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Anti virulence Strategies for Combating Antibiotic-Resistant Bacterial Infections and Their Application in the Poultry Sector

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Abstract

The escalating incidence of antimicrobial-resistant (AMR) significantly reduced the effectiveness of conventional antibiotics, necessitating the development of alternative therapeutic modalities. Anti virulence compounds represent a novel class of agents that neutralize bacterial pathogenicity by targeting virulence factors instead of inhibiting bacterial growth, thereby mitigating the selective pressure driving resistance evolution. This review synthesizes current knowledge on the mechanisms by which antivirulence agents disarm pathogens, with a specific focus on their applicability within the poultry industry. Key targets include the Type 3 Secretion System (T3SS), Two-Component Systems (TCSs), bacterial adhesion machinery, and Quorum Sensing (QS) networks. Therapeutic strategies targeting the T3SS primarily disrupt ATPase activity, needle assembly, or effector translocation, while QS inhibitors interfere with density-dependent signaling to suppress biofilm formation and toxin production. Despite their potential, the clinical translation of these agents is hindered by challenges in drug discovery, including computational complexity and the need for robust infrastructure. Nevertheless, integrating anti virulence strategies into poultry health management offers a viable solution to reduce antibiotic dependency and combat the growing threat of antimicrobial resistance. This review explore these mechanisms of antimicrobial resistance by attenuating pathogens rather than eliminating them, their startgies are believed to exert lower selective pressure and help preserve the beneficial commensal microflora.

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Introduction

Antibiotic resistance threatens global public health and the poultry industry's sustainability, promoting need for innovative therapeutic strategies **(McShan and De Guzman 2015).** Conventional antibiotics exert selective pressure by killing or inhibiting bacterial growth, often causing resistant strain emergence. In contrast, anti virulence therapies focus on target specific pathogen virulence factors, such as membrane-associated, and secreted components, or cytosolic molecules, attenuating pathogenicity without directly affecting bacterial viability **(Dehbanipour and Ghalavand, 2022; Bassetti *et al*., 2013).** These agents are hypothesized to exert minimal disruption on the host commensal microbiota, a significant advantage over broad-spectrum antibiotics by relying on the host immune system to clear attenuated pathogens, **(Baron 2010).** Antivirulence drugs are classified into several groups, including Type 3 Secretion System (T3SS) inhibitors, adhesion inhibitors, gene expression inhibitors, and communication and signaling pathway inhibitors **(Filipić *et al*. 2024).**

T3SS Inhibitors

The T3SS is a multi-megadalton syringe-like complex composed of more than 20 proteins, features ring structures embedded in the bacterial inner and outer membranes, as well as a translocation pore in the host cell membrane **(McShan and De Guzman, 2015).** T3SS is a key virulence mechanism utilized by several important pathogens. This apparatus enables bacteria injecting partially unfolded effector proteins directly into host cells, a mechanism conserved across major pathogens: *Salmonella*, *Shigella*, *Yersinia*, *Pseudomonas*, and enteropathogenic *E. coli* (EPEC) **(Coburn *et al*., 2007; McShan and De Guzman, 2015).** Therapeutic interference with the T3SS may occur at multiple stages, including transcriptional regulation, structural assembly, chaperone-effector interactions, or effector translocation **(Keyser *et al*., 2008; Baron, 2010).**

Salicylidene acylhydrazides (SAHs) constitute the most extensively characterized class of T3SS inhibitors. Initial high-throughput screening in *Yersinia* identified SAHs as potent inhibitors of T3SS gene expression, although early studies were unable to cleary between direct distinguish inhibition and upstream regulatory effects **(Kauppi *et al*., 2007).** However subsequent research confirmed that SAHs inhibit effector secretion in a dose-dependent manner, likely through three distinct mechanisms: First that disrup of the SPI-1 needle apparatus assembly, as evidenced by a ~40% reduction in Shigella needle formation **(Veenendaal *et al*., 2009);** second modulation of cellular metabolism via binding to partners such as WrbA, Tpx, and FolX, thereby altering T3SS gene expression (Wang *et al*., 2011); and third they act through iron chelation, which limits the availability of iron required for T3SS function in *Salmonella* **(DiGiandomenico *et al*., 2014).** Notably, most SAHs do not adversely affect bacterial growth, preserving the commensal flora **(Kauppi *et al*., 2003).**

In addition to Thiazolidinones represent another important class of inhibitors. They have been reported to block the T3SS in Yersinia, the Type II Secretion System (T2SS) in Pseudomonas, and Type IV pili in Francisella **(Felise *et al*., 2008).** These compounds are believed to target the conserved outer membrane Secretin protein, primarily interfering with effector translocation and needle-tip complex assembly **(Fasciano *et al*., 2019).**

Further more, specific molecular targets within the T3SS have been identified particulary that the T3SS ATPase as compounds targeting as EscN in EPEC and YscN in Yersinia, have shown promising results (Fasciano *et al*., 2019). For instance, omeprazole inhibits the ATPase activity of the Salmonella SPI-2 T3SS, potentially via nitric oxide regulation, while specific polypeptides target the CdsN ATPase in Chlamydia, preventing host cell invasion **(Stone *et al*., 2011; Fasciano *et al*., 2019).** Regarding structural components, phenoxyacetamide (PXA) inhibits the T3SS in *P. aeruginosa* by targeting the needle protein PscF, as resistance mutations map to this locus **(Bowlin *et al*., 2014).** Furthermore, benzimidazoles and

certain flavonoids, such as baicalein and quercetin, inhibit T3SS function by blocking the DNA binding of transcription factors (e.g., LcrF, ExsA, VirF) or covalently modifying translocases and effectors **(McShan and De Guzman, 2015; Tsou *et al*., 2016).** Lactoferrin has also been shown to degrade translocon proteins (IpaB/IpaC in Shigella; EspA/B/D in *E. coli*), thereby reducing pathogenicity **(Ochoa and Cleary, 2009).** Currently, MEDI3902, a bispecific antibody targeting the *P. aeruginosa* T3SS translocator PcrV and the biofilm exopolysaccharide Psl, is under clinical evaluation **(Ali *et al*., 2019).**

Two-Component Systems (TCSs) and Secretion Systems

TCSs enable bacterial to sense and response to environmental stimuli through a signaling mechanism comprising a membrane-bound histidine kinase and a cytoplasmic response regulator **(Rajput *et al*., 2021).** TCSs regulate critical virulence traits: including toxin production and host invasion, in response to signals such as osmolarity, temperature, and nutrient availability. Commonn examples of TCSS include DosR/DosS, EnvZ/OmpR, PhoP/PhoQ, and AgrC/AgrA **(Mühlen *et al*., 2016; Rajput *et al*., 2021).** In *Mycobacterium tuberculosis*, inhibitors as HC104A and HC106A downregulate the DosR regulon, disrupting signal transduction and impairing bacterial survival **(Zheng *et al*., 2020).** Similarly, savarin inhibits RNAIII production in *Staphylococcus aureus*, reducing virulence without affecting commensal strain *S. epidermidis* **(Salam *et al*., 2023).**

Beyond TCSs, the T2SS is essential for virulence in pathogens such as *Vibrio cholerae* and Klebsiella. High-throughput screening has identified virstatin, as a small-molecule inhibitor of the ToxT transcriptional regulator, which effectively suppresses cholera toxin production in murine models without causing cellular toxicity **(Lopatkin *et al*., 2021; Aburto-Rodríguez *et al*., 2021).**

Adhesion and Biofilm Inhibition

Bacterial adhesion to host tissues, mediated by surface adhesins: Microbial Surface Components Recognizing Adhesive Matrix Molecules in Gram-positive pathogens, is a prerequisite for colonization and infection **(Zrelovs *et al*., 2021).** Sortase A (SrtA), a cysteine transpeptidase responsible for anchoring these adhesins to the cell wall, lacks eukaryotic homologs, making it an attractive anti virulence target **(Cascioferro *et al*., 2014).** Numerous natural and synthetic SrtA inhibitors have been identified, offering a strategy to prevent initial colonization **(Zrelovs *et al*., 2021).**

Furthermore, biofilm formation confers significant protection against antimicrobial agents, with biofilm-embedded bacteria exhibiting up to 1,000-fold higher resistance than planktonic counterparts **(Uruén *et al*., 2020).** Biofilms contribute to chronic infections caused by *P. aeruginosa*, *Acinetobacter baumannii*, and *S. aureus* by limiting antibiotic penetration and harboring metabolically inactive persister cells. Consequently, agents that disrupt biofilm integrity or prevent its formation are being actively explored as adjunctive therapies **(Parrino *et al*., 2019).**

Quorum Sensing (QS) Inhibitors in Poultry Health

Quorum Sensing is a density-dependent regulatory system mediated by auto inducers (AIs) that coordinates virulence factor production and biofilm formation **(Bhardwaj *et al*., 2013).** In Gram-negative bacteria, LuxI-type enzyme-synthesized N-acyl-homoserine lactones bind to LuxR-type receptors, triggering the transcription of virulence genes **(Vashistha *et al*., 2023).** In Gram-positive bacteria, autoinducer peptides (ATPs) interact with histidine kinases to initiate phosphorylation cascades that regulate gene expression **(Bhate *et al*., 2015).** Quorum Sensing inhibitors (QSIs) disrupt this communication through three primary mechanisms: inhibiting AI synthesis, degrading secreted AIs, or competitively blocking receptor binding **(Rasmussen *et al*., 2005).**

In the poultry sector, Quorum Sensing plays a role in *Clostridium perfringens*-caused necrotic enteritis pathogenesis, particularly through the *Agr* system **(Yu *et al*., 2017).** Plant-derived

compounds have emerges as potent QSIs. Andrographolide, derived from *Andrographis paniculata*, significantly reduces positive adhesion and cytotoxicity of avian pathogenic *E. coli* (APEC) O78 in chicken pneumocytes **(Guo *et al*., 2014).** Similarly, synthetic QSIs (QSI-5/10) have been shown to decrease APEC-induced mortality and bacterial load in chickens **(Helmy *et al*., 2018; Helmy *et al*., 2022).** Tectorigenin, an isoflavone from *Belamcanda chinensis*, suppresses type IV pilus genes in *C. perfringens*, reducing motility and biofilm formation **(Liu *et al*., 2019).** Other phytochemicals, including rutin, gallic acid, protocatechuic acid, and baicalin, have demonstrated efficacy in downregulating virulence genes and inhibiting biofilm formation in *Salmonella Typhimurium,* APEC, and *C. perfringens* (**Peng *et al*., 2018; Alvarado-Martinez *et al*., 2020; Xu *et al*., 2020; Liu *et al*., 2023).** Additionally, peptide-containing cell-free spent media and quercetin have been reported to suppress QS-controlled virulence factors in *C. perfringens* and *P. aeruginosa*, respectively **(Jaramillo-Jaramillo *et al*., 2023; Ren *et al*., 2024).**

Conclusions

The development of anti virulence agents represents a paradigm shift in combating AMR, moving from bactericidal approaches to pathogenicity attenuation. While Computer-Aided Drug Design and High-Throughput Screening have accelerated discovery, the translation of these candidates into clinical practice remains constrained by high development costs and regulatory hurdles. To realize the potential of anti virulence therapies in the poultry industry, future research must focus on elucidating *in vivo* pharmacokinetics, assessing synergistic effects with conventional antibiotics, and demonstrating economic viability. Collaborative efforts between academia and the pharmaceutical industries are essential to overcome these barriers and integrate anti virulence strategies into sustainable poultry health management protocols.

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