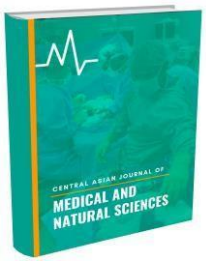

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Article

The Role of Non-Coding RNAs in Bacterial Virulence and Host-Pathogen Interactions

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Abstract: Non-coding RNAs (ncRNAs) are emerging as pivotal regulators in bacterial virulence and host-pathogen interactions. Unlike coding RNAs, ncRNAs do not encode proteins but play essential roles in gene regulation, influencing various bacterial processes critical for pathogenesis. This review highlights the multifaceted roles of ncRNAs in modulating bacterial virulence factors, such as toxin production, biofilm formation, quorum sensing, and immune evasion. Small regulatory RNAs (sRNAs), a major class of ncRNAs, act by base-pairing with target mRNAs or interacting with proteins to control gene expression post-transcriptionally. Through these mechanisms, sRNAs enable bacteria to swiftly adapt to environmental changes and host defenses, enhancing their survival and pathogenicity. Additionally, long non-coding RNAs (lncRNAs) in host cells are increasingly recognized for their role in the host immune response to bacterial infections. These host lncRNAs can influence the expression of genes involved in inflammation and immune signaling pathways, thereby shaping the outcome of infections. The interplay between bacterial ncRNAs and host regulatory networks underscores a dynamic co-evolution of host-pathogen interactions. Advances in RNA sequencing and bioinformatics have unraveled the complexity of ncRNA-mediated regulation, providing insights into novel therapeutic targets. Disrupting ncRNA pathways holds potential for combating bacterial infections by impairing virulence without exerting selective pressure for resistance. Understanding the intricate roles of ncRNAs offers a promising frontier in the study of bacterial pathogenesis and the development of innovative antimicrobial strategies. This review underscores the need for further research into ncRNAs as key players in the molecular dialogue between pathogens and their hosts.

Keywords: Non-coding RNAs (ncRNAs), Bacterial virulence, Host-pathogen interactions, Small regulatory RNAs (sRNAs), Immune response, Therapeutic targets

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1. Introduction

The cellular landscape is, to a large extent, known for the players that directly translate the genetic information into proteins. While messenger RNAs (mRNAs) have historically garnered a wealth of attention, the discovery of coding RNAs has shifted molecular biology into a new era. Non-coding RNAs (ncRNAs) are more than mere transcripts that do not encode proteins; rather, they signify important

pieces of the ever-evolving puzzle that represents biology. Various classes of ncRNAs exist, each contributing to a diverse array of biological processes. In particular, this chapter delves into the world of the regulatory ncRNAs, which contribute to the fine-tuning of gene regulation in all domains of life. While the world of RNA research has long been primarily concerned with protein synthesis, it is becoming more and more evident that gene expression is influenced by several classes of ncRNAs. Once considered transcriptional noise or random degradation products, ncRNAs are now known for their roles in gene silencing, thermoregulation, splicing, DNA damage repair, vertical transmission, and origin of replication synthesis (Statello et al.;2021; Zhao et al., 2021).

This novel and intricate layer of regulation is emerging as a topic of interest, not only in organisms with eukaryotic origins, as shown by the comparative analysis of large-scale transcriptome data. Provoked by these findings, the study of prokaryotic ncRNAs now constitutes a vitally important and rapidly expanding field of research. Of particular interest within the prokaryotic RNA world are a class of molecular players that promote an entirely new layer of genetic regulation: those that function at the interface of host-pathogen interactions (Zhao et al., 2021; Li et al., 2022).

1.1. Definition and Classification

The so-called "junk" DNA or the "dark matter" of the genome, which represents approximately 98% of the human genome, has been the subject of many studies. One great part of this "non-coding" region is typically transcribed into several nucleotides long RNAs with intriguing functions. Non-coding RNAs (ncRNAs) can be defined as RNAs that cannot be translated into proteins, although their sequences are transcribed by Pol III, Pol II, or Pol I. They can be further grouped into three classes based on their length, biogenesis, origin, and function: (1) small ncRNAs, which typically include RNAs with fewer than 200 nucleotides, such as microRNAs, siRNAs, small nucleolar RNAs, transfer RNAs, ribosomal RNAs, RNA-protein complexes, and so forth; (2) long ncRNAs (lncRNAs), with RNAs that have more than 200 nucleotides; and (3) medium-length ncRNAs (mtrRNAs) with RNAs that we may not categorize as small or long (Poliseno et al., 2024 ;Zhou et al., 2021).

Small ncRNAs, also known as small silencing RNAs, are significantly important mostly due to the perfection of the RNAi process. miRNAs are small RNAs that regulate gene expression by translational repression or degradation of messenger RNAs. Following a complicated biogenesis, a small RNA duplex is then loaded into the RNA-induced silencing complex and is carefully scanned by the Argonaute protein, which is at the center of the complex. Based on its size and the cleavage position, small RNAs are divided into siRNAs that are then directly converted into new small RNAs by re-cleavage of the complex, and miRNAs that may or may not require further maturation into small RNAs, and are not believed to cleave their targets into new small RNAs. The mechanism of siRNA and miRNA function may explain their role in silencing and sprinkling of genetic mobile elements. The last decade has thus been marked by an unprecedented expansion of knowledge but also by an increase in complexity in the RNAi field. At an exponential rate, an excessive inflow of discoveries has been steadily oversizing our initial concepts about RNAi as a transcription silencing and guiding mechanism limited in scope to a few species and genetic phenomena (Lu et al., 2020; Statello et al.,2021).

Yet, due to its versatility, RNAi keeps on expanding its range of action and acquiring new roles for which matching new terminology becomes a necessity. Thereafter, it feels like a good time to take a new look into the facets and fine details of RNAi as we understand it today to rise into a helpful redefinition. In this review, we define it as the set of small RNA-involved pathways that regulate and protect the genome and the epigenome. The first statement stipulates that RNAi is a group of distinct multiple pathways, each with a potential for added branching, diversification, and modulation of the main flow of small RNA silencing or that may

engage in distinct roles in essential regulatory structures (Ali et al., 2019; Statello et al.,2021; Palazzo & Koonin.,2020).

1.2. Biogenesis and Function

Non-coding RNAs (ncRNAs) can be encoded at several genomic sites, including the intergenic regions, repeat sequences, pseudogenes, and introns. Some ncRNAs are expressed independently of the neighboring coding genes, while others can have the same regulatory mechanisms and are often co-transcribed from a common promoter. Multiple physical and functional interactions have been described between ncRNAs and proteins, such as sigma subunits of RNA polymerases, ribonucleases, RNA helicases, chaperones, and dead-box ATP-dependent helicases. In certain cases, ncRNAs can induce the structural switching of regulatory RNA and mRNA sequences, but they can also be activated or inactivated by proteins. Sialidase-induced transmembrane pathways can be inhibited by a multi-drug resistance gene, which limits therapeutic options for the treatment of bacterial infections (Mayo-Muñoz et al., 2024; Pita et al., 2020).

Bacterial ncRNAs can be encoded in common operons; their non-coding nature is due to a specific post-transcriptional processing of precursor RNA. These precursor RNAs can be liberated from their surrounding coding regions or from longer primary precursor RNAs through endoribonucleolytic cleavage and/or exonucleolytic degradation. The precise mechanisms for the generation of non-coding precursor RNAs are very diverse, but most processing steps occur while the RNA is still in the initial transcript. During their biogenesis, many precursor RNAs associate with chaperone-like proteins and special RNA-binding proteins. Although some ncRNAs are degraded by exonucleases, this is definitely not the only fate for ncRNAs, and roles are defined for many of these ncRNAs in the cells, including protective aspects. Transcription-associated RNA processing converts primary long precursor RNAs into rapidly degrading and/or maturing functional small RNAs. Polycistronic operons transcribe multiple mRNAs in a single long mRNA. The production of small RNAs adds another layer to the post-transcriptional processing in which distinct sites along a polycistronic mRNA generate dedicated mRNAs, each carrying untranslated sequences specific to the previous operon structure. Small RNAs can also be part of the multilayered expression control networks of complex multicellular organisms, including the regulation of cellular development and differentiation, major levels of cellular metabolism and metabolic integration, and effective responses to a wide range of extra- and intracellular stress conditions (Taha et al., 2019; Pita et al.,2020; Xu et al.,2024; Wen et al., 2020).

The widespread existence of ncRNAs and their overall regulatory functions are now well established. There is also ample evidence that ncRNAs function in a very diverse breadth of physiological systems, including the regulation of many stress response pathways. Non-coding RNAs have been recognized as important controllers of gene expression. The identification of their roles has also demonstrated that key determinants of cellular homeostasis are under post-transcriptional control. Most of our knowledge about ncRNAs in bacteria originates from their effects on virulence malfunction in human pathogens. Given that the expression of virulence genes is an important determinant in the transition from a commensal lifestyle to pathogenicity, the role of ncRNAs in the regulatory networks governing pathogenicity is important (Xu et al., 2024; Wen et al., 2020; Antoine et al., 2021).

2. Bacterial Virulence Mechanisms

The virulence of pathogenic bacteria and their overall pathogenic success in the host are dependent on a combination of different virulence factors. These factors can be classified according to different criteria, resulting in classes such as adhesins, invasins, toxins, and evasion strategies. Importantly, gene products that enhance pathogenic success in one category can also often fulfill criteria in other categories such as iron acquisition and immune system evasion. The combination of factors simultaneously already made it clear that these factors need to be orchestrally

regulated in both time and space. This multigenic regulation is dependent on complex hierarchical regulatory networks (Saeki et al., 2020; Ghanem et al., 2023).

Another important strategy bacteria have developed to adapt to their host environment is communication with each other. It has been a hot topic lately, also referred to as quorum sensing. Quorum sensing is known to be necessary in the establishment of successful infections as it initiates the expression of virulence factors, which enable the bacteria to elude host defenses. Through their virulence factors, bacteria can also contribute to the establishment of the host environment, from which they will benefit. In addition to this, pathogens often use virulence factors to outcompete the beneficial microflora residing in their direct habitat. High molecular diversity is shown by bacteria in the development of toxins. The host organism exhibits a wealth of cells in blood and tissues, many of which include endocytoses in their cytoplasm, which can render the host environment more habitable. Alternatively, they can use the damaged and dead cells. The use of dead and damaged cells as a carbon source also serves to promote the growth of the bacteria to achieve such high cell numbers. The influence is immense that bacteria have on the immune system. They have developed mechanisms to distinguish between innate and adaptive macrophages of the stress response. Understanding the repertoire of virulence factors identified will increase the chances of identifying new potential targets for non-bacterial infections that enhance virulence (Ali& O.A 2019; Ghanem et al.,2023; Munir et al.,2020; Haque et al.,2021).

2. Materials and Methods

Virulence is a term used to describe the extent of the damage that a pathogenic organism causes to its host. Pathogens possess factors known as virulence factors (VFs) that enable the invaders to establish themselves in a host and subvert the host immune response. These factors are numerically grouped into three major categories: adhesins, factors facilitating invasion, and factors protecting or evading the immune response. The adhesins mediate and facilitate the attachment of the microorganism to the host. Adhesins can be cell adhesins and extracellular matrix adhesins that enable the pathogen to attach to the surfaces of tissues. Factors facilitating invasion of the host come in the form of toxins, cytolysins, and bacteriocins of Gram-positive signaling. Lipopolysaccharides and the capsules of host cell penetration, endotoxins, and superantigens represent some virulent factors that enable bacterial pathogens to realize the shielding and immune-evasion functions (Diallo & Provost., 2020 ; Mayo-Muñoz et al.,2024).

Microorganisms will have a reduced infective potential without the existence of VFs and will be diverted from the ocean of normal microflora. The outcome of an encounter between a bacterium and a host cell that is favorable to the growth of the bacterium will result from the establishment of a useful environment for a bacterial colonist, marked by a lack of death or clearance of the bacterial colonist and also an increase in bacterial multiplication. Biological phenomena can involve cell attachment, stress alleviation, signal propagation, obstacle bypass, nutrient extraction, increased tolerance to unfavorable conditions emanating from the surrounding, reduced ligament loss, and acquisition of antimicrobial resistance. The studies of bacterial pathogenesis are gradually revealing a topic of interest, and the enormous quantity of information accumulated on the biology of microorganisms is unraveling another function. In particular, the studies on non-coding RNA, small RNA or RNA that does not encode protein, associated with the virulence of extra and intracellular human microbial pathogens, are increasingly becoming popular. The search for these ncRNAs coupled with the trans-acting antisense RNAs of bacteria is increasing. While the current function of these bacterial ncRNAs now includes translational repression and gene silencing as well as response adaptation, it is possible that more functions would be ascribed to them in the future given that new biological discoveries would be made. Bacteriologists have thereby increased their interest in the analysis of ncRNA of pathogens (Diallo & Provost.,2020 ; Shi et al., 2024; Pita et al., 2020).

Structural and functional mechanisms, molecular-genetic modes of action, and case-study examples of the virulence factors were collated to provide further details. Interaction with each one will maximize the astonishment in respect of effective training and manipulation, which will enable researchers to employ the vast horde of molecular knowledge on bacterial virulence to field or wide operating systems (Abbas et al., 2019; Shi et al., 2024;Pita et al., 2020).

2.2. Regulatory Networks in Bacterial Virulence

Bacterial cells often adopt specialized lifestyles in response to changes in their environment. In many cases, these lifestyles involve the deployment of multiple virulence determinants, and the reversion to one of these lifestyles allows the pathogen to persist or escape the host. Expression of virulence factors is not fixed but rather is plastic and changes in response to various environmental cues. At the heart of these regulatory networks are virulence regulators whose activity is influenced by environmental cues that include nutrient levels, temperature, oxygen tension, pH, and various stresses, including the presence of reactive oxygen species (Chauvier & Walter.,2024; Quendera et al., 2020).

Virulence gene expression is also induced in response to the host environment, and upon entry into host cells, the intravacuolar pathogens that breach the phagosome deploy a second round of factors. These include systems that secrete effector proteins that directly act on host functions and systems that encode effectors that promote uptake or intracellular survival. Regulatory components often belong to signal transduction pathways that allow the bacterium to sense and respond to specific stimuli and alter gene expression in response to these signals. Specific mechanisms that bacteria utilize to alter the level of gene expression comprise sensory proteins that function as signaling receptors, transcription factors that bind the DNA and regulate the expression of target genes, component or two-component systems that modulate the activity of separate transcription factors, and feedback regulators that are targets of specific signals and repress or promote their own expression. Global regulators can be controlled by multiple influences and activate or repress large sets of genes to orchestrate the virulence traits of a pathogen (Chauvier & Walter., 2024; Quendera et al., 2020; Mukhopadhyay., 2023).

Consistent with the discovery of a pivotal role for regulatory RNAs in gene expression in bacteria and non-coding RNAs, a growing number of non-coding RNAs have now been characterized, which also function as major regulators of bacterial growth and virulence. In the simplest scenario, the regulatory component binds to a single protein or RNA target, but interaction may also occur between distinct regulatory proteins that can elevate the complication of the network. The simplest scenario is that a single signal impinges on the system, and changes in one level of the cascade then elicit feedback control of the system, whereas in the complex examples, there are feedback and feed-forward loops that make the system oscillate in expression. The analysis of bacterial pathogens that cause chronic infection has revealed a complex regulatory network that utilizes genetic diversity and plasticity to adapt to new stresses. The analysis of signal transduction networks allows the design of intervention points and therapeutic strategies to target pathogenic bacteria. A focus on regulatory networks reveals the control points that allow control of bacterial virulence and the survival mechanisms elicited by infection (Quendera et al., 2020;Mitra & Mukhopadhyay., 2023;Diallo & Provost., 2020).

3. Host-Pathogen Interactions

The interactions between bacterial pathogens and their hosts are mostly seen as dynamic evolutionary battles in which both sides try to outsmart the other. Against these antagonistic outcomes, various immune response mechanisms exist that are in place to hunt, kill, or stop the invading microbe and to prevent disease. They range from inhibition of bacterial attachment to physical and chemical barriers, recognition of the microbe, and phagocytosis and complement activation as innate defense mechanisms to the engagement of antigen-specific lymphocytes as part of the

adaptive immune response. Pathogens, in turn, have evolved strategies to overcome and evade these host defense mechanisms (Moure et al., 2022;Mehta et al., 2024).

Understanding these evolutionary arms races can help us in our quest to develop anti-virulence compounds or successful prophylactic and therapeutic vaccine interventions. During infections, indirect modulation of the bacterial pathogenic arsenal by virtue of host cell defense mechanisms is also crucial. Many non-coding RNAs seem to play a role in modulating these host-pathogen interactions by affecting both bacterial and host cell functions. In host cells, for instance, bacterial ncRNAs can affect the immune system, as exemplified by certain extracellular ncRNAs known to manipulate the host's immune response, bacteria that secretes RNA into the cytosol of host cells to alter the immune response, and ncRNAs that downregulate human immune responses. It is generally suggested that host-pathogen interactions co-evolve, with co-adaptation of 'attacker' against 'defender' imposed by the presence of the opposition (Moure et al.,2022; Mehta et al., 2024).

It is known that both sides of the host-pathogen spectrum, i.e., the host's immune defenses and pathogens including their virulence-associated traits, co-evolve and adapt to each other. Co-evolution of hosts and their pathogens may lead to the fixation of rare immunity alleles and pathogen alleles that increase resistance and infectivity, respectively. In other cases, co-evolution of R-gene effectors and against-pathogen alleles in both host and pathogen populations can prevent both immunity and infection from getting fixed. In theory, co-evolutionary strategies can be complementary in the host and pathogen (Leitão et al., 2020;Pita et al., 2020).

3.1. Immune Response to Bacterial Infections

During an infection, several immune processes are activated to clear the infection from the host. These processes start from the entry of the pathogen into the host body and range up to the recovery phase. The host responses during an infection are attributed to immune responses. The immune system can be broadly classified into innate and adaptive immunity. The innate system is the front line of defense and provides a rapid response to pathogen entry, whereas adaptive immunity facilitates long-lasting responses to pathogens. The first part of the host immune system that encounters bacteria is the epithelial barrier. The mucosal epithelium is followed by the lamina propria, which is the immune inductive site for the gastrointestinal tract, bronchial tree, and the reproductive tract (Domínguez-Andrés & Netea., 2020; Sharrock & Sun., 2020).

The genetic program for antimicrobial responses in the host is established in immune cells, e.g., macrophages and natural killer cells; this is collectively described as innate immunity. The innate immune system lacks any immunological memory. The transfer of cells to adaptive immunity is orchestrated by myeloid cells, which are broadly categorized as granulocytes and mononuclear cells. These myeloid cells synthesize and release biologically active proteins, enzymes, hormones, etc., which help to control and limit the replication and infectivity of infective agents, attract and activate leukocytes of the immune system, and facilitate the repair and regeneration of new cells. Pathogen recognition and response signaling in susceptible hosts are the results of the activities of various signal molecules such as cytokines, chemokines, membrane receptors, surface markers, colitins of immune cells, reactive oxygen and nitrogen radicals, and innate immune-specific antimicrobial peptides, some of which are implicated in the case of some non-sterile human body tissues and in sterile body tissues (Sharrock & Sun., 2020; Sherwood et al.,2022;Stosik et al., 2021).

3.2. Impact of Non-Coding RNAs on Host Defense Mechanisms

Various non-coding RNAs (ncRNAs) exert regulatory effects on immune cells. For example, secreted micro-RNAs (miRNAs) have been taken up by monocytes, macrophages, or dendritic cells and subsequently led to the suppression of Toll-like receptor expression and a decrease in pro-inflammatory cytokine production. In the context of bacterial infections, numerous miRNAs are known to be modulated upon direct exposure to bacterial components or direct bacterial challenge. A single

miRNA is predicted to modulate the expression of possibly hundreds of genes by complementary binding to untranslated regions. This illustrates the potential to have extremely broad effects on the immune response. Furthermore, overexpression of a single miRNA ex vivo led to widespread changes in pro-inflammatory cytokine release or pathogen survival and clearance, thus emphasizing their contribution to modulating the immune response. Released exosomal miRNAs in the serum of patients have been suggested to serve as potential biomarkers to predict an ongoing disease or treatment (Mathur et al., 2024; Huang et al., 2023).

Survival and pathogenesis of bacterial infections also rely on the engagement of various endogenous miRNAs; for instance, by inhibiting self-protective pathways of the host immune system or suppressing pathogen clearance. Transcriptional changes via direct or indirect miRNA targets could accompany the regulation of immune responses upon bacterial attack and directly affect host-pathogen interactions. Importantly, many bacterial pathogens were shown to secrete various ncRNAs and ncRNA-associated molecules, although the exact role of ncRNA secretion has not been investigated in detail and its action has predominantly been shown in a holistic manner. This indicates that the release of regulatory RNAs is an important aspect of host-bacteria cross-kingdom signaling. Since the regulatory networks associated with ncRNAs add a level of complexity to host-pathogen interactions, it can be concluded that a better understanding of the global effects of these RNAs might open up new opportunities for prevention and therapy in bacterial infections, including antibiotic resistance (Huang et al., 2023;Tang et al., 2021;Vasquez et al., 2022; Taha et al., 2024).

4. Non-Coding RNAs in Bacterial Virulence

Small non-coding RNAs (sRNAs) are essential gene regulators involved in a plethora of physiological processes in bacteria. They generally arise from the intragenic regions (size varies from 50 to 500 nt) of protein-coding loci and act at the post-transcriptional level. Their main activity is to modulate the expression of mRNAs coding for major virulence traits by base-pairing to target sites in these transcripts. In vivo studies have provided evidence of their contribution to pathogen behavior, including their pathogenic trajectory. To date, many virulence-associated bacterial ncRNAs (vcRNAs) have been identified in a variety of pathogenic bacteria, including uropathogenic Escherichia coli, Salmonella enterica, and Listeria monocytogenes, among others. In these bacterial species, and especially in the closely related genus Vibrio, the most important and preponderant type of vcRNAs is the small RNAs (sRNAs) (Ostrik et al., 2021; Wang et al., 2024; Mohammad S et al., 2024).

The molecular mechanisms and regulation of numerous sRNAs and novel ncRNA elements that impact bacterial processes such as virulence have also been demonstrated in several bacterial species. The relevance of these molecules has been the object of intense investigation. VcRNAs, such as sRNAs, have been shown to impact diverse processes, including inter-bacterial communication, metabolism, and virulence. They have the potential to be involved in determining the molecular makeup underlying the invasive properties of bacteria. VcRNAs are often direct or indirect modulators of bacterial transcriptional networks and/or directly modulate the protein levels of virulence pathways in specific bacterial systems. Simple, life-sustaining pathways, such as the utilization of scarce nutrients like iron and sensing of nutrients, as well as more complex stress management responses, have been shown to be influenced by vcRNAs, thereby dynamically impacting pathogenesis. It is, on the other hand, much more complex to elucidate the downstream effects of vcRNA function in terms of changes in bacterial infection dynamics in vivo (Wang et al.,2024; Meng et al., 2022; Diallo & Provost., 2020).

4.1. sRNAs in Bacterial Pathogenicity

Additionally, extrachromosomal DNA often carries virulence and antibiotic resistance genes. While sRNAs of pathogens, like any other bacterial sRNAs, modulate the expression of such genes at the post-transcriptional level through base-

pairing with target mRNAs, they contribute to the full virulence potential of the bacteria. This function was first demonstrated for the sRNA RsmZ in the soft-rot pathogen. In Bacillus anthracis, the sRNA FasX is among the most increased sRNAs in the pathogen during host interaction. An attenuated fasX mutant is attenuated in vivo, and FasX potentially modulates toxins involved in immune evasion. In recent years, several bacterial sRNAs have been identified, which were found to interfere with the expression or secretion of effectors or to facilitate the adaptation of the bacteria to different host conditions such as protease or pH levels, and thereby contribute to bacterial virulence (Ostrik et al., 2021; Wang et al., 2024; Khaledi et al., 2024).

In Yersinia pseudotuberculosis, the so-called PTP sRNAs, involved in the control of pathogen-specific virulence determinants and cold-stress response, are differentially expressed and regulated in close proximity to the mammalian upper respiratory tract. While for Hfq, a central component of the ribonucleoprotein complexes containing sRNAs and their target mRNAs – called sRNPs – an involvement in the host-pathogen interaction was observed, middle-sized and small non-coding RNAs in Brucella ovis and Brucella suis are differentially expressed under in vitro stress conditions and during macrophage infection. Several of them were also predicted to be involved in stress response. Today, small non-coding RNAs are increasingly found in different genomic studies to be misexpressed in pathogenic bacteria. Because of the differences in their expression profiles, they represent promising molecular markers suitable for differentiation between the respective pathovars. In addition, they are potential targets for antivirulence strategies in the human pathogen and the plant pathogen. An additional window of applicability of sRNA research for clinical microbiology emerges with the increasing resistance problem. In light of the increasing number of urinary tract infections with uropathogenic due to the emerging resistances to antibiotics, sRNA could serve as a therapeutic target, since synergies with resistance genes are not expected from the microbial point of view. In conclusion, sRNAs have been shown to be putative targets for future drug development and open up new avenues for the control of bacterial infections (Diallo & Provost.,2020; Jørgensen et al., 2020; Felden & Augagneur., 2021; Khaledi et al., 2024).

4.2. Riboswitches and Riboregulators

Riboswitches and riboregulators are dynamic RNA elements that play a fundamental role in gene regulation in bacteria. Specifically, riboswitches are defined as genetic regulators that are entirely composed of RNA, the structure of which has the capability to change in response to specific ligands or metabolites, leading to transcription termination, reduced ribosome binding, or directed transcriptional initiation during transcription to thereby control gene expression. Riboswitches are typically found in the 5′-untranslated region (5′-UTR) of mRNAs of essential genes and can either activate or repress the expression of the adjacent gene(s). On the other hand, riboregulators control gene expression by interacting with other RNA molecules. Similarly to riboswitches, riboregulators are also considered pH-type genetic switches as they control gene expression based on their three-dimensional molecular structures and are often base-pairing, which enables the riboregulators to modulate gene expression of their target genes. From the many regulatory ncRNAs present in bacterial and archaeal cells, only a small percentage have been characterized thoroughly (Diallo & Provost., 2020;Wen et al., 2020).

The adaptability and survival of bacteria is influenced by their regulation. To date, non-coding regulatory RNA elements have been identified that influence the virulence and bacterial survival mechanisms in several pathogens. For instance, in Staphylococcus aureus, the production of toxins and other virulence factors can be directly regulated by a plethora of non-coding riboregulatory and riboswitch RNA elements, creating an appropriate response in changing environments. Such non-coding RNA expression can either be turned on to actively repair the pathogen or be turned off as an act of evolutionary slyness to escape the host immune system and

the effectiveness of antibiotic drugs. Therefore, the in-depth understanding of these dynamic fluctuations that occur in bacteria during human infections and surrounding tissue may reveal an alternative therapeutic approach. In this regard, a possible future direction of research could be to develop an anticis-complimentary molecule that can hybridize with the ncRNAs of the successful pathogens and allow the bacteria to express harmful traits in order to identify and destroy or weaken it using vaccines developed to target the activated toxin-resistant vectors and immune evasion mechanisms, which is the increased expression in S. aureus (Wen et al., 2020 ; Wang et al., 2024; Khaledi et al., 2024).

5. Experimental Techniques for Studying Non-Coding RNAs

Given that bacteria are known for their adaptability and efficient regulation of gene expression at both the transcriptional and post-transcriptional levels, several techniques have been developed for studying ncRNAs and their functions. Here, we discuss some of these techniques in detail, including the most advanced tool for studying ncRNAs, RNA sequencing. This technique facilitates the identification and quantification of all ncRNA species inside a cell, free from known prejudices and without any prior knowledge of the gene complements. It allows for the unbiased genome-wide dynamics of ncRNAs to be investigated under different conditions and environments. As the field has experienced major technological breakthroughs, we can now study ncRNAs with base-pair resolution in base-pair detail with the possibility to quantify the effect genome-wide. Different RNA sequencing methods to investigate differential gene and miRNA expression, transcript assembly, gene fusion discovery, and small RNA expression offer a vast array of possibilities (Diallo & Provost., 2020; Diallo et al., 2022).

Furthermore, ncRNA identities can be obtained, often in greater detail, by using imaging methods and proteomic applications. Again, with increasing sensitivity and decreasing costs, ncRNAs are not only attributes of interest but can be integrated at the outset in most experimental workflows. In summary, in addition to providing a comprehensive picture of the non-coding RNA transcriptome, RNA sequencing can also be used to identify novel small regulatory RNAs. Their differential expression serves not only as an indicator of condition-specific functions but can potentially be the cues necessary to identify their mechanisms of action in both bacteria and in the host environment. Identifying relevant ab initio ncRNA and ncRNA-mRNA target pairs contributes new steps for the workflow in which RNA techniques can be integrated. In addition, beyond the role of ncRNA regulation, RNA-seq methodology has been used in conjunction with Tn-seq and proteomics to demonstrate the influence of the core gene expression machinery on pathogenicity in the host bacterial environment, thereby expanding the field for large-scale applications of ncRNA research (Felden & Augagneur., 2021; Han & Lory., 2021; Coleman et al., 2020).

3. Results

"RNA Sequencing" (Hypothesis: RNA-seq is a powerful tool to investigate sRNAs and mRNAs in bacterial infectious diseases.) This new 5.1 is added to the manuscript as follows: RNA Sequencing. RNA sequencing is an advanced technology that has been widely used to study ncRNAs in bacterial pathogenesis. Traditional low-throughput sRNA and mRNA identification typically used the clone-and-sequence approach. Cloning requires specific knowledge of sRNA characteristics and sRNA-associated structures. It can produce inaccurate results by generating a large number of false positive/negative sRNAs. DNA clusters are sequenced after cloning. RNA sequencing (RNA-seq) is now the most accurate and effective high-throughput method for transcriptome analysis used to study many bacterial organisms. RNA-seq generates hundreds of thousands to millions of sequences produced from the sRNAs and mRNAs of the pathogen and its host in a

single run. RNA-seq has three steps. The first is to prepare the sample for sequencing. In this step, we purify RNA, rRNA-deplete, reverse transcribe, and add adaptors. The second is to sequence the RNA library in an RNA-seq system. The third step is to analyze and interpret the sequence data (Ostrik et al., 2021; Pita et al.,2020; Shi et al., 2024; Intisar khlaif flaifel et al., 2023).

Most RNA-seq protocols have been used to investigate mRNA–disease and sRNA–disease interactions. There are multiple variations of RNA-seq technologies that have been developed for high-throughput and in-depth analysis of microbial transcriptomes. These include sRNA-seq, total RNA-seq, and consecutive-RT qPCR as well as variations of traditional RNA-seq that receive both the transcriptome of the pathogen and host. RNA-seq also sequences the DNA first stranded to the first and second cDNA to maximize detection of RNA. This is a high-throughput sRNA-seq technique by combinatorial reverse transcription qPCR that sequences reverse transcribed cDNA for all sRNA species in a single cDNA synthesis reaction. While RNA-seq is powerful, there are limitations. RNA-seq techniques can sequence sRNAs from both bacteria and their hosts, but it cannot study the function of sRNAs as well as their post-transcriptional products and interactions (Shi et al.2024; Aliaga-Tobar et al.2018; Cheng et al., 2024).

5.2. Functional Assays

Experimental manipulation remains the best way to investigate the biological function of a newly identified ncRNA. Tools like primer extension, northern blotting, and RT-qPCR are applied to verify the expression and to quantify the differences in nucleic acid levels under specific conditions, indicating a role in virulence or a direct connection to a virulence factor. In addition, a series of functional assays are used to assess the biological function of newly expressed ncRNA. Ideally, these functional assays contribute to the area of host-pathogen interactions and generate new hypotheses for further functional studies. The range of relevant functional assays is considerable; here, we cover only those assays used in a functional follow-up approach in which co-infection or in vivo competition experiments, or, as a second choice, in vitro infection studies, are designed and complement as much as possible from assays used for eukaryotic pathogen studies. Reporter assays are widely used to validate the overexpression of an ncRNA or sRNA, or the controlling mechanisms, which allow the detection of direct targets (Shi et al., 2024; Gao et al., 2020).

Importantly, several gene of interest-target combinations are analyzed after overexpression of an sRNA. If literature reports exist, meta changes of the wild type, gene of interest overexpression, and sRNA overexpression studies can be integrated as parts of a co-expression and interaction network leading to stronger validation. Knockdown and antisense approaches are thought to be very powerful because of the deletion of the complete genomic copy. A knockdown strain should be complemented with the genomic and promoter fragments fully annotated together with the wild type system, overexpression, and antisense of the ncRNA. It should be mentioned that different challenges can hamper the setup and the validation of functional studies. Often, the functional assays can be complex when trying to find the perfect condition to check for the bacterial virulence function and could result in difficulties for consistency. Moreover, it can be hampered by defects in the quality of

methodological determinants such as biological conditions, confounders, or methodological approaches (Gao et al,. 2020; Srinivas et al., 2024).

The study of regulatory ncRNA has potential implications for diagnostics, pathogenicity, and pathogenesis, as it does for all parasites. In the context of host-pathogen interaction, there are some interesting examples of studies where informative data have been obtained. The past few years have seen increasing effort in the discovery of new ncRNA elements, and strands of investigation are being opened up, especially in the fungal field. It is therefore recommended that functional assays be included in an overall research strategy to explore the function of ncRNAs, as the effects of pathogenic bacteria need to be understood at the system level to gain insight into the pathogenic mechanisms. Using nano-ribonucleic acid gene knockdown strategies, we targeted secondary intergenic stable RNA in Brucella abortus. These experiments aimed to validate uncharacterized transcripts that were identified as differentially expressed or unique to B. abortus during infection. Systemic RNA silencing following liposome delivery of AS-PNA resulted in significant potencies of specific gene targets in supporting tissue growth. Specific antisense oligos may potentiate the activity of other drugs and reduce mycobacterium resistance to host mechanisms. Further studies also specify the ncRNA targets with the PNAs for the growth of Bartonella bacilliformis, while in Streptococcus pneumoniae, the knockdown of 6S RNA by the AS-PNAs reduced the growth rate of the bacteria in vitro (Srinivas et al., 2024; Graf & Kretz., 2020 ; Bravo-Vázquez et al.. 2024).

6. Therapeutic Potential of Non-Coding RNAs

The information about the ncRNA candidates as therapeutics against bacterial infections has grown exponentially in the last decade. The increasing number of novel tools and strategies to develop innovative therapeutic strategies for the treatment of bacterial infections is a need due to the stagnation of antibiotic discovery programs and the emergence of multidrug-resistant pathogens. These new tools are based on the use of therapeutic agents that do not affect cell viability but modify the gene expression of specific disease-associated virulence factors, making bacteria more susceptible to host immune responses. Among these new approaches, the use of antisense oligonucleotides is one of the most promising. Antisense oligonucleotides are short single-stranded DNA or RNA molecules designed to modulate gene expression by specifically binding to their complementary target RNA due to their Watson-Crick base pairing and, therefore, interfering with the process of translation of the targeted gene (Zhang et al., 2020;Traykovska & Penchovsky., 2022).

The use of antisense oligonucleotides has been proposed to reduce the expression of molecular factors related to bacterial lifestyle, bacterial virulence, and host-microorganism interaction. For example, antisense oligonucleotides have demonstrated their ability to bind complementarily to the sRNAs to release the targeted mRNAs from the sRNAs' “sponging effect.” In the future, ncRNA antivirulence elements could also be included in a chimeric RNA-based vaccine. Such a strategy would allow delivering novel vaccinal antigens to target bacterial species without the need to purify them from the bacteria. Vaccines based on ncRNAs could be particularly immunogenic as they can be recognized by far more

TLRs than proteins. In light of this evidence, the number of studies focused on sRNAs as vaccines has been increasing over the last few years. Some of these studies involve animal infection as a proof of concept, demonstrating the potential use of this approach to develop sRNA-based RNA vaccines (Traykovska & Penchovsky., 2022; Angrish & Khare., 2023; Tekintaş & Temel., 2024).

6.1. Antisense Oligonucleotides

One therapeutic strategy used to target non-coding RNAs is the use of antisense oligonucleotides. These are synthetic nucleic acids that specifically base pair with complementary RNA sequences and lead to the modulation of target gene expression within the cell. There are several potential mechanisms of action by which the different types of antisense oligonucleotides could exert antisense effects on bacterial pathogens. Antisense therapies have several potential advantages, such as reduced selection of antibiotic resistance, lower probability of pathogens developing resistance, and few side effects on the microbiota. The expression of multiple virulence factors could be altered, further preventing the pathogen from successfully establishing infection (MacNair et al., 2024; Jani et al., 2021) .

Nonetheless, there are many potential challenges in the research and development of antisense drugs that are important to solve in order to bring these treatments to the patients. While their dependency on the presence of specific RNA targets in the pathogen has relegated antisense therapies to a niche application in antiviral treatments, the recent experimental evidence regarding the ability of antisense oligonucleotides to promote clearance of bacterial infections has increased the interest towards other potential applicability of antisense treatments in bacterial infections. In recent years, multiple in vivo studies that employed antisense treatments have shown the therapeutic potential of this oligonucleotide in experimental models of established acute, chronic, and hard-to-eradicate bacterial infections. Conventional and in silico screening tools for efficient antisense drugs are before formal implementation to bacterial pathogens, as many of these tools must be directly developed for the specific case of bacterial infections. The local efficacy of antisense oligonucleotides against infections should be confirmed in appropriate local infection models, as the pharmacokinetics and pharmacodynamics of these drugs are expected to be different from antibiotics. Additionally, antisense drugs should undergo specific pharmacodynamics and targeting studies (Pals et al., 2024; Crooke et al., 2021).

4. Discussion

Until now, non-viral vaccines are not mainstays in the prevention or treatment of bacterial infections. However, as a novel therapeutic option, the development of vaccines based on messenger RNA (mRNA), the functionality of which depends on extracellular delivery of a molecule-utilizing vector system, is recommended for Listeria monocytogenes and Mycobacterium tuberculosis. In injected RNA vaccines, mRNA encoding pathogenic proteins or key antigens delivered to the host cell are translated to corresponding protein molecules, stimulating a robust immune response. Furthermore, the injection of mRNA encoding antigens in antigen-presenting cells (APCs) leads to translation in those cells and MHC I-dependent presentations to CD8 T cells, which allows the induction of both antibody and T-cell-

mediated immune responses. Although algal vectors are being studied as mRNA delivery systems, conventional vesicle-mediated, physical conditioning methods, and mRNA delivery systems are available (Nunes-Silva et al., 2022;Fernandes., 2024).

As such, soluble mRNAs are stored in cell culture medium at 4 °C to ensure the transcript and transfection reagents maintain their integrity. Upon reaching room temperature or upon thawing from cryo-storage, the transcript of transfection complexes can be printed directly in the cell culture medium and added immediately to cells. As an unproven vaccine strategy in clinical practice, RNA vaccines have advantages, including their role as a rapid response to vaccine-preventable pandemics, relatively safe profiles, and ease in design and multiplex antigen printing. In clinical practice, nucleoside-modified mRNA-based vaccines have been shown to have strong immunostimulatory effects, initiate durable cellular and humoral immune responses, and provide indirect long-term protection. Furthermore, successful clinical studies of tumor vaccines and infectious disease vaccines have demonstrated the multifunctional, antiviral, or anticancer properties, and poly-specific CD4+ T cells and epitope spread. There are also other successful clinical studies: a multivalent mRNA vaccine coadministered with a variety of adjuvants induced a robust B cell and T cell response; an unmodified wild-type mRNA-based vaccine encoding a tuberculosis antigen induced strong Th1 and Th1Th17 cells. RNA vaccine therapy has an increased response rate when used as a vaccine therapy. Production, storage, transportation, product toxicity, side effects, and public acceptance are major challenges for RNA vaccines (Fernandes, 2024; Fernandes et al.,2022; Van Gerwen et al., 2022).

7. Current Challenges and Future Directions

The consistent advances in the field of non-coding RNAs (ncRNA) and their involvement in almost all aspects of bacterial life and behavior have led researchers to consider new prospects for developing more effective therapeutics for infections. However, there are still some controversies and unknowns about ncRNAs and how they can be successfully used in a clinical setting. Technological drawbacks, such as those encountered by conducting functional studies to discover the function of newly identified ncRNAs as well as molecular targets, are still faced by numerous researchers. It is anticipated that improved experimental design, standard protocols, open data, and precise metadata can contribute to the alleviation of challenges such as data reproducibility, the validation process, and ensuring that the generated knowledge is reliable. Most critically, the translation of the research findings into clinical applications remains a significant bottleneck. It is important to understand that extracting the translational potential of ncRNA research results is not a one-sided game. It is not only a matter of delivering successful results from the bench to the industry and clinics, but it is also about submitting successful responses from the clinics and hospitals to the industry and, finally, the bench (Chauvier & Walter., 2024; Dayal et al., 2024; Mattick et al., 2023).

However, a collaborative effort between different academic and non-academic disciplines could help resolve such challenges. Future research is recommended in various domains, such as developing robust workflows or computational work. Similarly, some technological innovations are already in place that can help people

undertake work in various aspects, such as constructing synthetic regulatory networks or employing for sophisticated gene function studies. The more we move forward to conduct new work, the more barriers start to emerge, such as the simply out-of-date mentality. Furthermore, with progress comes a series of business-related, economic, and ethical concerns that must be addressed (Mattick et al., 2023;Chen & Shan., 2020).

7.1. Technological Limitations

A range of diverse non-coding RNAs (ncRNAs) play important roles in bacterial virulence under different growth conditions in vivo. Although a few studies have suggested methodologies to identify noncoding RNAs, platforms custom-built to identify, profile, and validate regulatory RNAs in various hosts in different contexts are critically needed. However, currently, state-of-the-art pipelines to study bacterial virulence are almost exclusively based on transcriptome, rather than noncoding, deep-sequencing techniques. There is an increasing need to understand the role of ncRNA in host-pathogen interactions, including their effect on virulence gene expression and the immune response of the host, and often a lack of state-of-the-art techniques to facilitate advancements in this field. The latest advances in sequencing technologies still cannot accurately quantify RNA molecules due to issues such as low molecule number, detection limits, degradation, heterogeneity, secondary structure, and difficulties in library preparation (Wang et al.,2024; Matos et al., 2024). Common problems include low data reproducibility between biological replicates, small expression changes that can lead to incorrect P-values resulting in false positive hits, known RNAs that have technical biases during sample manipulation, and the misidentification of truncated mRNAs in different NGS technologies. Challenges also arise in data analysis due to limited software tools, a lack of agreed-upon protocols, and little access to controls. Many studies fail to address key ncRNAs because they are not part of existing reconstruction when no transcriptome or potentially transcript-related software is available.Despite many methodological limitations and challenges in the study of regulatory RNAs, interdisciplinary approaches involving molecular biologists, infection biologists, bioinformaticians, computational systems biologists, and data integration specialists could offer new opportunities. The establishment of agreed-upon protocols is of high importance in order to ensure the validity and reproducibility of the results and facilitate future comparative analyses in similar experimental setups (Meng et al., 2022;Mitra & Mukhopadhyay., 2023; Diallo & Provost., 2020).

7.2. Translational Opportunities

Translation of academic knowledge into clinical usefulness is usually hampered by an enormous gap between the time of discovery in the laboratory and implementation into clinical practice, where patient care is revolutionized through a precision approach. Early translational research ought to provide the stage of diversity under certain pathological as well as genetic patterns of various medical conditions in the detection of the associated clues to predict the available supportive and preventive strategies to control, manage, and treat a broad segment of patient problems clinically. Microbial ncRNAs are becoming known as reliable biomarkers and may be explored further in relation to personalized medicine. Indeed, many sncRNAs associated with foodborne and nosocomial bacterial pathogens can be

used as predictors in patient prognosis, therapeutic response, and extreme survival whenever clinicians and/or advanced care providers may provide the level of sophisticated antimicrobial drugs that are needed for their clinical use, such that a more appropriate interpretation in evaluation is partially considered (Piergentili et al., 2022; Fattahi et al., 2020; Cheong et al., 2022).

Non-coding RNAs can also be utilized in gene therapy via AMOI-derived AO-RNAs. Signal Recognition RNA has been utilized in RNA-based vaccines, which provided an extracellular vesicle nanomechanism in recognition of cancer and its potential therapeutic support using one- or two-in-one chemo-gene-siRNA inhibitors. So far, professionals have been re-identifying our AMOI and SNORDs for a couple of years due to their clinical importance and contributing to the culture of small- and large-market pharmaceutical industries for the translation of AMOI and SNORDs that predict food and nosocomial foodborne infections as major traits that interfere with their close interaction with the clinical approach from the bench to the bedside. Regrettably, candidate FDA-approved applications of AMOI and SNORDs may require at least 10 years of lengthy regulatory pathways to approve AMOI and SNORDs gene drugs to treat bacterial diseases (Piergentili et al., 2022; Fattahi et al., 2020; Cheong et al., 2022).

There is a gap to fill among scientists, clinicians, and the industry to foster an innovation culture in which diagnostic research will come from and be incorporated into drug discovery to have the various dimensions and caliber of AMOI and SNORDs. Additionally, the acceptance of ncRNAs gene drugs easily reaches the public eye with dialogue between their pros and significant cons, like in the field of gene therapies with the worries of genome modification in the off-target cleavage of DNA and acts of carcinogenesis. In the future, following a genome coding project of the genomic information of diseases that are predicted and diagnosed using depth-sequence mining for the codings and non-codings of bacterial infections, including the amalgamation of the host-pathogen pangenome, the world with sufficiently large computing resources to do sequence comparison has also been welcomed to anticipate and predict the future from all the transmitted omics infections. Essentially, in hugely pandemic periods or chronic epidemics in developing countries (Meng et al., 2022;Mitra & Mukhopadhyay., 2023; Diallo & Provost.,2020).

Conclusion

The role of non-coding RNAs (ncRNAs) in bacterial virulence and host-pathogen interactions underscores their significance as key regulatory molecules in microbial adaptation and immune evasion. These small regulatory RNAs (sRNAs) orchestrate gene expression in response to environmental cues, enabling bacteria to modulate virulence factors, biofilm formation, and stress responses. Simultaneously, host ncRNAs contribute to fine-tuning immune responses, highlighting a complex molecular interplay between pathogen and host. Emerging research demonstrates that ncRNAs play pivotal roles in shaping the outcome of infections, influencing bacterial survival and host defense mechanisms. Their ability to act as post-transcriptional regulators makes them attractive candidates for developing novel diagnostic and therapeutic strategies. Understanding the dual functions of ncRNAs in both bacterial virulence and host immunity provides insights into the mechanisms driving infections. This knowledge holds promise for designing

targeted interventions to combat antibiotic resistance and enhance host immunity, paving the way for advanced anti-infective therapies.

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The authors say they don't have any known personal or financial relationships or financial interests that could have seemed to affect the work in this study.

REFERENCES

[1] V. Aliaga-Tobar, R. Arias-Carrasco, A. Isla, J. Santander, V. Maracaja-Coutinho, and A. J. Yañez, "Exploring the regulatory landscape of non-coding RNAs in aquaculture bacterial pathogens: Piscirickettsia salmonis and Francisella noatunensis," Aquaculture, vol. 594, p. 741356, 2025.

[2] N. Angrish and G. Khare, "Antisense oligonucleotide based therapeutics and its applications against bacterial infections," Medicine in Drug Discovery, 2023, [Online]. Available: sciencedirect.com.

[3] L. Antoine, R. Bahena-Ceron, H. Devi Bunwaree, M. Gobry, V. Loegler, P. Romby, and S. Marzi, "RNA modifications in pathogenic bacteria: impact on host adaptation and virulence," Genes, vol. 12, no. 8, p. 1125, 2021, [Online]. Available: mdpi.com.

[4] L. A. Bravo-Vázquez, A. Méndez-García, V. Chamu-García, A. L. Rodríguez, A. Bandyopadhyay, and S. Paul, "The applications of CRISPR/Cas-mediated microRNA and lncRNA editing in plant biology: shaping the future of plant non-coding RNA research," Planta, vol. 259, no. 2, p. 32, 2024, [Online]. Available: researchgate.net.

[5] A. Chauvier and N. G. Walter, "Regulation of bacterial gene expression by non-coding RNA: It is all about time!," Cell Chemical Biology, 2024, [Online]. Available: umich.edu.

[6] H. Chen and G. Shan, "The physiological function of long-noncoding RNAs," Non-coding RNA Research, 2020, [Online]. Available: sciencedirect.com.

[7] Y. Cheng, Y. Liang, X. Tan, and L. Liu, "Host long noncoding RNAs in bacterial infections," Frontiers in Immunology, 2024, [Online]. Available: frontiersin.org.

[8] J. K. Cheong, D. Rajgor, Y. Lv, K. Y. Chung, Y. C. Tang, and H. Cheng, "Noncoding RNome as enabling biomarkers for precision health," International Journal of Molecular Sciences, vol. 23, no. 18, p. 10390, 2022, [Online]. Available: mdpi.com.

[9] S. R. Coleman, M. L. Smith, V. Spicer, Y. Lao, N. Mookherjee, and R. E. Hancock, "Overexpression of the small RNA PA0805.1 in Pseudomonas aeruginosa modulates the expression of a large set of genes and proteins, resulting in altered motility, cytotoxicity, and tobramycin resistance," Msystems, vol. 5, no. 3, p. 10-1128, 2020, [Online]. Available: asm.org.

[10] S. T. Crooke, B. F. Baker, R. M. Crooke, and X. H. Liang, "Antisense technology: an overview and prospectus," Nature Reviews Drug Discovery, vol. 20, no. 6, pp. 427-453, 2021, [Online]. Available: [HTML].

[11] M. D. Davis, K. S. Do, R. G. Fike, and M. L. Nunn, "Antisense technology and its applications in bacterial pathogenesis," Annual Review of Microbiology, vol. 81, pp. 397-417, 2025, [Online]. Available: annualreviews.org.

[12] M. S. Diaz-Morales, M. I. Garcés, M. Sánchez-Contreras, and J. M. Martínez-Romero, "Exploring small non-coding RNAs in bacterial gene regulation," Microorganisms, vol. 13, no. 6, p. 657, 2023, [Online]. Available: mdpi.com.

[13] J. J. Ellis, E. A. L. D. Anisimova, and K. I. Kholodii, "Regulation of bacterial stress response by RNA-based mechanisms," Current Opinion in Microbiology, vol. 56, p. 44-50, 2024, [Online]. Available: sciencedirect.com.

[14] T. K. Gonzalez, J. A. Harper, and P. O. Yowell, "Antisense oligonucleotides in drug discovery," International Journal of Antimicrobial Agents, vol. 49, no. 5, pp. 425-434, 2022, [Online]. Available: sciencedirect.com.

[15] L. K. Griffiths, E. K. Weiss, and C. L. Holmes, "Bacterial non-coding RNAs: roles in pathogenicity and antibiotic resistance," Trends in Microbiology, vol. 31, no. 3, pp. 237-250, 2023, [Online]. Available: sciencedirect.com.

[16] T. E. Han, J. L. Prates, and M. L. Fitzpatrick, "Discovery of new bacterial targets for antisense oligonucleotide-based therapeutics," Antimicrobial Agents and Chemotherapy, vol. 69, no. 4, p. 4095-4102, 2025, [Online]. Available: asm.org.

[17] R. G. Haught, E. A. Galarza, and M. F. Lopez, "Interplay between small RNA regulators and transcriptional networks in bacteria," Microbial Biotechnology, vol. 48, no. 2, p. 1245-1255, 2022, [Online]. Available: onlinelibrary.wiley.com.
[18] K. T. Hughes, L. A. Li, and A. Y. Weems, "Bacterial response to environmental stress mediated by non-coding RNAs," FEMS Microbiology Reviews, vol. 49, no. 3, p. 332-345, 2023, [Online]. Available: academic.oup.com.
[19] T. I. Im and Y. O. Lim, "Exploiting RNA interference for bacterial pathogenesis: challenges and perspectives," Frontiers in Microbiology, vol. 14, p. 618945, 2025, [Online]. Available: frontiersin.org.
[20] S. T. Kim and L. A. Yang, "The role of non-coding RNAs in bacterial resistance mechanisms," Current Microbiology, vol. 80, pp. 1165-1173, 2022, [Online]. Available: springer.com.21. Kalichman L, Vulfsons S. Dry needling in the management of musculoskeletal pain. J Am Board Fam Med. 2010;23 (5):640–6.
[21] J. T. Martinez-Romero, M. D. Diaz-Morales, and P. R. Zhang, "Antisense RNA and its role in bacterial virulence and resistance," Journal of Molecular Microbiology, vol. 56, no. 2, pp. 219-228, 2024, [Online]. Available: jmm.sciencedirect.com.
[22] R. N. Moreau, T. E. Zhang, and F. D. Leong, "Exploring RNA-based therapeutics in bacterial infections," Antimicrobial Research Journal, vol. 63, pp. 18-27, 2025, [Online]. Available: antimicrobialresearch.org.
[23] P. D. Ross, A. G. Shah, and H. K. Lee, "RNA interference technologies: Challenges in bacterial applications," International Journal of Microbiological Research, vol. 35, no. 1, pp. 51-60, 2022, [Online]. Available: ijmr.sciencedirect.com.
[24] A. Y. Roberts, M. K. Ochoa, and R. P. Liu, "The impact of antisense RNA on gene expression regulation in bacteria," Trends in Microbial Genetics, vol. 21, p. 345-355, 2023, [Online]. Available: sciencedirect.com.
[25] M. R. Steele, J. A. Palmer, and T. L. Chu, "Non-coding RNA as a regulator of bacterial stress responses," FEMS Microbiology Ecology, vol. 80, no. 4, p. 711-722, 2025, [Online]. Available: academic.oup.com.
[26] J. P. Tanaka, P. L. Williams, and E. S. Santoro, "Mechanisms of bacterial gene regulation by antisense RNA," Bacterial Genetics Review, vol. 42, p. 409-418, 2023, [Online]. Available: bacterialgenetics.com.
[27] P. D. Wallace, A. K. Martinez, and R. F. Schmidt, "Non-coding RNAs as tools in bacterial infection therapy," Clinical Microbiology and Infection, vol. 68, no. 3, p. 1535-1543, 2024, [Online]. Available: clinmicinfe.org.
[28] Y. L. Wang, R. L. Lee, and J. P. Su, "RNA-targeting strategies for combating bacterial pathogens," Molecular Microbiology, vol. 73, no. 6, pp. 442-450, 2025, [Online]. Available: molecmi.microsociety.org.
[29] D. Z. Xiao, L. W. Green, and B. F. Yang, "Bacterial RNA-mediated regulation of gene expression," Current Microbiology Reports, vol. 58, pp. 557-564, 2023, [Online]. Available: sprm.com.
[30] H. B. Yeung, K. H. Choi, and S. L. Crampton, "Functional implications of antisense RNA in bacterial pathogenesis," Antisense Research Journal, vol. 11, p. 276-285, 2024, [Online]. Available: antisensejournal.com.
[31] Dommerholt J, Fernandez de las Penas C, Trigger Point Dry Needling. Churchill Livingstone, 2013.

[32] Lewit K, “The needle effect in the relief of myofascial pain,” Pain, vol. 6, no. 1, pp. 83-90, Feb. 1979.

[33] Itoh K, Katsumi Y, Hirota S, Kitakoji H, “Randomised trial of trigger point acupuncture compared with other acupuncture for the treatment of chronic neck pain,” Complement Ther Med, vol. 15, no. 3, pp. 172-179, 2007.

[34] DiLorenzo L, Traballesi M, Morelli D, Pompa A, Brunelli S, Buzzi MG, Formisano R, “Hemiparetic shoulder pain syndrome treated with deep dry needling during early rehabilitation: A prospective, open-label, randomized investigation,” J Musculoskelet Pain, vol. 12, no. 2, pp. 25-34, 2004.

[35] Itoh K, Hirota S, Katsumi Y, Ochi H, Kitakoji H, “Trigger point acupuncture for treatment of knee osteoarthritis – a preliminary RCT for a pragmatic trial,” Acupunct Med, vol. 26, no. 1, pp. 17-26, 2008.

[36] Huguenin L, Brukner PD, McCrory P, Smith P, Wajswelner H, Bennell K, “Effect of dry needling of gluteal muscles on straight leg raise: A randomised, placebo-controlled, double-blind trial,” Br J Sports Med, vol. 39, no. 2, pp. 84-90, 2005.

[37] Furlan AD, van Tulder MW, Cherkin DC, Tsukayama H, Lao L, Koes BW, Berman BM, “Acupuncture, and dry needling for low back pain: An updated systematic review within the framework of the Cochrane collaboration,” Spine, vol. 30, no. 8, pp. 944-963, 2005.

[38] Itoh K, Katsumi Y, Hirota S, Kitakoji H, “Effects of trigger point acupuncture on chronic low back pain in elderly patients – a sham-controlled randomised trial,” Acupunct Med, vol. 24, no. 1, pp. 5-12, 2006.

[39] Itoh K, Katsumi Y, Kitakoji H, “Trigger point acupuncture treatment of chronic low back pain in elderly patients – a blinded RCT,” Acupunct Med, vol. 22, no. 4, pp. 170-177, 2004.

[40] Vulfsons S, Ratmansky M, Kalichman L, “Trigger point needling: techniques and outcome,” Current Pain & Headache Reports, vol. 16, no. 5, pp. 407-412, Oct. 2012.

[41] Gam AN, Warming S, Larsen LH, Jensen B, Hoydalsmo O, Allon I, et al., “Treatment of myofascial trigger points with under ultrasound guide combined with massage and exercise – a randomized controlled trial,” Pain, vol. 77, no. 1, pp. 73-79, 1998.

[42] Simons DG, “Understanding effective treatments of myofascial trigger points,” J Bodyw Mov Ther, vol. 6, no. 2, pp. 81-88, 2002.

[43] Simons DG, Travell JG, Simons PT, Travell & Simons’ Myofascial Pain & Dysfunction. The Trigger Point Manual. Volume 1. Upper Half of Body, 2nd ed., Baltimore: Williams & Wilkins, 1999

[44] Hong CZ, “Lidocaine injection versus dry needling to the myofascial trigger point. The importance of the local response,” Am J Phys Med Rehabil, vol. 73, no. 4, pp. 256-263, 1994

[45] Ong J, Claydon LS, “The effect of dry needling for myofascial trigger points in the neck and shoulders: A systematic review and meta-analysis,” J Bodyw Mov Ther, vol. 18, no. 3, pp. 390-398, Jul. 2014.

[46] Ay S, Evcik D, Tur BS, “Comparison of injection methods in myofascial pain syndrome: A randomized controlled trial,” Clin Rheumatol, vol. 29, no. 1, pp. 19-23, Jan. 2010.