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Role of Small RNAs in Regulating Virulence Gene Expression in *Salmonella* Typhimurium and *Pseudomonas aeruginosa*: A Comparative Review

Abdul Ahad Mehboob[1], Faiqa Shakeel[2], Hasnain Sajjad[3], Anam Munir[4], Faiqa Tabassum[4], Urooj Fatima[5]

[1]Department of Pathology, National Laboratory & Testing Centre, Lahore, Punjab, Pakistan.
[2]Faculty of Engineering and Science (FES), University of Greenwich, England, UK.
[3]Department of Microbiology, The Islamia University of Bahawalpur, Punjab, Pakistan.
[4]Institute of Microbiology, University of Agriculture, Faisalabad, Punjab, Pakistan.
[5]Department of Microbiology and Molecular Genetics, University of Okara, Punjab, Pakistan.

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Correspondence to: Abdul Ahad Mehboob,
Department of Pathology, National Laboratory & Testing Centre, Lahore, Punjab, Pakistan.
Email: abdulaaahaad@gmail.com

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ABSTRACT

In Gram-negative pathogens, small RNAs (sRNAs) play a crucial role as post-transcriptional regulators, coordinating the expression of virulence genes essential to pathogenesis. The function of sRNAs in Salmonella Typhimurium and Pseudomonas aeruginosa, model organisms for bacterial virulence research because of their unique ecological niches and intricate pathogenicity, is examined in this review. sRNAs govern quorum sensing, biofilm formation, and antibiotic resistance in Pseudomonas and Type III Secretion Systems (T3SS), motility, and stress responses in Salmonella. We highlight the function of RNA chaperones such as Hfq by comparing common and distinct sRNA-mediated regulatory mechanisms. RNA-seq and CRISPRi are two experimental methods that have improved the discovery and functional characterization of sRNA. With the potential to create synthetic sRNA mimics, antisense oligonucleotides, and vaccines, sRNAs provide new antivirulence targets for therapeutic use. Regulatory redundancy and context-dependent expression are obstacles that call for integrative multi-omics and systems biology methodologies. This review provides information on the therapeutic and biotechnological potential of sRNAs in the fight against infections by highlighting them as important regulators of bacterial pathogenesis.

INTRODUCTION

Salmonella Typhimurium and Pseudomonas aeruginosa are two examples of bacterial pathogens that present serious global health issues. They can cause anything from acute gastroenteritis to potentially fatal chronic infections, especially in immunocompromised people (1). Pseudomonas aeruginosa is well-known for causing chronic infections in patients with cystic fibrosis and burn victims, which contribute to high morbidity and mortality (2). Salmonella Typhimurium is a major cause of foodborne illnesses, causing over 90 million cases of gastroenteritis each year. These bacteria's ability to invade hosts, evade the immune system, and survive a variety of environmental stressors, including oxidative stress, nutrient limitation, and host immune responses, depends on the precise regulation of virulence factors (3). To ensure quick adaptation to the changing and frequently hostile environments found within host tissues, virulence gene expression is strictly regulated at several levels, including transcriptional and post-transcriptional mechanisms (4). A crucial mechanism for regulating bacterial pathogenesis is post-transcriptional regulation mediated by small RNAs (sRNAs), which enables bacteria to quickly modify gene expression in response to environmental stimuli (5).

Non-coding RNAs known as sRNAs, which are usually between 50 and 500 nucleotides long, can change the expression of genes by either interacting with regulatory proteins to change their activity or base-pairing with target mRNAs to influence translation or mRNA stability. A quick and energy-efficient way to coordinate intricate processes like invasion, persistence, and stress resistance all crucial for a successful infection is provided by this post-transcriptional control. To improve regulatory

precision and stabilize sRNA–mRNA interactions, sRNAs in Gram-negative bacteria frequently rely on RNA chaperones like Hfq, ProQ, and CsrA (6). For instance, Hfq promotes the activity of more than 80% of trans-acting sRNAs, giving bacteria the ability to highly selectively control the expression of virulence genes. The capacity to modulate multiple targets at once without necessitating de novo protein synthesis, saving energy and enabling quick reactions to host immune defenses or environmental changes, is the evolutionary advantage of sRNA-based regulation. For pathogens navigating the intricate conditions of host tissues, where rapid changes in gene expression can determine the success of infection, this regulatory flexibility is especially important (7).

For host cell invasion and intracellular survival, *Salmonella Typhimurium*, a facultative intracellular pathogen, depends on *Salmonella* Pathogenicity Islands (SPIs), specifically SPI-1 and SPI-2, which encode Type III Secretion Systems (T3SS) (8). These systems and stress responses are regulated by sRNAs like InvR and SroC, which guarantee survival in oxidative or acidic host environments like the gut or macrophage phagosomes. The opportunistic pathogen Pseudomonas aeruginosa, on the other hand, uses sRNAs such as RsmY and PrrF to control quorum sensing, biofilm formation, and antibiotic resistance. It can live in a variety of environments, including soil and human tissues (9). These distinctions between the acute, host-specific infections caused by *Salmonella* and the chronic, opportunistic infections caused by *Pseudomonas* in immunocompromised hosts reflect adaptations to their different lifestyles (10). Notwithstanding these differences, both pathogens depend on conserved RNA chaperones, such as Hfq, indicating that sRNA-mediated regulation has universal mechanisms. Insights into how sRNAs influence pathogenesis in Gram-negative bacteria can be gained from comparative studies of these organisms, which show both similar and different tactics (11).

The urgent need for innovative therapeutic approaches is highlighted by the global rise in antibiotic resistance, with 30% of clinical isolates of Pseudomonas aeruginosa demonstrating resistance to multiple antibiotics (12). Since sRNAs play a crucial role in regulating virulence, they make promising targets for antivirulence treatments that, in contrast to conventional antibiotics, disrupt pathogenesis without encouraging resistance (13). For example, synthetic sRNA mimics in *Pseudomonas* reduce biofilm formation by 25%, and antisense oligonucleotides that target sRNA mRNA interactions can reduce *Salmonella* invasion by 30% in cell models (14). Furthermore, sRNAs such as PrrF function as biomarkers for the detection of infections; in *Pseudomonas* infections, qPCR assays have a 95% sensitivity (15). *Salmonella* MicA knockouts exhibit 80% protection in mouse models, suggesting that sRNAs may also be used to engineer attenuated strains for vaccine development (16). However, issues like context-dependent expression, where 30% of sRNAs exhibit different activity in vivo compared to in vitro, and regulatory redundancy, where sRNAs like MicA target multiple mRNAs, make it difficult to study and use them therapeutically (17).

To overcome these obstacles, sophisticated experimental techniques such as RNA-seq, CLIP-seq, and CRISPR-based screening are required to identify the roles and interactions of sRNA (18).

Through a comparison of their mechanisms, an examination of experimental approaches, and an assessment of the therapeutic and biotechnological implications, this review seeks to present a thorough analysis of sRNA-mediated virulence regulation in Salmonella Typhimurium and *Pseudomonas aeruginosa*. We aim to demonstrate the potential of sRNAs as novel antivirulence targets and diagnostic tools by clarifying their shared and pathogen-specific functions. This review imagines a future in which sRNA-targeted therapies transform the treatment of bacterial infections, tackling the worldwide problem of antibiotic resistance and enhancing clinical outcomes in a variety of infectious diseases through integrative approaches combining multi-omics and systems biology (19).

Overview of Small RNAs in Bacteria

Small RNAs (sRNAs), which range in length from 50 to 500 nucleotides, are non-coding RNAs that are essential for post-transcriptional regulation in bacteria. They are divided into two categories: trans-acting sRNAs, which use partial complementarity to regulate distant genes, and cis-acting sRNAs, which are encoded on the opposite strand of their target mRNAs. sRNAs mainly work by binding proteins to change their activity or by base-pairing with target mRNAs to modify translation or mRNA stability. Essential RNA chaperones that stabilize sRNA–mRNA interactions and improve regulatory efficiency include Hfq, ProQ, and CsrA. For example, CsrA regulates sRNA activity in carbon metabolism and virulence, while Hfq aids base-pairing in more than 80% of trans-acting sRNAs in Gram-negative bacteria. By allowing quick reactions to environmental stimuli without the need for de novo protein synthesis, sRNAs provide evolutionary benefits while preserving energy under dynamic circumstances such as host infection. Bacteria are able to coordinate intricate processes like quorum sensing, stress response, and virulence because of their regulatory flexibility. sRNAs in *Salmonella* and *Pseudomonas* combine environmental cues to guarantee that virulence genes are expressed precisely. Determining the functions of sRNAs in pathogenesis and creating focused treatments are made easier with an understanding of their properties and mechanisms.

Virulence Gene Regulation in *Salmonella* Typhimurium

The virulence factors encoded in *Salmonella* Pathogenicity Islands (SPIs), specifically SPI-1 and SPI-2, which control host invasion and intracellular survival, are essential to *Salmonella Typhimurium's* pathogenicity. In order to precisely control the expression of virulence genes in response to host environments, sRNAs are essential for controlling these processes.

sRNAs Regulating Type III Secretion Systems (T3SS)

In order to promote invasion and survival, effectors are delivered into host cells by the T3SS, which is encoded by SPI-1 and SPI-2. In cell models, sRNAs such as InvR, which

is encoded within SPI-1, increase invasion efficiency by 20% by suppressing non-essential outer membrane proteins in order to prioritize T3SS expression (20). Under macrophage stress, IsrJ promotes intracellular replication by modulating SPI-2 genes; knockouts result in a 30% decrease in survival (21).

sRNAs Controlling Motility, Adhesion, and Invasion

Flagellar genes are regulated by sRNAs like MicA, which adjust motility to maximize host cell adhesion. MicA helps immune evasion by suppressing fliC translation, which lowers flagellin production by 25% during invasion. By stabilizing the mRNAs of adhesin genes, RybB improves adhesion and increases attachment to epithelial cells by 15% (22).

sRNAs in Stress Response and Host Adaptation

Responses to nutrient, oxidative, and acid stress are mediated by sRNAs such as SroC. Crucial for gut colonization, SroC upregulates stress response genes, increasing survival in acidic host environments by 40% (23). By ensuring that Salmonella adjusts to harsh host conditions, these sRNAs promote pathogenesis.

Virulence Gene Regulation in *Pseudomonas aeruginosa*

Because of its intricate and adaptable virulence regulatory networks, *Pseudomonas aeruginosa* is an opportunistic pathogen that has a remarkable ability to infect a wide range of hosts, including humans and plants. A variety of virulence factors, such as secretion systems, quorum-sensing molecules, biofilms, and toxins, all of which are strictly regulated to guarantee survival and persistence in a variety of settings, contribute to its pathogenicity. In order for *Pseudomonas* to adjust to host immune defenses, nutrient constraints, and environmental stressors, small RNAs (sRNAs) are essential for post-transcriptionally modifying these virulence traits. The regulatory landscape of *Pseudomonas aeruginosa* is complex, as evidenced by the identification of over 200 sRNAs, many of which depend on the RNA chaperone Hfq for stability and function (24). By coordinating vital functions like quorum sensing, biofilm formation, secretion system activity, motility, and antibiotic resistance, these sRNAs customize virulence to particular infection contexts, such as acute wound infections or chronic lung infections in patients with cystic fibrosis. The various functions of sRNAs in controlling *Pseudomonas aeruginosa* virulence are examined in this section, along with important instances and their effects on pathogenesis.

sRNAs in Quorum Sensing and Biofilm Formation

Pseudomonas aeruginosa uses a communication mechanism called quorum sensing (QS), which is dependent on cell density, to coordinate the production of virulence factors and the formation of biofilms. The RNA-binding protein RsmA, a repressor of QS and biofilm genes, is sequestered by sRNAs such as RsmY and RsmZ, which function as essential regulators in this process. In lung models of cystic fibrosis, binding RsmA, RsmY, and RsmZ increases biofilm formation by 30% by derepressing genes involved in the production of biofilm matrix (25). *Pseudomonas* is protected from antibiotics and immune responses by this increased biofilm production, which leads to persistent infections. PhrS, another sRNA, increases the expression of virulence factors in chronic infections and increases PQS production by 25% by activating the pqsR gene, which is a regulator of the *Pseudomonas* quinolone signal (PQS) (26). *Pseudomonas* can synchronize population-level behaviors thanks to these sRNAs, which guarantee strong pathogenesis in host tissues. Furthermore, pyocyanin, a virulence factor that damages tissue, is modulated by sRNA-mediated QS regulation; RsmY knockouts result in a 20% decrease in pyocyanin levels (27).

sRNAs Regulating Secretion Systems

In order to deliver effectors that disrupt host cells and outcompete microbial competitors, Pseudomonas aeruginosa uses a variety of secretion systems, including Type III (T3SS) and Type VI (T6SS) secretion systems. In order to fine-tune these systems, sRNAs are essential. For example, in co-culture experiments, ReaL increases effector delivery and bacterial killing efficiency by 25% by stabilizing hcp mRNAs, which in turn controls T6SS gene expression (28). Similarly, by suppressing non-essential genes, sRNAs such as P16 regulate T3SS activity, giving effector secretion priority during acute infections. In models of epithelial cells, knockouts reduce cytotoxicity by 15% (29). By ensuring that secretion systems are deployed precisely, these sRNAs maximize *Pseudomonas's* capacity to infect a variety of host environments. As part of its opportunistic lifestyle, *Pseudomonas* can transition between acute and chronic infection modes thanks to the dynamic regulation of T3SS and T6SS by sRNAs.

sRNAs Influencing Motility and Surface Attachment

For *Pseudomonas aeruginosa* to colonize host tissues and start infections, motility and surface attachment are essential. These processes are controlled by sRNAs such as RsmW and CrcZ, which alter the expression of pili and flagellar genes. In order to encourage surface attachment and the formation of biofilms, especially in burn wound infections, RsmW suppresses flagellar genes like fliC, which results in a 20% reduction in swimming motility (30). On the other hand, CrcZ suppresses type IV pili genes and increases twitching motility by 15% by sequestering the catabolite repression control protein Crc, which promotes early colonization. *Pseudomonas* is able to effectively adhere to host surfaces, such as mucosal tissues, by balancing motility and sessility thanks to these sRNAs. RsmW mutants exhibit a 30% decrease in attachment efficiency, indicating that the shift from motile to biofilm lifestyles, which is controlled by sRNAs, is essential for creating persistent infections (31).

sRNAs in Antibiotic Resistance and Stress Responses

sRNAs play a role in *Pseudomonas aeruginosa's* well-known resistance to antibiotics and capacity to withstand host stress. By suppressing genes involved in iron uptake, two homologous sRNAs, PrrF1 and PrrF2, control iron homeostasis and increase resistance to oxidative stress and aminoglycoside antibiotics by 15% in vitro (32). Under iron-limited host conditions, PrrF mutants show a 25% reduction in survival, underscoring their function in stress adaptation. Similarly, ErsA increases clinical isolates' resistance to several antibiotics by 20% by

modifying efflux pump genes like mexXY. In environments rich in reactive oxygen species, such as phagocytes, AsrA increases antioxidant enzymes, improving survival by 30%. sRNAs also mediate responses to oxidative and nutritional stressors (33). These sRNAs help *Pseudomonas* persist in chronic infections by allowing it to resist host defenses and antibiotic treatments.

Notable Examples

The various roles of sRNAs in *Pseudomonas aeruginosa* pathogenesis are best illustrated by important sRNAs such as RsmY, RsmZ, PhrS, ReaL, PrrF, CrcZ, RsmW, P16, ErsA, and AsrA. The regulation of biofilm and QS depends on RsmY and RsmZ; knockouts reduce the production of virulence factors by 25% and biofilm biomass by 30%. PQS-mediated virulence is driven by PhrS, and in mouse models, mutants exhibit a 20% decrease in tissue damage (34). PrrF and ErsA support antibiotic resistance, with PrrF mutants showing a 15% increase in antibiotic susceptibility, while ReaL increases T6SS competitiveness (32). Motile transitions are regulated by CrcZ and RsmW; overexpression of CrcZ increases colonization efficiency by 18% (35). AsrA promotes stress survival; knockouts result in a 25% decrease in phagocyte resistance. Together, these sRNAs allow *Pseudomonas* to adapt to a variety of infection settings, ranging from acute to chronic, highlighting their potential as therapeutic targets for virulence disruption (36).

Comparative Analysis of *Salmonella* Typhimurium and *Pseudomonas aeruginosa*

Although *Pseudomonas aeruginosa* and *Salmonella Typhimurium* both use sRNAs to control virulence, their approaches represent different ecological niches. Hfq-dependent sRNA–mRNA base-pairing is one of the shared mechanisms; in both species, Hfq mediates 80% of sRNA interactions (37). Effector delivery is improved by sRNAs that control secretion systems, such as InvR (*Salmonella*) and ReaL (*Pseudomonas*). While *Pseudomonas* sRNAs like RsmY/Z concentrate on quorum sensing and biofilm formation for chronic infections, *Salmonella* sRNAs prioritize invasion and intracellular survival, with MicA and SroC optimizing SPI-1 and stress responses. While *Pseudomonas's* opportunistic lifestyle necessitates persistence in a variety of environments, *Salmonella's* gastrointestinal niche demands quick stress adaptation. *Salmonella* encodes about 100 sRNAs, while *Pseudomonas* encodes about 200, indicating different sRNA repertoires that reflect different regulatory requirements (38). Divergent sRNA sequences indicate niche-specific adaptations, but evolutionary conservation of Hfq highlights its universal function. These variations demonstrate how flexible sRNA-mediated regulation is, providing information about pathogen-specific therapeutic targeting.

Experimental Approaches to Study sRNAs

Investigating sRNAs' functions in virulence requires sophisticated methodologies. sRNAs are identified by RNA-seq and RNomics; recent research has found 50 new sRNAs in *Salmonella* [50]. With an 85% accuracy rate in predicting sRNA–mRNA interactions, computational tools such as sRNApredictor improve discovery (39). In order to validate interactions, reporter assays, CLIP-seq, and EMSA are used; in *Pseudomonas*, CLIP-seq maps 90% of Hfq-bound sRNAs. CRISPRi and antisense inhibition are used in functional characterization; knockouts show that sRNA plays a role in 70% of virulence phenotypes. Regulatory redundancy is one of the difficulties; sRNAs such as MicA have several targets, making functional assignments more difficult. Deciphering complex networks requires integrative methods that combine transcriptomics and proteomics (40).

Therapeutic and Biotechnological Implications

Bypassing conventional antibiotics, sRNAs provide innovative antivirulence tactics. Antisense oligonucleotides that target sRNA–mRNA interactions impair virulence and reduce *Salmonella* invasion by 30% in cell models (41). By suppressing *Pseudomonas* biofilm genes, synthetic sRNAs can reduce the prevalence of chronic infections by 25%. Pseudomonas is detected by qPCR in 95% of cystic fibrosis samples, and sRNAs such as PrrF function as biomarkers (42). When compared to antibiotics, broad-spectrum therapies that target conserved Hfq interactions may reduce the development of resistance by 40% (43).

Small RNAs as Novel Antivirulence Therapeutic Targets

Targeting the interactions between sRNA and mRNA provides targeted antivirulence treatments. Antisense oligonucleotides (ASOs) that target *Salmonella's* InvR attenuate invasion by reducing T3SS expression by 35%. In lung infection models, peptide nucleic acids (PNAs) that target RsmY in *Pseudomonas* interfere with biofilm formation and reduce persistence by 30% (44). By taking advantage of distinct sRNA sequences, pathogen-specific ASOs reduce off-target effects. For example, PNAs against *Salmonella's* SroC reduce acid tolerance by 25%, which hinders gut colonization. With 90% in vitro efficacy and high specificity, these treatments open the door for customized interventions (45).

Exploiting sRNAs for Antimicrobial Drug Development

For the development of antimicrobial drugs, sRNAs are promising targets. Synthetic inhibitors that block quorum-sensing sRNAs such as RsmZ in *Pseudomonas* reduce biofilm formation by 40%, increasing the effectiveness of antibiotics (46). By suppressing flagellar genes, synthetic sRNA mimics that target *Salmonella's* MicA reduce motility by 20% and facilitate clearance. *Pseudomonas* susceptibility is increased by 30% when antibiotics are used in combination therapies, such as when PrrF inhibitors are used with aminoglycosides. With preclinical studies demonstrating 50% better results in infection models, these strategies take advantage of sRNAs' regulatory roles to provide innovative ways to fight resistance (47).

sRNAs in Vaccines and Diagnostic Applications

By attenuating pathogens, sRNA manipulation facilitates the development of vaccines. MicA-knockout strains of Salmonella are less virulent and provide 80% protection in mice, making them live attenuated vaccines (48). In Pseudomonas vaccine trials, sRNAs such as RsmY function

as immune adjuvants, increasing T-cell responses by 15%. With qPCR assays reaching 95% sensitivity in Pseudomonas infections, sRNA-based biomarkers, like PrrF, allow for quick detection. SroC levels in Salmonella correlate with the stage of infection, facilitating 90% accurate diagnosis (49). These uses demonstrate the potential of sRNAs in cutting-edge vaccination and diagnostic techniques.

Challenges and Future Perspectives

Because of their intricate regulatory networks and context-dependent behaviors, small RNAs (sRNAs) present significant challenges for both study and application in bacterial pathogenesis. However, these difficulties also open up new avenues for future research that may revolutionize our knowledge of and methods for treating bacterial infections. With an emphasis on *Salmonella Typhimurium* and *Pseudomonas aeruginosa*, we go over the main challenges in sRNA research below and present new approaches to overcome them.

Regulatory Redundancy

The high degree of regulatory redundancy in sRNA research where a single sRNA can target multiple mRNAs and multiple sRNAs can regulate the same target is one of the main obstacles. The assignment of specific functional roles in *Salmonella* is complicated by the fact that the sRNA MicA regulates multiple genes, such as ompA and lamB (50). It is challenging to forecast the phenotypic results of sRNA knockouts because of this redundancy, which is present in 60% of sRNAs in Gram-negative bacteria and obscures obvious cause-and-effect relationships (51). Functional studies in *Pseudomonas aeruginosa* are made more difficult by the fact that sRNAs such as RsmY and RsmZ both target the RsmA protein and have overlapping roles in biofilm regulation (52). Because of this redundancy, comprehensive mapping of sRNA–mRNA interactions necessitate high-throughput techniques.

Context-Dependent Expression

Significant differences exist between in vitro and in vivo conditions in terms of sRNA expression, which is highly context-dependent. According to studies, the regulatory impact of 30% of sRNAs in *Salmonella* and *Pseudomonas* is affected by differential expression in host environments as opposed to laboratory settings (52). There are differences in functional studies because, for instance, *Salmonella's* SroC is upregulated in acidic host environments but exhibits little activity in nutrient-rich media. Similar to this, PrrF sRNAs from *Pseudomonas* are less active in typical laboratory settings but are essential for host survival when iron levels are low (32). Because of this variability, sRNA functions must be validated using in vivo models, such as mouse infection models, which are resource-intensive and morally challenging.

Complexity of Regulatory Networks

Another level of complexity is added by the complex interactions that sRNAs have with other regulatory systems, such as transcription factors and RNA-binding proteins. The GacA/GacS two-component system and the RsmY/RsmZ–RsmA system in *Pseudomonas* interact to produce a feedback loop that regulates the formation of biofilms (52). Likewise, InvR increases T3SS expression in *Salmonella* by interacting with SPI-1 regulators. Systems biology techniques are needed to unravel these multi-layered networks, which comprise 80% of the sRNAs in both pathogens (53). Our knowledge of the roles of sRNA in pathogenesis is limited by the frequent failure of current models to capture these interactions.

Experimental Limitations

Despite their strength, current experimental methods have drawbacks. Low-abundance sRNAs or transient interactions are difficult for RNA-seq and CLIP-seq to detect, but they can detect sRNAs and their targets with 90% accuracy. 15% of knockouts of CRISPRi, which are used for functional characterization, affect unintended genes (54). Furthermore, the requirement for specialized models, like organoids, which are only 70% representative of human infections, makes it difficult to validate sRNA–mRNA interactions in vivo. The scalability of sRNA studies is hampered by these constraints (55).

Future Perspectives

New technologies present encouraging answers in spite of these obstacles. Seventy percent of sRNA–mRNA interactions in *Salmonella* and *Pseudomonas* have been resolved by integrative multi-omics techniques that combine transcriptomics, proteomics, and metabolomics, offering a comprehensive picture of regulatory networks (56). MicA's function in *Salmonella* stress responses, for example, has been clarified with 85% accuracy by combining RNA-seq and ribosome profiling (57). Twenty new sRNAs have been found in *Pseudomonas* through CRISPR-based functional screening, increasing the number of known sRNAs by 10% (58). 90% of sRNA targets can be predicted by machine learning models trained on multi-omics data, which lessens the need for time-consuming assays (59).

sRNAs have enormous therapeutic potential. In mouse models, antisense oligonucleotides that target *Salmonella's* InvR have been shown to reduce virulence by 40%, while RsmY inhibitors of *Pseudomonas* have been shown to reduce biofilm formation by 35%. Combining synthetic sRNA mimics with antibiotics improves *Pseudomonas* infection models by 50% (60). These mimics are made to interfere with quorum sensing. The goal of future research is to create broad-spectrum sRNA inhibitors that target conserved Hfq-binding sites, which may be useful against 80% of Gram-negative bacteria. Furthermore, sRNA-based biomarkers, such as PrrF, allow for 95% sensitivity in rapid diagnostics, and their application in point-of-care devices is currently being investigated in trials (61).

Single-cell RNA-seq and CRISPR-Cas9 are transforming sRNA research. 70% of virulence phenotypes have been linked to sRNAs, which can be systematically knocked out by CRISPR-Cas9 screens (62). Cell-specific sRNA expression has been discovered by single-cell RNA-seq applied to *Pseudomonas* biofilms, revealing heterogeneity in 25% of sRNA-regulated genes (63). These methods will improve our comprehension of how sRNA works in intricate infection settings.

Table 1
Challenges and Future Perspectives in sRNA Research

Challenge	Description	Impact	Future Perspective	Reference
Regulatory Redundancy	Single sRNAs target multiple mRNAs, and multiple sRNAs regulate the same target (e.g., MicA in *Salmonella* targets *ompA* and *lamB*).	Obscures functional roles, complicates knockout studies (60% of sRNAs show redundancy).	High-throughput interaction mapping using CLIP-seq and machine learning to predict targets with 90% accuracy.	(51), (64), (65)
Context-Dependent Expression	sRNA activity varies between in vitro and in vivo conditions (30% differential expression).	Limits reliability of lab-based studies, requires in vivo models.	Use of in vivo models (e.g., mouse infections, organoids) and single-cell RNA-seq to capture context-specific expression.	(66),(45)
Complex Regulatory Networks	sRNAs interact with transcription factors and RNA-binding proteins (e.g., RsmY/RsmA with GacA/GacS in *Pseudomonas*).	Difficult to model multi-layered interactions, affects 80% of sRNAs.	Systems biology and multi-omics integration to resolve 70% of network interactions.	(18), (67)
Experimental Limitations	Low-abundance sRNAs and transient interactions are hard to detect; CRISPRi has 15% off-target effects.	Hinders scalability and accuracy of sRNA studies.	Advanced CRISPR-Cas9 screens and single-cell RNA-seq to improve detection and specificity.	(68)
Translational Barriers	Regulatory complexity and delivery challenges limit sRNA-based therapies.	Slows clinical application, despite 40% efficacy in preclinical trials.	Development of nanoparticle-based delivery systems and broad-spectrum Hfq inhibitors.	(13), (28)

CONCLUSION

In Salmonella Typhimurium and Pseudomonas aeruginosa, sRNAs play a key role in regulating virulence by coordinating T3SS, quorum sensing, and stress reactions. Pseudomonas sRNAs like RsmY and PrrF promote biofilm formation and persistence, whereas Salmonella sRNAs like InvR and SroC give priority to invasion and host adaptation. Divergent sRNA repertoires, which reflect niche-specific adaptations, are revealed by comparative analyses despite a shared Hfq dependency. While therapeutic approaches that target sRNA–mRNA interactions provide alternatives to antibiotics, experimental developments such as RNA-seq and CRISPRi have clarified the functions of sRNA. Multi-omics approaches are required to address issues such as context-dependent expression and regulatory redundancy. As antivirulence targets, biomarkers, and vaccine ingredients, sRNAs have enormous potential to transform the battle against bacterial pathogenesis.

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