Anti-virulence therapeutic strategies against bacterial infections: recent advances

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Abstract

The emergence and increasing prevalence of multidrug-resistant pathogens has become a major global healthcare problem. According to the World Health Organization if these trends continue, mortality from infection in 2050 will be higher than that from cancer. Microorganisms have various resistance mechanisms against different classes of antibiotics that emphasize the need for discovery of new antimicrobial compounds to treat bacterial infections. An interesting and new strategy for disarming pathogens is antivirulence therapy by blocking bacterial virulence factors or pathogenicity. Therefore, the use of these new pathoblockers could reduce the administration of broad-spectrum antimicrobials and prevalence of resistant strains.

This review provides an overview of the antivirulence strategies published studies between years 2017 and 2021. Most antivirulence strategies focused on adhesins, toxins and bacterial communication. Additionally, targeting two-component systems and ncRNA elements were also examined in some studies. These new strategies have the potential to replace traditional antimicrobial agents and can be used to treat infections, especially infections caused by resistant pathogens, by targeting virulence factors.

Keywords **Antivirulence therapeutics, pathoblockers, antimicrobial resistance, virulence factors, antiadherence strategies, antibiofilm activity, quorum sensing, secretion systems, two-component systems, non-coding RNA.**

Introduction

Antimicrobial resistance is a significant global public health threat. The emergence of multidrug resistance in ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), responsible for serious chronic nosocomial infections, has become a serious challenge worldwide.[1] On the other hand, new classes of resistance mechanisms such as metabolic gene alterations may be a new concern to human health.[2] Resistance expansion prevents efficient treatment of infected patients, especially in the hospital setting. All of these threats emphasize the need to identify new strategies for the treatment of bacterial infectious diseases. In this review we highlight antivirulence approaches that are being investigated for the prevention and treatment of bacterial infections. It should be noted that, among the compounds identified and studied for antivirulence therapy, a few have been tested using *in vivo* models.

Review criteria

This review provides an overview of the antivirulence strategies research. Published works on antivirulence therapy studies from years 2017 to 2021 were identified using the following search terms "antivirulence therapeutics", "pathoblockers", "new therapeutic strategies" in Google Scholar, PubMed, Medline and Scopus. The final sample consisted of 77 articles after

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processing and applying exclusion criteria, and are presented as follows.

Antivirulence strategies

Virulence is a microorganism's ability to produce disease. Virulence factors are molecules produced by a variety of microbial pathogens that assist in colonization, immunoevasion, immunosuppression, obtaining nutrition and damaging host cells. These factors are often classified in three forms, including membrane associated, secretory or cytosolic.[3] Blocking the activities of virulence factors is a new approach that has emerged over the last decade. Antivirulence drugs, the new class of drugs, target virulence factors of pathogens instead of killing or stopping their growth and consequently disarm infectious pathogens. Bactericidal antibiotics may also cause the selective pressure that drives resistance. Antivirulence drugs interfere with the interaction of the pathogen with its host, and thereby reduce damage to the host and impair the organism's ability to cause disease without killing it or creating selective pressure.[4] Research on the inactivation of diphtheria and tetanus toxins are the first examples of the antivirulence approach.[5,6] Also, bezlotoxumab is the first antivirulence agent approved by the US food and drug administration (FDA). This agent blocks TcdB in *Clostridioides difficile*.[7] There are a variety of bacterial targets for antivirulence therapy, however some of the most attractive targets are adhesins, toxins, bacterial communication, two component systems and non-coding RNAs. Studies in recent years have suggested a variety of compounds as candidates for antivirulence therapies (Table 1).

Antiadherence strategies

Adhesion to host tissues is the initial step of infectious diseases caused by pathogenic bacteria. Therefore, inhibiting bacterial adherence by antivirulence drugs can be a promising strategy to prevent infection.

Gram-positive pathogens including staphylococci, enterococci and streptococci can express various surface adhesion proteins known as MSCRAMM (microbial surface component recognizing adhesive matrix molecules). In addition to bacterial adhesion, MSCRAMM play important roles in immune evasion and biofilm formation. On the other hand, sortaseA (SrtA), a membrane-localized cysteine transpeptidase in Gram-positive pathogens, is crucial for the assembly and anchoring of aforementioned cell-surface adhesins to the cell wall envelope. Due to its easy accessibility and lack of homologous sortase in eukaryotes, it is a promising drug target for the development of antivirulence therapeutics against Gram-positive bacterial infections. Additionally, if the selective pressure induced by inhibitors results in mutation in the SrtA gene or an increase in enzyme production to counteract the decrease in enzymatic activity, it finally leads to a reduction in pathogenicity of the pathogen through decreasing enzyme activity and increasing metabolic burden, respectively. To date, various natural products and synthetic small molecules have been discovered as SrtA inhibitory compounds.[8,9]

Moreover, one of the most significant strategies for bacteria to counteract antimicrobials is biofilm formation. Bacterial cells are embedded in a self-produced extracellular matrix within biofilms. The antimicrobial resistance of biofilm cells is up to a thousand times higher than that of planktonic forms, and it may be linked to accumulation of resistance mechanisms of single cells within biofilms. Biofilms are involved in chronic and recurrent infections caused by pathogens such as *P. aeruginosa*, *A. baumannii*, *S. aureus*, *E. coli*, *K. pneumoniae* and *Streptococcus pneumoniae* (Figure 1). Furthermore, the presence of bacterial cells in the depth of metabolically inactive cell layers makes it difficult for antibiotics to access them and penetrate the matrix. Given the importance of biofilm in bacterial pathogenicity, it seems to be an attractive target for anti-virulence drug development. The main approach in this regard is based on two strategies: 1) inhibition of biofilm formation by preventing bacterial cells from adhesion to the host tissue and 2) disruption of biofilm communities. To date, various inhibitory compounds have been identified and introduced to achieve these goals.[10] For example, a large number of synthetic small molecules have been



Figure 1. Biofilm-associated infections

identified in recent years that have an interesting capability to target the biofilm formation at different stages.[11]

Targeting toxins and secretion systems

The type III secretion system (T3SS) is one of the most important virulence factors used by some Gram-negative pathogens such as *P. aeruginosa*, *Salmonella enterica* serovar Typhimurium, *Yersinia pestis* and *Chlamydia* spp. *P. aeruginosa* is capable to infect a wide host range and lead to high mortality rates, especially in patients with cystic fibrosis (CF). This pathogen utilizes the type III secretion system to transport bacterial effectors, ExoS, ExoU, ExoT and ExoY, directly into host cells. T3SS consists of a series of regulated genes that encode components of the secretion apparatus and a translocon, and are important for intoxication of eukaryotic cells.[12] Since the T3SS machinery is evolutionarily conserved, it can be used as a potential target for pathoblockers. In addition, because T3SS is required for pathogenesis but not for survival, the use of inhibitors results in attenuated virulence as well as less selective pressure for resistance. INP0341, a salicylidene acylhydrazide, is a promising substance that has been shown to inhibit T3SS in a range of Gram-negative bacteria, resulting in preventing toxin delivery and, hence, decreasing bacterial virulence.[13]

Aggregatibacter actinomycetemcomitans is a member of the HACEK group of bacteria together with *Haemophilus* spp., *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*. Although these fastidious Gram-negative organisms are found in the human oral cavity and known as the normal flora, they can cause various invasive infections, particularly endocarditis and localized aggressive periodontitis (LAP). *A. actinomycetemcomitans* colonizes the gingival sulcus and invades the epithelial tissues and stimulates a pathophysiologic inflammatory response.[14] This organism has different virulence factors including adhesive type IV pili, surface-exposed autotransporter proteins, type V collagen, leukotoxin and lipopolysaccharide. The operon of leukotoxin is comprised of four coding genes including *ltxC*, *ltxA*, *ltxB*, and *ltxD*. LtxA is a large pore-forming toxin and a key virulence factor, which has the ability to annihilate host immune tissues.[15] Since LtxA has strong affinity for plasma membrane cholesterol-rich lipid rafts, inhibitors such as catechins can prohibit LtxA-mediated cytotoxicity in white blood cells through altering LtxA structure and reducing affinity for cholesterol. Catechin is one group of

flavonoids derived from plants, with a variety of health beneficial effects through antioxidant, anticancer, antimicrobial and antiviral properties. Therefore, catechin and its derivatives are potential compounds to be used as antivirulence agents.[16] In addition, MEDI4893 (suvratoxumab) and AR-301 are two monoclonal neutralizing antibodies against α-hemolysin (Hla) in *S. aureus*.[17,18] MEDI4893 has completed phase 2 clinical trials and AR-301 had entered phase 3 trials for the prevention of *S. aureus* pneumonia.[19]

Targeting quorum sensing

Bacterial quorum sensing (QS) is a comprehensive phenomenon that involves the ability to react to cell-population density via gene regulation. QS involves extracellular signaling molecules, also called autoinducers (AIs). This chemical communication is a critical pathway for survival in a competitive environment, for nutrient uptake and cell growth.[20] Gram-positive bacteria use a two-component system that is comprised of membrane-bound sensor kinase receptors and cytoplasmic transcription factors phosphorylated by kinase which then regulate gene expression. Gram-negative bacteria, including *Pseudomonas* spp., *Acinetobacter* spp., or *Burkholderia* spp. employ another type of autoinducers, the acyl-homoserine lactones (AHLs) which, after binding to a regulatory protein in the cell, operate as a transcription factor for various enzymes and virulence factor secretion genes.[21] Many virulence traits are influenced by QS and thus targeting QS can be a hopeful strategy to inhibit bacterial infections and combat the growing problem of antibiotic resistance. Interference with QS, called quorum quenching (QQ), is a process of obstructing QS by impeding signaling. This process consists of several parts, including inhibition of signaling molecules, blocking the receptor of signaling molecules by mimic molecules, degradation of signaling molecules and amendment of the QS signals by the enzyme activity. The use of nanotechnology in combination with antivirulence therapy in the treatment of diseases seems to be a promising strategy to confront pathogens. Selenium nanoparticles (SeNPs) as a drug carrier have shown significant effects on the intracellular delivery of antivirulence compounds such as polyphenols of honey with anti-QS activity against *P. aeruginosa*.[22]

Four essential oils (clove, cinnamon, thyme and marjoram) have been reported to have anti-biofilm and anti-QS activities against Gram-negative and Gram-positive multidrug-resistant pathogens such as *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii* and *S. aureus*.[23]

Targeting two-component systems

Two-component systems (TCSs) are involved in sensing environmental changes and expression of genes responding to environmental signals. They consist of a membrane-bound histidine kinase and a corresponding cytoplasmic response regulator that accomplish signal transduction by phosphorylation. TCSs play important roles in bacterial functions including drug resistance and host invasion. Studies characterized some TCSs that control gene networks in reaction to osmolarity, secondary metabolites, temperature, nutrients and ions. These include: DosR/DosS, EnvZ/OmpR, RcsB/RcsC, PhoP/PhoQ, BarA/SirA, CpxR/CpxS, AgrC/AgrA, and QseC/QseB.[24,25]

The DosRST two-component regulatory system has an important role in virulence in *Mycobacterium tuberculosis* (Mtb). Studies on two chemical inhibitors including HC104A and HC106A demonstrated that the compounds downregulate the DosR regulon genes and disrupt signal transduction leading to decreased Mtb survival.[26] Savirin, a small molecule inhibitor, reduces the expression of AgrCA-regulated genes and also inhibits RNAIII production in *S. aureus*, thus leading to a reduction of virulence. However, savarin has no effect on skin commensal *Staphylococcus epidermidis*.[27]

Non-coding RNAs as novel antimicrobial targets

A non-coding RNA (ncRNA), is a functional RNA molecule that operates without the need for being translated. In general, ncRNAs are involved in biofilm formation, regulation of gene

expression responding to extracellular stress and preserving homeostasis of the microbial cell at the transcriptional and post-transcriptional level. Some small non-coding RNAs (sRNAs) function to regulate genes that confer antibiotic resistance. CsiR in *Proteus vulgaris* were found to regulate EmrB multidrug efflux pump and are involved in the regulation of ciprofloxacin resistance.[28] On the other hand, since sRNAs are expressed in different growth phases of the bacterial life, it is suggested that the presence of sRNAs in the disease progression process is necessary to reconcile with the environmental changes.[29]

Since ncRNA can control biofilm formation, antibiotic resistance and bacterial stress responses, targeting the ncRNA pathways is a promising strategy for overcoming bacterial infections.

Discussion

The use of antibiotics has greatly helped in the treatment and control of infections during the 20th century but the widespread usage of conventional antibiotics has led to alarming increases in antibiotic resistance, even last-resort antibiotics like colistin, and failure to treat persistent infections. In recent years, an approach based on antivirulence therapeutics has become a promising strategy to counteract human pathogens. Interestingly, synergy between different antivirulence compounds with distinct targets is an attractive approach to use combination therapy to enhance the anti-pathogenic effect.[30]

However, one of the limitations of antivirulence therapies is the inability to completely clear the infection, which is challenging in clinical applications especially for immunocompromised patients. It seems that antivirulence compounds in combination with antibiotics can overcome this limitation. In fact, the use of antivirulence compound makes it possible to use lower concentrations of antibiotics, minimizing side effects and reducing the generation of antibiotic resistance in pathogens as well as effective pathogen removal. A study on the combination of gallium and furanone (as antivirulence compounds) together with antibiotics (colistin, ciprofloxacin, tobramycin and meropenem) in *P. aeruginosa* showed a significant effect of combination therapy in limiting the spread of antibiotic resistance. Interestingly, it has been suggested that the effectiveness of combination therapy is linked to the molecular mechanism of antibiotic resistance.[31] However, proving this hypothesis requires more detailed and comprehensive studies. Although these studies promise new therapeutic approaches, more research is needed for clinical applications. Since drug interactions are concentration dependent, pharmacodynamics and pharmacokinetics studies are required. In addition, there is evidence that antivirulence compounds may, contrary to expectations, in certain instances act in vivo as signals for activation of virulence factors in pathogens.[32] Hence, animal models must be used to evaluate the effectiveness of antivirulence compounds, alone or in combination with antibiotics, in the host environment.

Conclusions

Here, we reviewed current antivirulence strategies to disarm pathogens by targeting bacterial virulence factors. It should be noted that this strategy, like the use of antibiotics, also has disadvantages including lack of effect on all forms of the disease and low therapeutic effects compared with antibiotics. Because many of the pathogenicity mechanisms in pathogens are unknown, extensive studies on the virulence factors of pathogens and host-pathogen interactions are needed to advance this strategy. Appropriate and ongoing cooperation between governments and pharmaceutical companies can create a great future for new treatment strategies.

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Table 1. Inhibitors of bacterial adherence, biofilm formation, quorum sensing systems, toxin production and function, and two component systems

Substance	Inhibitory activity	Targets	Virulence factors affected	First author, year
MEDI4893 (mAb)	Anti-toxin	*S. aureus*	• Inhibition of oligomerization and pore formation by α-hemolysin	Yu et al., 2017[17]
Sclareol	Anti-toxin Anti-QS	*S. aureus*	• Reduced a-hemolysin production • Downregulation of *hla* and *RNAIII* expression • Reduced hemolysis	Ouyang et al., 2017[33]
Dracorhodin perchlorate	Anti-toxin Anti-QS	*S. aureus*	• Reduced α-hemolysin production • Downregulation of *hla* and *RNAIII* expression • Reduced hemolysis	Liu et al., 2017[34]
Chalcone	Anti-toxin Anti-SrtA Anti-QS Anti-biofilm	*S. aureus*	• Downregulation of *hla* and *agr*A expression • Reduced α-hemolysin production • Inhibition of SrtA activity • Reduced adherence to fibronectin • Reduced hemolysis • Reduced biofilm formation	Zhang et al., 2017[35]
Lysionotin	Anti-toxin Anti-QS	*S. aureus*	• Downregulation of *hla*, and *agr* expression • Reduced α-hemolysin production	Teng et al., 2017[36]
5-benzylidene-4-oxazolidinones	Anti-biofilm	*S. aureus*	• Reduced biofilm production • Biofilm dispersion	Edwards et al., 2017[37]
Kaempferol	Anti-SrtA Anti-biofilm	*S. aureus*	• Reduced biofilm production (inhibition of initial attachment) • Inhibition of SrtA activity • Downregulation of *clfA*, *clfB*, *fnbA* and *fnbB* expression	Ming et al., 2017[38]
3F1 compound	Anti-biofilm	*Streptococcus mutans*	• Biofilm dispersion	Garcia et al., 2017[39]
Bicyclo [2.2.1] hept-5-ene-2,3-dicarboxylic acid 2,6-dimethylpyridine 1-oxide	Anti-QS Anti-biofilm	*Vibrio harveyi*	• Reduced biofilm production • Disintegrated mature biofilm • Reduced swarming and swimming	Rajamanikandan et al., 2017[40]
AIP-II peptidomimetics	Anti-QS	*S. aureus*	• Inhibition of Agr-system activity and quorum sensing	Vasquez et al., 2017[41]

Substance	Inhibitory activity	Targets	Virulence factors affected	First author, year
Metformin	Anti-QS Anti-biofilm Anti-toxin	*P. aeruginosa* PAO1	• Reduced biofilm, pyocyanin, proteases, hemolysin and elastase production • Reduced swimming and twitching motility	Abbas et al., 2017[42]
Phenyllactic acid	Anti-QS Anti-biofilm Anti-toxin	*P. aeruginosa* PAO1 and clinical isolates	• Reduced pyocyanin, proteases, rhamnolipid, and hemolysin production • Reduced swarming motility • Reduced biofilm production	Chatterjee et al., 2017[43]
Zeaxanthin	Anti-QS Anti-biofilm	*P. aeruginosa* PAO1	• Reduced biofilm formation • Downregulated *rhlA* and *lasB* expression	Gökalsın et al., 2017[44]
Triaryl derivatives	Anti-QS	*P. aeruginosa*	• Inhibition of quorum-sensing receptor LasR	Capilato et al., 2017[45]
Pyridoxal lactohydrazone	Anti-QS Anti-biofilm	*P. aeruginosa* PAO1	• Reduced biofilm, alginate and pyocyanin production • Reduced swarming and twitching motility	Heidari et al., 2017[46]
N-(4-{fluoroanilno}-butanoyl)-L-homoserine lactone N-(4-{chlororoanilno} butanoyl)-L-homoserine lactone	Anti-QS Anti-biofilm	*P. aeruginosa* PA330 *P. aeruginosa* PA282	• Reduced biofilm production	Kalaiarasan et al., 2017[47]
Flavonoids	Anti-QS	*P. aeruginosa* PA14	• Reduced pyocyanin production and swarming motility • *rhlA* transcription inhibition	Paczkowski et al., 2017[48]
3-(2,4-dichlorophenyl)-1-(1Hpyrrol-2-yl)-2-propen-1-one	Anti-QS Anti-biofilm	*V. harveyi*	• Reduced biofilm production • Biofilm disintegration • Swimming and swarming motility reduction	Rajamanikandan et al., 2017[49]
(KFF)3 K peptide-conjugated locked nucleic acids	Anti-QS Anti-toxin	*S. aureus*	• Reduced expression of *RNAIII*, *psmα*, *psmβ*, *hla*, and *pvl*	Da et al., 2017[50]
CRISPR interference	Anti-QS Anti-biofilm	*E. coli* AK-117	• Reduced biofilm formation	Zuberi et al., 2017[51]
CRISPR-Cas9	Anti-QS Anti-biofilm	*E. coli* SE15	• Reduced biofilm formation • Downregulation of *mqsR*, *pgaB*, *pgaC*, *csgE*, and *csgF*	Kang et al., 2017[52]
AR-301 (mAb)	Anti-toxin	*S. aureus*	•Neutralization of α-hemolysin	Francois et al., 2018[18]
Biaryl hydroxyketones	Anti-QS Anti-toxin	*S. aureus*	• Reduced *RNAIII*, *psmα* and *hla* transcription	Greenberg et al., 2018[53]

Substance	Inhibitory activity	Targets	Virulence factors affected	First author, year
Savirin	Anti-TCS	*S. aureus*	• Downregulation of AgrCA-regulated genes expression • Inhibition of RNAIII production	Salam et al., 2018[27]
Terrein	Anti-QS Anti-biofilm	*P. aeruginosa* PAO1	• Reduced elastase, pyocyanin, rhamnolipid, and biofilm production • Attenuated in vivo virulence of *P. aeruginosa* PAO1 toward *C. elegans* and mice	Kim et al., 2018[54]
Parthenolide	Anti-QS Anti-biofilm	*P. aeruginosa* PAO1	• Reduced pyocyanin, proteases, and biofilm production • Reduced swarming motility	Kalia et al., 2018[55]
4-amino-quinolone-based compounds	Anti-QS Anti-biofilm	*P. aeruginosa* PAO1-L *P. aeruginosa* PA14	• Reduced biofilm and pyocyanin production	Soukarieh et al., 2018[56]
Lactam hybrids of solonamide B and AIP	Anti-QS	*S. aureus* RN10829 reporter strain	• Inhibition of AgrC	Hansen et al., 2018[57]
Linear peptidomimetics	Anti-QS Anti-toxin	*S. aureus* 8325-4 *S. aureus* reporter strains	• Reduced expression of *RNAIII* • Reduced *hla* expression	Karathanasi et al., 2018[58]
Coumarin	Anti-QS Anti-biofilm	*P. aeruginosa* PAO1 and clinical isolates	• Reduced biofilm production • Down-regulation of *lasI*, *rhlI*, *rhlR*, *pqsB*, *pqsC*, *pqsH*, *ambBCDE* • Reduced protease and pyocyanin production • Reduced expression of T3SS secretion system-associated genes	Zhang et al., 2018[59]
T315 compound	Anti-biofilm	*S. enterica* serovar Typhimurium, *S. enterica* serovar Typhi, *A. baumannii*	• Reduced biofilm production	Moshiri et al., 2018[60]
2-aminobenzimidazole derivatives	Anti-biofilm	*S. enterica* serovar Typhimurium	• Reduced biofilm production	Huggins et al., 2018[61]
Peptidomimetic compounds	Anti-biofilm	*Porphyromonas gingivalis*	• Three-species biofilm inhibition	Tan et al., 2018[62]
Eriodictyol	Anti-toxin Anti-QS	*S. aureus*	• Downregulation of *hla* and *RNAIII* expression • Reduced α-hemolysin production • Reduced hemolysis	Xuewen et al., 2018[63]

Substance	Inhibitory activity	Targets	Virulence factors affected	First author, year
Prim-O-Glucosylcimifugin	Anti-toxin Anti-QS	*S. aureus*	• Reduced α-hemolysin production • Downregulation of *hla* and *RNAIII* expression • Reduced hemolysis	Ping et al., 2018[64]
2-aminoimidazole derivatives	Anti-toxin	*C. difficile*	• Reduced toxin activity	Thanissery et al., 2018[65]
Peptides	Anti-toxin	*A. actinomycetemcomitans*	• Inhibition of LtxA-mediated cytotoxicity	Krueger et al., 2018[66]
Resveratrol	Anti-toxin Anti-QS	*S. aureus*	• Downregulation of *hla*, *RNAIII* and *saeRS* expression • Reduced α-hemolysin production	Duan et al., 2018[67]-Tang et al., 2019[68]
Galloylated catechins	Anti-toxin	*A. actynomycetemcomitans*	• Inhibition of LtxA-mediated cytotoxicity	Chang et al., 2019[69]
2,6-Disubstituted imidazo[2,1-b][1,3,4]thiadiazole derivatives	Anti-SrtA Anti-biofilm	*Staphylococcus*	• Inhibition of SrtA • Inhibition of biofilm formation	Cascioferro et al., 2019[70]
HC104A and HC106A	Anti-TCS	*M. tuberculosis*	• Downregulation of the DosR regulon genes and disruption of signal transduction	Zheng et al., 2019[26]
Japonicin-2LF	Anti-biofilm	*S. aureus*, methicillin-resistant *S. aureus*, *E. coli*	• Inhibition and eradication of biofilms	Yuan et al., 2019[71]
LL-37	Anti-biofilm	*S. aureus*	• Eradication of biofilms	Kang et al., 2019[72]
vB_PaeM_LS1	Anti-biofilm	*P. aeruginosa*	• Inhibition and eradication of biofilms	Yuan et al., 2019[73]
Dpo48	Anti-biofilm	*A. baumannii*	• Eradication of biofilms	Liu et al., 2019[74]
Flavonoids (e.g., quercetin)	Anti-biofilm	*Enterococcus faecalis*	• Inhibition of biofilm formation	Qayyum et al., 2019[75]
Paraoxonases (e.g., acylase I)	Anti-QS	*Aeromonas hydrophila*, *Pseudomonas putida*, *P. aeruginosa*	• Inhibition of AHL-mediated biofilm formation	Kalia et al., 2019[76]
Carvacrol + eugenol	Anti-biofilm	*P. aeruginosa*	• Inhibition of biofilm formation	Namivandi-Zangeneh et al., 2020[77]
Auranofin	Anti-adhesion	Vancomycin-resistant enterococci (VRE)	• Adherence-inhibition activity by protease and lipase inhibitions	Abutaleb et al., 2020[78]
Clove	Anti-QS Anti-biofilm	*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *S. aureus*	• Disruption of QS communication • Inhibition of AHL synthesis	Alibi et al., 2020[23]

Substance	Inhibitory activity	Targets	Virulence factors affected	First author, year
Thyme (thymol)	Anti-biofilm	*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *S. aureus*	• Downregulation of *sar*A gene • Eradication of biofilms	Alibi et al., 2020[23]- Valliammai et al., 2020[79]
Catechin-7-xyloside/ sappanol/ butein (plant-based natural products)	Anti-biofilm Anti-QS	*P. aeruginosa*	• Suppression of quorum sensing by interaction with LasR	Zhong et al., 2020[80]
INP0341 (a salicylidene acylhydrazide)	Anti-toxin	*P. aeruginosa*	• Prevention of toxin delivery	Sharma et al., 2020[13]
vB_EfaH_EF1TV	Anti-biofilm	*E. faecalis*	• Biofilm eradication	D'Andrea et al., 2020[81]
Baicalin	Anti-toxin	*C. difficile*	• Reduction of toxin synthesis	Pellissery et al., 2020[82]
Thiazole derivatives	Anti-biofilm	Gram positive-Gram negative pathogens	• Inhibition of biofilm formation	Cascioferro et al., 2020[83]- Carbone et al., 2021[84]
Phenyl-argininebnaphthylamide (PAbN)	Anti-toxin	*Vibrio cholerae*	• Reduce cholera toxin (CT) and the toxin-coregulated pilus (TCP) production by activating a ToxR-dependent metabolic feedback mechanism	Weng et al., 2021[85]
Hibiscus acid	Anti-QS Anti-biofilm	*P. aeruginosa*	• Inhibition of violacein production • Reduction of biofilm formation	Cortes-López et al., 2021[86]
1,2,4-Oxadiazole topsentin analogs	Anti-SrtA Anti-biofilm	*S. aureus*	• Inhibition of SrtA • Inhibition of biofilm formation	Parrino et al., 2021[87]
DEXT-3 + C-30	Anti-biofilm Anti-toxin Anti-QS	*P. aeruginosa*	• Inhibition of biofilm production • Inhibition of type III secretion • Inhibition of LasR QS-system	Aburto-Rodríguez et al., 2021[30]
Lignans (sesamin and sesamolin)	Anti-biofilm Anti-QS	*P. aeruginosa*	• Inhibition of biofilm formation • Attenuation of QS pathways	Anju et al., 2021[88]
Epigallocatechin gallate	Anti-toxin	*A. actinomycetemcomitans*	• Inhibition of toxin secretion	Wu et al., 2021[16]- Chang et al., 2021[89]
Azan-7	Anti-QS	MRSA	• Inhibition of agr quorum sensing signaling	Bernabe et al., 2021[90]
Staquorsin	Anti-QS	*S. aureus*	• Inhibition of Agr-system activity and quorum sensing • Downregulation of the alpha and delta-hemolytic activities, lipolytic activity • Downregulation of the RNA III transcript	Mahdally et al., 2021[91]