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Regulation of virulence and antibiotic resistance in Gram-positive microbes in response to cell wall-active antibiotics

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Abstract

Purpose of review: Antibiotic stress can evoke considerable genotypic and phenotypic changes in Gram-positive bacteria. Here, we review recent studies describing altered virulence expression in response to cell wall-acting antibiotics and discuss mechanisms that coordinate regulation of the antibiotic response.

Recent findings: Pleiotropic effects induced by antibiotic exposure include alterations to bacterial metabolism, cell wall structure and antibiotic resistance. In addition, subinhibitory concentrations of cell wall-active antibiotics have increasingly been shown to induce the production of exotoxins and biofilm formation that may influence virulence. Remarkably, phenotypes associated with comparable antibiotic stresses can vary considerably, emphasizing the need to better understand the response to cell wall-active antibiotics. Recent studies support both direct antibiotic recognition and recognition of antibiotic-induced stress to the bacterial cell wall. Specifically, bacterial two-component systems, PASTA kinases and conserved oxidative-stress sensors each contribute to modulating the antibiotic stress response.

Summary: Bacterial sensory systems and global regulators coordinate signaling in response to cell wall-active antibiotics. Regulation of the antibiotic response is complex and involves integration of signals from multiple response pathways. A better definition of the antibiotic stress response among Gram-positive pathogens may yield novel therapeutic targets to counter antibiotic resistance and virulence factor expression.

Keywords

Antibiotic; cell wall; resistance; stress response

INTRODUCTION

In Gram-positive bacteria, a thick peptidoglycan layer comprises the cell wall and provides structure for the organism. The cell wall also serves a sensory function as bacteria have evolved intricate mechanisms to perceive environmental change including heat, pH, nutrient

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limitation, and chemical and oxidative stress. Cell wall-active (CWA) antibiotics target biosynthesis of the bacterial cell wall and it is well established that these agents can act as bacterial signaling molecules, evoking diverse biological responses in a concentration-dependent manner (1, 2). Specifically, CWA antibiotics can alter bacterial metabolism, antibiotic resistance and virulence through a coordinated response involving conserved signaling pathways. Considerable heterogeneity exists in the response to antibiotic stress and mechanisms controlling this response remain largely unknown. The current review highlights recent findings of altered virulence potential in response to CWA antibiotics and discusses current insights into signaling pathways that influence the antibiotic stress response.

Altered virulence potential in response to cell wall-active antibiotics

Antibiotics can remarkably influence the production of bacterial toxins. Studies in *Staphylococcus aureus* have demonstrated that subinhibitory concentrations of CWA antibiotics induce production of potent exotoxins including alpha-hemolysin (3, 4), PVL and TSST-1 (5-7). In *Clostridium difficile*, subinhibitory CWA antibiotics similarly induce toxin expression (8) including Toxin A and Toxin B (9, 10). Regulation of toxin expression is undoubtedly complex and factors that control toxin expression in response to CWA antibiotics are not completely understood. Recent studies suggest that two-component signaling (TCS) systems, global regulators and certain penicillin-binding protein utilization can influence toxin induction (6, 11). Long noncoding RNA also contributes to the control of antibiotic-induced toxin production in *S. aureus* (12), indicating an integration of various regulatory pathways in virulence expression.

In addition to toxins, the expression and architecture of bacterial biofilm is modified in response to CWA antibiotics. In *S. aureus and Enterococcus faecalis*, subinhibitory concentrations of β-lactam and glycopeptide antibiotics induce biofilm formation in a manner dependent upon cell lysis and extracellular DNA (13-16). Recent studies highlight that CWA antibiotics increase bacterial attachment under both static and flow conditions and produce thicker biofilms containing more pillar and channel structures (17). Exposure of existing biofilms to subinhibitory concentrations of antibiotics can similarly lead to biofilm restructuring (18).

Many of the studies assessing the impact of subinhibitory antibiotics on virulence have been conducted *in vitro* and require validation of antibiotic effects *in vivo* and in a polymicrobial context. Importantly, strain- and antibiotic-dependent behavior is often observed when monitoring the effects of CWA antibiotics on virulence expression. This points toward a multifaceted response to CWA antibiotics that is dependent upon genetic background, context of the interaction, and multiple interacting signaling pathways, discussed further in the present review.

Two-component signaling systems orchestrate the response to CWA antibiotics

Antibiotic stress can induce an adaptive response in bacteria that comprises the cell wall stress stimulon (CWSS). Notably, the CWSS in Gram-positive organisms is controlled by one or more TCS systems. Although some CWSS TCS systems are triggered by specific

classes of antibiotics, others appear to be activated less selectively in response to diverse CWA antibiotic stress (19). Genome-wide transcriptional profiling studies in several Gram-positive pathogens have demonstrated that the CWSS includes a subset of genes that rapidly modulate cell wall metabolism, providing a mechanism of tolerance to antibiotic-induced stress (20-24) (Figure 1A). A systems-wide proteomic analysis in *S. aureus* similarly found an upregulation of the peptidoglycan biosynthetic pathway following CWA antibiotic stress (25). Activation of CWSS TCS systems results in transcriptional profiles that are strain-dependent (26), and induction kinetics reflect antibiotic- and concentration-dependent growth inhibition (19). CWSS TCS systems provide specificity to the CWA antibiotic response and function in response to disrupted cell wall synthesis, or cell wall hydrolysis, but not toward other external stresses.

Modulation of the CWA antibiotic response by PASTA kinases

Recently, serine/threonine kinases, containing extracellular penicillin-binding protein and serine/threonine kinase-associated (PASTA) domains, and their cognate phosphatases have emerged as critical factors regulating the bacterial response to CWA antibiotics. As regulators of cell wall metabolism, PASTA kinases are important in perceiving environmental stress. Genetic deletion of a PASTA kinase sensitizes Gram-positive organisms to CWA antibiotics, supporting an integral role for these kinases in the cell wall stress response and antibiotic resistance (27-32). Likewise, selectively targeting PASTA kinase activity with chemical inhibitors increases susceptibility to β-lactam antibiotics (31, 33-36). Conversely, mutation of an antagonistic Ser/Thr phosphatase in *S. aureus* contributes to reduced vancomycin susceptibility (37-39) and recapitulates the characteristic thick cell wall phenotype of vancomycin resistance (27).

PASTA kinases coordinate the response to cell wall stress through interactions with bacterial TCS systems and global regulators. For example, *S. aureus* PASTA kinase signaling targets VraRS, WalKR, GraSR, CcpA, MgrA and SarA (reviewed in (40)) and an *E. faecalis* PASTA kinase targets CroSR (41), involved in CWA antibiotic resistance. Crosstalk among these independent stress response pathways demonstrates an appreciable level of complexity in the signaling network that underlies the bacterial response to CWA antibiotics. Gene expression studies demonstrate that PASTA kinase activity regulates broad cellular functions including cell wall biosynthesis, metabolism and virulence expression (Figure 1B) (42). Their involvement in toxin expression support that PASTA kinases play a role in the response to subinhibitory concentrations of CWA antibiotics. Remarkably, the contributions of PASTA kinase activity to CWA antibiotic stress are bacteria- and strain-specific. Sequence divergence of PASTA domains (43) and dissimilar interactions with TCS systems among bacteria confound our current understanding of PASTA kinase signaling.

The mechanism by which PASTA kinases recognize cell wall stress remains unclear. Extracellular PASTA domains can recognize the cell wall precursor lipid II and soluble muropeptides (44-47) supporting the notion that cell wall stress could be sensed by altered peptidoglycan precursor pools. Recent evidence also points toward the direct recognition of β-lactam antibiotics by PASTA kinases (45, 46, 48). Intriguingly, a *S. aureus* isolate that encodes a truncated PASTA kinase, lacking the extracellular PASTA domains, still provides

resistance to CWA antibiotics, signifying kinase activation can occur via a mechanism independent of PASTA ligand recognition (32). Further studies are required to clarify the mechanism of PASTA kinase activation, signaling interactions and observed phenotypes in response to CWA antibiotic stress.

Activation of the Spx regulon by CWA antibiotic stress

In addition to TCS systems and PASTA kinases, the Spx stress regulator is highly conserved among Gram-positive bacteria and controls the expression of genes involved in stress tolerance and virulence. Spx is recognized as a transcriptional regulator in the oxidative stress response and *spx* mutants were recently found to exhibit sensitivity to CWA antibiotic stress (49, 50). Consistent with a role for Spx in the antibiotic stress response, mutation of *yjbH*, a negative regulator of Spx, reduces bacterial susceptibility to both glycopeptide and β-lactam antibiotics (39, 51).

Induction of the Spx regulon by CWA antibiotics suggests a link between the antibiotic and oxidative stress response. Remarkably, studies in *Bacillus subtilus* demonstrate that mechanisms of Spx activation during CWA antibiotic stress are distinct from the oxidative stress response (Figure 1C) (50, 52). Specifically, CWA antibiotic stress requires upregulation of *spx* from a σ^M promoter, whereas several promoters can activate *spx* expression during disulfide stress (50). Contrary to oxidized Spx (redox switch) present during disulfide stress, Spx was maintained in a reduced form during antibiotic stress, permitting differential gene regulation in response to different stresses (50). Oxidation of the YjbH regulator was also dispensable for *S. aureus* β-lactam resistance, but not disulfide stress (51). Thus, Spx is a common regulator in both oxidative and antibiotic stress, but independent signaling interactions provide specificity to each response.

Proteolytic activity regulates adaptation to the CWA antibiotic response by eliminating stress-damaged proteins, controlling cell wall metabolism and regulating virulence (53). In addition to complex transcriptional regulation, Spx is subject to posttranslational oxidation and proteolysis (54). Under non-stress conditions, Spx is targeted to the ClpP protease by YjbH. CWA antibiotic stress induces *spx* expression but also stabilizes Spx protein levels by the anti-YjbH factor, YirB (52). YirB is induced by the CssRS TCS system in response to CWA antibiotics (52), pointing toward interaction of the Spx pathway with TCS sensory systems. Further, TrfA, a factor contributing to glycopeptide and oxacillin resistance, is induced by Spx in response to CWA antibiotics (55-57). TrfA imparts both specificity and timing to protease-mediated degradation of antitoxins and other factors implicated in the response to antibiotic stress (58). Together, the Spx regulon actively controls both gene expression and protein turnover during the CWA antibiotic stress response.

CONCLUSION

The bacterial response to CWA antibiotics is well appreciated; however, mechanisms directing the outcome of such interactions remain less clear. Multiple conserved sensory systems have now been shown to contribute to perception of CWA antibiotic stress. Alternative sigma factors (20, 59) and the SOS response (60, 61) also play a role in response to antibiotic stress. Interestingly, considerable variation is evident in the global antibiotic

response. Strains of the same pathogen can exhibit fundamentally dissimilar responses to the same antibiotic stress (62), highlighting a dependence upon genetic background, the context of the antibiotic interaction and possible epigenetic effects. Phenotypic variations among bacterial subpopulations ultimately contribute to pathogen success and persistence during environmental stress. In the context of genome-wide responses, a strikingly poor correlation between transcriptional and phenotypic fitness suggests that CWA antibiotic stress is quite complex (63). Together, the overall significance of antibiotics toward bacterial evolution and virulence merits the continued investigation of mechanisms controlling CWA antibiotic-induced stress.

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ABBREVIATIONS

CWA	cell wall-active
CWSS	cell wall stress stimulon
PASTA	penicillin-binding protein and serine/threonine kinase-associated
TCS	two-component signaling

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KEY POINTS

- Cell wall-active antibiotics can induce the expression of potent exotoxins and alter biofilm formation in Gram-positive bacteria.
- Strain-specific responses to cell wall-active antibiotics indicate that complex mechanisms regulate the antibiotic-induced stress response.
- Bacterial two-component signaling systems, PASTA kinases and the global Spx stress regulator coordinate a response to cell wall-active antibiotics.
- Antibiotic-induced cell wall stress utilizes conserved signaling pathways but independent interactions provide specificity to the response.

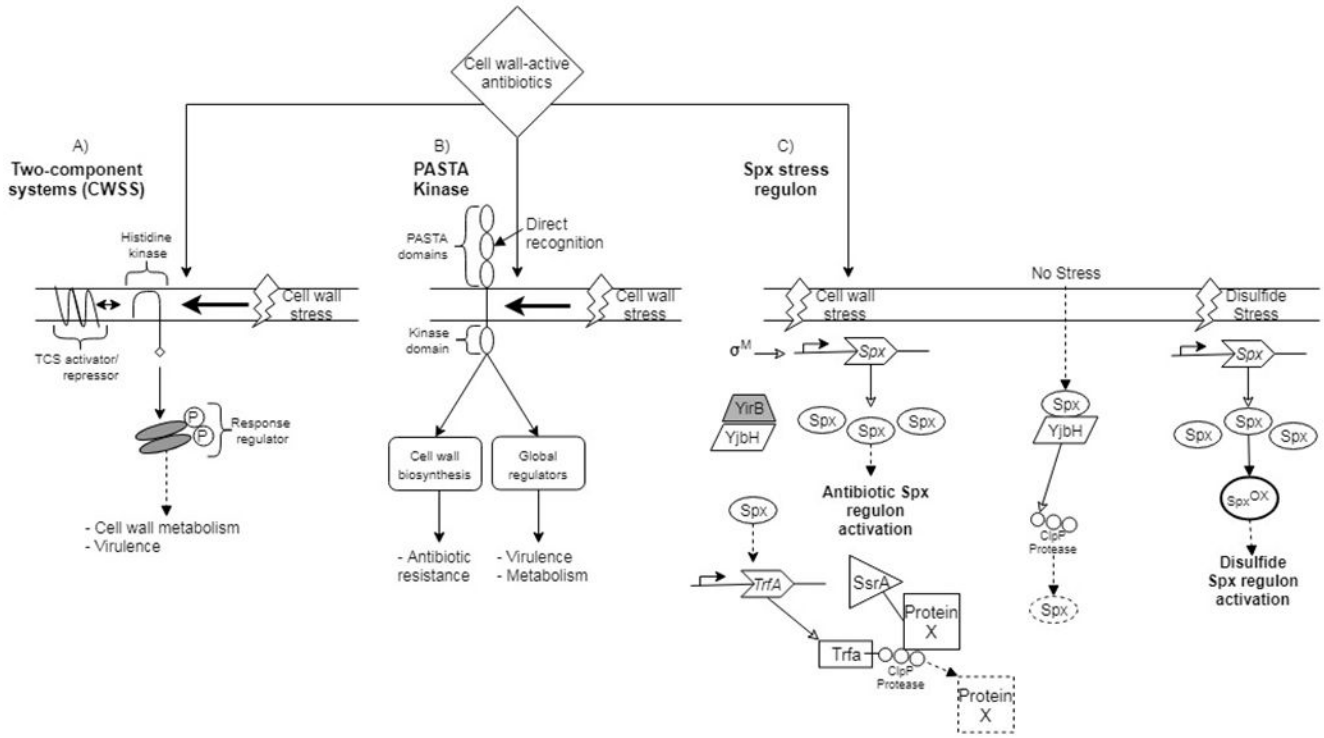

Figure 1. An integrated signaling network coordinates the Gram-positive bacterial response to cell wall-active antibiotics.

The response to cell wall-active antibiotics involves multiple sensory systems including TCS systems, PASTA kinases and the Spx stress regulon. (*A*) One of more TCS systems comprise the CWSS. A TCS histidine kinase responds to cell wall stress by autophosphorylation and subsequent transphosphorylation of its response regulator. A third TCS component can act as an activator, or repressor, in regulating TCS signaling. (*B*) PASTA kinase signaling occurs by direct recognition of antibiotics or, alternatively, by responding to cell wall stress. PASTA kinase activation contributes to modulation of several cellular functions including cell wall biosynthesis, metabolism and virulence by acting on global regulators. (*C*) The Spx pathway responds to independent stresses. Under no-stress conditions, Spx is bound by YjbH and targeted for degradation. Disulfide stress increases Spx levels and oxidizes Spx to control activation of gene expression. In contrast, antibiotic stress increases Spx, in a manner dependent upon σ^M, and YirB sequesters YjbH allowing a reduced form of Spx to activate target gene expression. TrfA is induced by Spx during antibiotic stress and serves as a proteasome adapter for recognition of SsrA-tagged substrates. Notably, cross-talk exists among PASTA kinases and TCS systems and between the Spx and TCS systems, coordinating activities of the antibiotic response. Dashed boxes indicated degraded protein.