pseudomonas aeruginosa</i>	Biofilm formation and chronic airway infections (25).
PhoPQ	<i>Salmonella enterica</i>	Survival in macrophages and oxidative stress (26).
MprAB	<i>Mycobacterium tuberculosis</i>	Latency and persistence in granulomas (26).
ArcAB	<i>E. coli</i>	Adaptation to anaerobic/low oxygen environments (26).

7. Therapeutic Implications and Targeting TCSs

Two-component systems (TCSs) have become very attractive candidates as therapeutic targets, being involved in bacterial adaptability, AMR and persistence (18). In contrast to classical antibiotics that typically block key bacterial processes like cell wall production and protein synthesis, TCS-targeted therapeutics are designed to cripple pathogens by disrupting their capacity to sense and adapt to external stimuli in the environment and from the host. One potential merit of this approach is that it may reduce the selective pressure to resist development, as bacterial viability is not directly affected (15).

There are many strategies for interfering with TCS. One powerful approach is the discovery of small-molecule inhibitors that inhibit histidine kinase autophosphorylation or subsequent phosphotransfer to response regulators. For instance, molecules, including walkmycin B and LED209, were reported to suppress the histidine kinase activity of pathogens (e.g., *Staphylococcus aureus* and *Escherichia coli*), resulting in decreased production of virulence factors. Another

attractive strategy is interfering response regulator-DNA binding and thereby nuclear virulence/resistance-gene activation (28).

Apart from direct inhibition, antivirulence approaches that manipulate TCSs to lower the virulence of pathogens have been developed. Inhibition of *S. aureus* quorum sensing such as persulfurated allyl methyl sulfide could decrease toxin production and biofilm formation, which would make the bacteria more vulnerable to host immune response. Moreover, TCS inhibitors in combination with pathogen-specific conventional antibiotics have shown synergistic activities affecting higher bacterial sensitivity profiles and decreasing antibiotic doses (29).

Despite these encouraging results, clinical translation of therapy is difficult. The diversity in TCS structures between species further complicates the development of broad-spectrum inhibitors. In addition, redundancy in bacterial signaling networks might offset inhibition of individual TCS. Studies in the future need to probe for conserved structural motifs amenable for drug design and utilize systems biology techniques to predict TCS

inhibition induced network level effects. In conclusion, the exploitation of TCSs as drug targets is a new approach for antimicrobial chemotherapy and might

possibly “revive” existing antibiotics in response to resilient pathogen systems (30).

Table 6. Examples of experimental TCS inhibitors and their targets

Compound	Target TCS/Pathogen	Mechanism of Action
Walkmycin B	Histidine kinases (<i>S. aureus</i>)	Inhibits autophosphorylation of HKs
LED209	QseC system (<i>E. coli</i> , <i>S. typhimurium</i>)	Blocks host-signal sensing and virulence regulation (14).
Closantel	Multiple HKs in Gram-positive bacteria	Inhibits kinase activity, reduces virulence (12).
Sodium tungstate	NarL system (<i>E. coli</i>)	Interferes with response regulator function (29).

8. Future Perspectives

While detectably great and intriguing progress has been made with respect to the role of TCSs in bacterial pathogenesis and immune modulation, many questions still remain open, providing interesting perspectives on future research. One such focus is the integration of systems biology and omics technologies. High-throughput transcriptomics, proteomics, and metabolomics of TCS mutants can reveal the global networks and hidden cross-regulatory circuits (14). These observations may identify new therapeutic molecular targets and offer a clearer understanding of how bacteria integrate a myriad of host signals and organize opposing responses (28).

The effect of the host microbiome on TCS activity is yet another important aspect. The commensal bacteria use TCSs to coordinate communication with the host, and their signals can also affect the behaviour of the pathogen indirectly. Understanding how pathogenic TCSs integrate immune- and microbiome- derived signals may help to explain differences in host outcomes following infection (30).

Moreover, the structural biology of TCSs and especially that of membranous His kinases is largely still elusive and future investigations should address this aspect. Interestingly, advancements in cryo-electron microscopy and computational modelling may provide structural templates for computational drug design. Protein targets that are necessary for the pathogenicity of the organism but are also conserved in other strains might allow the

design of chemically mutagenized organism libraries, which would allow large numbers of strains to be screened before choosing specific targets for the construction of live vaccines (15), or would permit more extensive attenuation if the organism was closely related genetically to an organism that was not pathogenic to humans (16).

Testing TCS inhibitors in vivo is an urgent clinical imperative. Most candidate molecules remain in the preclinical phase of development with insufficient data in the literature on pharmacokinetics, toxicity and therapeutic efficacy. TCS inhibitors in combination with antibiotics or immunomodulators represent a potential new approach to overcome multidrug resistance and treatment failures ().

Finally, the future of TCS research will probably rely on interdisciplinary collaboration between microbiology, immunology, structural biology and computational sciences. Such concerted efforts will not only elucidate TCS-immune interactions but also facilitate the translation of basic research to translational antimicrobial approaches.

Conclusion

Indeed, TCSs directly act here as key players in the interplay between pathogen and host, high-lighting the crucial role of the TCS signaling networks in fancy decisions on whether the path to infection leads to victory or despair. Pathogen-encoded two component systems (TCSs) transmit external signals (e.g., antimicrobial peptides, ion starvation, oxidative stress

and immune signaling molecules) and convert these signals into adaptive gene expression regimes. This regulatory flexibility is not only essential for the survival of the bacteria under high immune pressure but also plays a major role in key biological functions such as virulence factor regulation, biofilm generation, antimicrobial resistance and persistence in hostile environments in the host.

On the host side, the evolved immune responses sense and fight invading bacteria while on the pathogen side, a TCS-mediated adaptation is frequently how invading pathogens evade clearance, become entrenched in chronic infection, and trigger therapeutic failures. PhoPQ, PmrAB, Agr and CsrRS are representative of individual TCSs known to promote immune evasion and resistance toward frontline antibiotics (31,32). Collectively, these observations underscore that TCSs are not merely bacterial survival tactics but rather actual virulence regulators affecting infection progression and clinical outcome (33,34,35).

Therapeutic perspective: today, these compounds come in 5th module of TCSS and are an attractive target for new generation of antimicrobials. Small molecule inhibitors, antivirulence approaches and combined therapies hold the potential to combat bacterial virulence with less propensity for resistance selection. Nonetheless, at the level of the pathways, the diversity, redundancy and complexity of TCS signalling among bacterial species still hinder the decoding of TCS signalling (36,37).

Future interdisciplinary structural biology, omics approaches and systems modeling based clinical TCSs evaluation will be required for optimal harnessing of TCSs as drug targets. Future TCS-targeting therapies that exploit these vulnerabilities in TCSs may complement existing antibiotics or act as adjunct therapies, through the integration of mechanistic foundational knowledge within a translational context. Hence, the molecular cross-talk between bacterial TCSs and host immunity provides valuable insights and new tools in combatting AMR and chronic infections impact in post-antibiotic era (38,15,4,7).

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