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Original Article

Small interfering RNAs targeting *agrA* and *sarA* attenuate pathogenesis of *Staphylococcus aureus* in *Caenorhabditis elegans*

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Abstract

Introduction: The use of small interfering RNA (siRNA) gene silencing is a promising therapeutic option as it does not impose selective pressure on bacteria that is often associated with the development of resistance. The study assessed the effect of siRNA targeted to *sarA* and *agrA* in *S. aureus* and the relationship between the transcriptional response, biofilm formation and pathogenicity.

Methodology: siRNAs designed against *agrA* and *sarA* were electroporated into methicillin-resistant and methicillin-susceptible *S. aureus* strains. mRNA levels, growth kinetics, biofilm formation and minimal inhibitory concentration were measured. Efficacy of siRNA in bacteria was assessed using survival assays in a *C. elegans* model. Differences in gene expression before and after siRNA treatment were anaysed using the paired t-test, while the log rank test was used to assess the significance of any difference among survival rates of nematodes.

Results: Biofilm formation decreased significantly in siRNA treated strains and growth rates of siRNA treated strains were significantly higher compared to untreated strains. We observed significant decreases in the transcriptional response in siRNA treated strains, with concomitant significant increases in the lifespan of *C. elegans* worms exposed to siRNA-treated versus untreated strains.

Conclusions: siRNA targeted to *agrA* and *sarA* lowered mRNA transcription and pathogenicity of *S. aureus.*

Key words: *Staphylococcus aureus*; small interfering RNA; gene silencing; *C. elegans*; pathogenicity.

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Introduction

Given the growing incidence of multidrug resistance and the difficulty in treating infections, novel treatment strategies that might abolish or attenuate expression of virulence genes are critical for disarming the pathogen during infection [1-4]. Knowledge of the signal transduction systems involved in bacterial pathogenesis have provided insights regarding gene expression at different stages of infection [5]. Cell to cell communication and virulence gene expression in *S. aureus* [6] are mediated by a 2-component signal transduction system (TCSTS) that coordinates cooperative gene expression in *S. aureus* to enhance survival during infection. The accessory gene regulator (*agr*) operon is a TCSTS in *S. aureus* and is one of the most well studied systems of bacterial human pathogenesis. *S. aureus* occupies numerous niches within its host, a feature that is tightly regulated by the selective expression of virulence genes [7], and the *agr* operon is central to this regulation [8,9].

Operationally, the *agr* operon comprises two divergent promoters, P2 and P3, which control production of two transcripts, *RNAII* and *RNAIII* respectively. *RNAII* produces the components of the *agr* circuit (*agrBDCA*). Briefly, AgrB is responsible for the processing of the signalling propeptide AgrD. Once propeptide processing is complete, the mature AgrD is exported extracellularly where it functions as the system's signal molecule. AgrC serves as the system's sensor and autophosphorylates AgrA once critical concentrations of AgrD are attained. The phosphorylated AgrA activates P2 and P3 [10,11]. Activation of P2 ensures the circuit remains active providing the auto-inducing peptide is at critical concentration. P3 activation leads to *RNAIII* production that results in the up-regulation of secreted toxins to favour infection and the down-regulation of adhesins and surface proteins to facilitate the switch from colonization to invasion [5,12].

The *S. aureus* accessory gene regulator, while not as extensively studied as the *agr* TCSTS, is important in virulence gene regulation. SarA is a DNA binding protein whose locus consists of three overlapping genes (*sarB, sarC and sarA*), which together encodes the SarA protein [13]. The action of SarA assists in the coordination of virulence gene expression in conjunction with the *agr* loci [14]. Given the importance of these regulatory systems to *S. aureus*'

virulence, their blockade could result in attenuated expression of virulence genes and pathogenicity. While biofilm formation might not be affected by perturbation of *agr* expression [15], it is important that *sarA* is functional [16]

RNA mediated interference (RNAi) was first discovered as an antiviral mechanism in plants, and later in the nematode *Caenorhabditis elegans* and the insect *Drosophila melanogaster* [17,18]. RNAi was identified as a proceed of restriction digestion of mRNA to form smaller pieces of RNAs known as small interfering RNAs (siRNAs) [19]. A similar mechanism was identified in prokaryotes, aimed primarily at protecting bacterial cells from bacteriophage infections using the CRISPR/*cas* system [20]. Incorporation of these RNAs into the CRISPR loci result in the provision of a sort of 'immunity' should the bacterium encounter similar sequences in the future [21]. The system effectively suppresses gene expression using double stranded siRNA as a trigger.

C. elegans is a genetically tractable nematode that has been shown to be a useful model for studying microbial pathogenesis [22,23]. Its use has been supported by the fact that virulence factors involved in pathogenesis in *C. elegans* were also required for pathogenesis in mammals [24]. These, along with the ease of handling and the statistical advantage offered when using lower order model organisms, make *C. elegans* suitable for studying *S. aureus* pathogenesis. In the current study, the efficacies of siRNA targeted to *agrA* ($siRNA^{agrA}$), *sarA* ($siRNA^{sarA}$) and both *agrA* and *sarA* ($siRNA^{agrA\ and\ sarA}$) on the transcriptional response, biofilm formation and pathogenicity of *S. aureus* were examined.

Methodology

Strains and culture conditions

Staphylococcus aureus subsp. *aureus* Rosenbach (ATCC 25923; Hospital-acquired methicillin-resistant MRSA; HA-MRSA), *S. aureus* subsp. *aureus* Rosenbach (ATCC 1556; Community-acquired MRSA; CA-MRSA) and *Staphylococcus aureus* strain Newman (Methicillin-sensitive; MSSA) were used in this study. Strains were grown at 37 °C in Tryptic soy broth with agitation.

Small interfering RNA (siRNA) design and labelling

Small interfering RNAs (siRNA) were designed using the *agrA* and *sarA* genes of the above-mentioned strains as templates. Homology screens were conducted on siRNA to determine sequence homology to other *S. aureus* genes using NCBI BLAST tool. siRNA sequences used in the study are shown in Table 1 and were obtained from Integrated DNA Technologies (Coralville, IA, USA). siRNAs were labelled using the Silencer®siRNA Labelling Kit-Cy™3 (Ambion®, Bioanalytical Instruments) according to the manufacturer's specifications. Briefly, 5 µg siRNA was mixed with nuclease free water, 10x loading buffer and Cy™3 labelling reagent, and the mixture was vortexed and incubated in the dark at 37 °C. Subsequently, the labelled oligonucleotides were ethanol precipitated and electroporated into the bacteria.

Table 1. Nucleotide sequences of small interfering RNAs (siRNAs) used in this study.

siRNA (polarity)	Sequence (5'-3')
sarA (sense)	AGUUAAAAUUUUAUCUCAAGU
agrA (sense)	UUCGUAAGCAUGACCCAGUUGGUAACAU

Preparation of electro-competent S. aureus cells and electroporation

S. aureus (10 mL) was grown to $OD_{600} \approx 0.2$ and placed in 90 mL of Luria-Bertani (LB) broth. The bacteria were grown until $OD_{600} = 0.6$ after which they were pelleted at 4 °C, washed twice with sterile ice-cold water and centrifuged as before. The supernatant was decanted, and the pellet was subsequently washed with ice-cold water and centrifuged at 4 °C. The pellet was then washed twice with ice-cold 10% glycerol and centrifuged at 4 °C. Ice-cold glycerol was used to resuspend the pellet, and aliquots were stored at -70 °C for later use.

Electrocompetent cells were thawed on ice and then incubated at room temperature for 10 minutes. The bacteria were re-suspended in EC buffer (0.5 M sucrose in 10% glycerol) and centrifuged, and the pellet re-suspended in EC buffer. To the mixture, 2 µg siRNA was added and after gentle agitation, was transferred to a sterile 1 mm gap cuvette and electroporated for 5 ms at 23 kV (Eppendorf Electroporator 2510). Immediately following electroporation, LB broth at 37 °C was added to the cuvette. The contents were mixed by gently pipetting and transferred to a sterile tube and incubated at 37 °C for 2 hours before being spread on LB agar plates and incubated overnight at 37 °C.

Growth kinetics

Stationary cultures of *S. aureus* strains obtained by overnight growth at 37 °C were inoculated into LB broth. The OD_{600} was measured and recorded as T_0. The cultures were incubated at 37 °C and the optical density

measured every 20 minutes until the cultures reached stationary phase. All growth curves were done in triplicates, and the mean values ± standard error plotted.

RNA isolation

S. aureus was grown to OD_{600} = 0.5 and used as starting culture for RNA extraction using the hot phenol method. Briefly, to each tube, 1/10 volume of stop solution (10% phenol in absolute ethanol) was added. Each tube was vigorously shaken, placed on ice, and then centrifuged at 4 °C. After centrifugation, the supernatant was decanted and the cell pellet, flash frozen with liquid nitrogen, and subsequently re-suspended in RNAse-free water. Each sample was split equally into two tubes, and to each was added hot phenol solution (20 mM Tris, 400 mM NaCl, 40 mM EDTA, 1% SDS, 1% β-mercaptoethanol and 1-part phenol). Tubes were vortexed, placed in a 95 °C water bath for 1 min, then centrifuged, and the supernatant was discarded and to the pellet, phenol/chloroform (1:1) solution was added followed by brief vortexing. Tubes were then centrifuged, and the top aqueous layer was extracted twice with chloroform. Subsequently, 2.5 volumes of absolute ethanol and 1/10 volume of sodium acetate were added to the extracted aqueous layer, and after mixing by inversion, tubes were incubated at -20 °C overnight. Following this, tubes were centrifuged at 4 °C, the pellet washed with 70% ethanol at -20 °C and re-suspended in RNAse-free water. RNA concentration was determined using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and agarose electrophoresis was used to estimate genomic DNA in samples. DNAse treatment was done using 1U DNase and 1 µg Ribolock per 1 µg of DNA present, after which, samples were extracted with phenol/chloroform, re-suspended in DEPC treated water and stored at -80 °C.

mRNA semi-quantification

cDNA synthesis was conducted using the RevertAid H-Minus cDNA synthesis kit (Thermo Scientific, Wilmington, DE, USA). Test reactions were set up with 5 µg template RNA, 1 µL random hexamer primer, 1 µL Ribolock RNAse Inhibitor and 1 µL 10 mM dNTP mix at 25 °C for 5 minutes, 42 °C for 60 minutes followed by 70 °C for 5 minutes. Singleplex reactions were conducted using 2 µL of the cDNA using the primers and annealing temperatures in Table 2. A no-template reaction was set up using 5 µL DEPC treated water instead of RNA template. Gels were analyzed using ImageJ software [25], and differences in mRNA expression determined. Changes in gene expression of *agrA*, *sarA*, *RNAII* and *RNAIII*, relative to the 16S rRNA content, were calculated using the following formula:

$$Cr = x - \frac{y}{x + \frac{y}{2}}$$

Where: C_r = relative change in mRNA production, x = 16S rRNA expression value, and y = *sarA*/*agrA*/*RNAII*/*RNAIII* mRNA expression value.

Microtitre plate assay biofilm

Biofilm assays were conducted using the method described by Zmantar *et al*. [26]. After planktonic *S. aureus* cells and media were aspirated from the wells, biofilms were washed and stained with 0.1% crystal violet and the optical density for wells recorded at 570 nm.

C. elegans lifespan assay

C. elegans strain CF512 (Caenorhabditis Genetics Center [CGC], University of Minnesota, MN, USA) was grown on lawns of *E. coli* OP50 (CGC) at 15 °C and subsequently harvested to obtain L4 stage nematodes using the hypochlorite method [24]. Strains treated with $siRNA^{agrA}$, $siRNA^{sarA}$, or $siRNA^{agrA\ and\ sarA}$ were plated in triplicate on nematode growth medium and incubated at 37 °C for 10 hours. Each plate was subsequently seeded with 30 L4 *C. elegans* worms and incubated at 24 °C. Similar incubations were carried out with plates containing L4 worms inoculated with

Table 2. Primer sequences and annealing temperatures for genes examined in gene expression.

Gene/Target	Primer Name	Sequence (5' → 3')	Annealing Temperature (°C)
16S rRNA	16S rRNA1	ATGCAAGTCGAGCGAAC	57
	16S rRNA2	TGTCTCAGTTCCAGTGTGGC	
agrA	AgrA1	GGTGAAGGTCGTGGTTTAGGT	54.5
	AgrA2	TCACCGATGCATACGAGTGTT	
sarA	SarA1	TGACTAACCAAATGCTAACCCA	55
	SarA2	GCCATTAGTGCAAAACCTCTT	
RNAIII	RNAIII fwd	AGATCACAGAGATGTGATGG	53
	RNAIII rev	CAAAAGGCCGCGAGCTTGGG	
RNAII	RNAII fwd	CGAAATGCGCAAGTTCCG	51.5
	RNAII rev	CCAACTGGGTCATGGTTAGCA	

Table 3. Optical Density at 550 nm of cytoplasmic contents of labelled and unlabeled siRNA with silencer siRNA labelling.

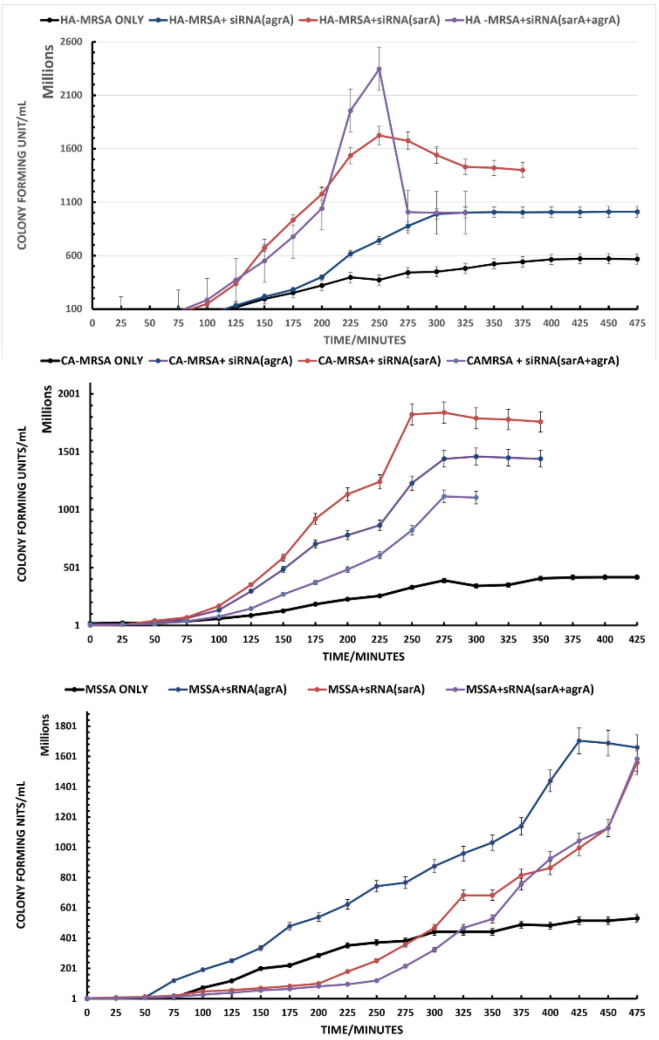
siRNA	CA-MRSA (ATCC 1556)		HA-MRSA (ATCC 25923)		MSSA (Newman)	
	Labelled	Unlabelled	Labelled	Unlabelled	Labelled	Unlabelled
sarA	0.192	0.001	0.201	0.002	0.098	0.001
agrA	0.172	0.002	0.179	0.001	0.095	0.001
agrA and sarA	0.198	0.002	0.221	0001	0.240	0.003

untreated *S. aureus* strains. Plates were scored each day for responsive (live) and unresponsive (dead) worms until all worms were unresponsive.

Statistical analyses

Statistical analyses were done using the Statistical Package for Social Sciences (SPSS) version 18. The paired t-test was used to determine any differences in gene expression, growth kinetics and biofilm production with and without siRNA treatment. Statistical significance was identified at $p < 0.05$. *C. elegans* survival was determined using the Kaplan-Meier test and any significant difference in lifespan was assessed using the log-rank test.

Figure 1. Growth curves for HA-MRSA, CA-MRSA, and MSSA strains treated with siRNA targeted to agrA and/or sarA.



Results

Growth kinetics

The growth kinetics of strains exposed to siRNA treatment showed shorter lag phases and longer exponential phases. All treatment groups except for MSSA+siRNA$^{agrA\ and\ sarA}$, had significantly higher growth rates compared their untreated counterparts (Figure 1).

Effect on gene expression

The extent of incorporation of labelling of cytoplasmic contents siRNA in the various strains are given in Table 3. mRNA production (relative to 16S rRNA) in HA-MRSA+siRNA^{agrA} and HA-MRSA+siRNA$^{agrA\ and\ sarA}$ decreased when compared to untreated HA-MRSA (Figure 2, top), with the lowering in the latter treatment group being significant ($p < 0.05$). This was observable by an observable 'fold' increase in the calculated C_r value, which has an inverse relationship with the measured variable. Consequently, as the mRNA for the targeted gene decreases, there is recorded a measurable increase in relative change in pixel area when compared to the 16S rRNA content in the bacteria. Paired t-test showed that the treatment that caused a significant difference in mRNA production in the community strain was CA-MRSA+siRNA^{agrA} ($p < 0.05$). While treatment with siRNA$^{agrA\ and\ sarA}$ caused a lowering in transcriptional response, this change was not statistically significant (Figure 2, middle). *RNAII* expression increased when CA-MRSA was treated with siRNA^{sarA} and siRNA^{agrA}. MSSA treated with siRNA did not result in any significant lowering of mRNA production. In fact, there were notable increases in transcriptional response when the strain was treated with siRNA^{agrA} and siRNA$^{agrA\ and\ sarA}$ (Figure 2, bottom).

Effect on biofilm formation

Biofilm formation was significantly reduced in strains that were treated with the various siRNAs (Figure 3). Significant lowering of biofilm formation upon siRNA treatment was observed for HA-

MRSA+siRNA^{agrA} and HA-MRSA+siRNA$^{agrA\ and\ sarA}$ ($p < 0.05$); for HA-MRSA+siRNA^{sarA}, p = 0.05. Reductions in biofilm formation in MSSA+siRNA^{sarA} ($p < 0.01$) and CA+siRNA$^{agrA\ and\ sarA}$ ($p < 0.05$) were also significant. While reductions were noted in other treatment groups, they were not statistically significant.

Effect on infectivity and survival in C. Elegans

Survival analysis for *C. elegans* worms fed HA-MRSA showed worms having a longer probability of survival with treated strain when compared to untreated strain (Figure 4, top). Median lifespan for worms fed untreated strain was 6 days while for siRNA treated strain, the cumulative survival probability at 0.5 (q = 0.5) was 10.5 days. Differences in lifespan were found to be significant by log rank test ($p < 0.05$), with 90% probability of *C. elegans* survival being obtained at 3 days while for worms fed HA-MRSA+siRNA^{agrA}, HA-MRSA+siRNA^{sarA} and HA-MRSA+siRNA$^{agrA\ and\ sarA}$, it was 6.3, 3.5 and 7 days, respectively (Table 4). The mean-time-to-death (TDmean) of worms fed HA-MRSA was 10.9 ± 1.3 days, and this was increased to maximum of 16.4 ± 1.2 days when worms were fed HA-MRSA+siRNA^{sarA}. Differences between TDmeans of worms fed untreated vs treated strains were approaching significance ($p = 0.0059$).

Figure 2. Difference in pixel area coverage in agrA, sarA, RNAIII and RNAII genes of HA-MRSA, CA-MRSA, and MSSA strains treated with siRNA targeted to agrA and/or sarA.

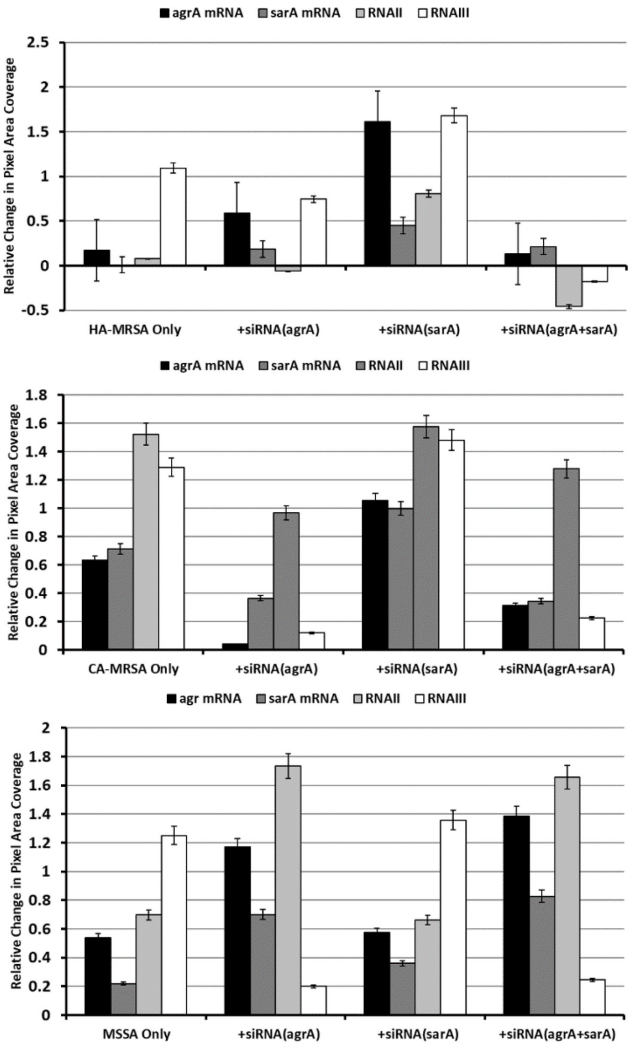
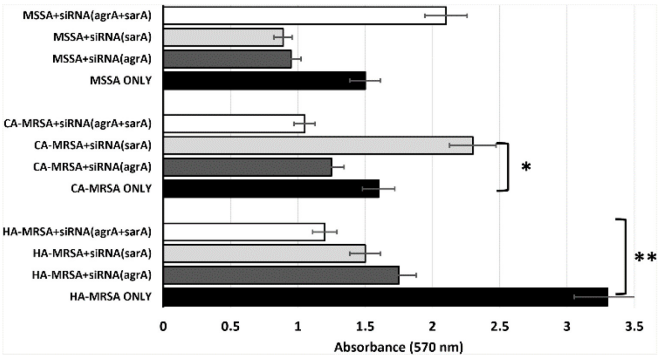


Figure 3. Optical density (biofilm staining) at 570 nm for HA-MRSA, CA-MRSA and MSSA strains treated with siRNA targeted to agrA and/or sarA. * p < 0.05; ** p < 0.01.



Survival analysis of *C. elegans* fed CA-MRSA showed significant differences ($p < 0.01$) in the survival probability of worms fed untreated strains compared to those fed CA-MRSA+siRNA (Figure 4, middle). Median lifespan life-span survival (q = 0.5) was longer in treated *vs*. untreated strain (untreated CA-MRSA was 2.8 days; CA-MRSA+siRNA^{agrA}, 11 days; CA-MRSA+siRNA^{sarA}, 10.8 days; CA-MRSA+siRNA$^{agrA\ and\ sarA}$, 12.7 days). There was a greater probability for survival of worms to survive at the beginning of the experiment (q = 0.9) when they were treated (CA-MRSA+siRNA^{agrA} was 7 days; CA-MRSA+siRNA^{sarA}, 3 days; CA-MRSA+siRNA$^{agrA\ and\ sarA}$, 7.5 days) compared to the untreated strain that had a 90% probability of survival of 2.8 days (Table 4). The TDmean of worms fed CA-MRSA was 12.8 ± 2.5 days, and this was extended to 17.9 ± 1.6 days when worms were fed CA-MRSA+siRNA$^{agrA\ and\ sarA}$. Differences between TDmeans of worms fed untreated vs treated CA-MRSA strains not significant ($p = 0.0533$).

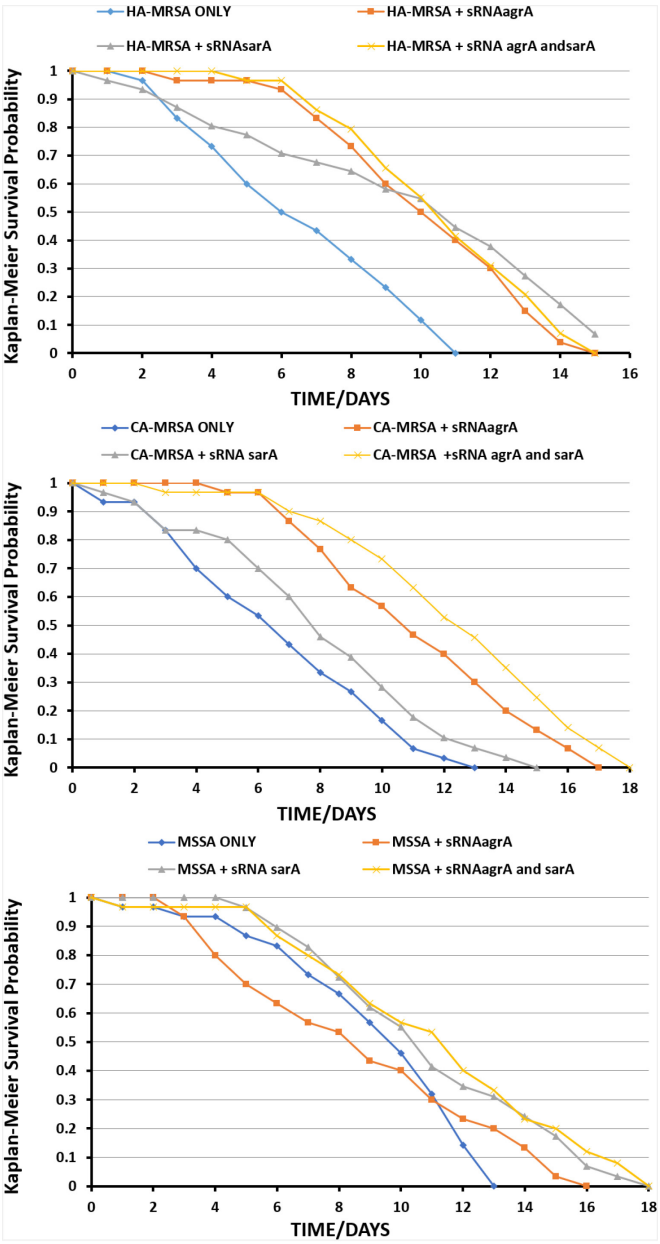
Survival analysis of *C. elegans* fed MSSA showed that there was a significant difference ($p < 0.01$) in the survival probability of worms fed untreated MSSA compared to MSSA+siRNA$^{agrA\ and\ sarA}$ (Figure 4, bottom). There was a greater probability for worms fed MSSA+siRNA$^{agrA\ and\ sarA}$ or MSSA+siRNA^{sarA} to survive at the beginning of the experiment (q = 0.9) compared to the untreated MSSA strain that had a 90%

Table 4. q values for *C. elegans* fed treated and untreated HA-MRSA, CA-MRSA and MSSA strains.

	q	Strain Only	+siRNA^{agrA}	+siRNA^{sarA}	+siRNA$^{agrA\ and\ sarA}$
HA-MRSA	0.9	3	6.3	3.5	7
	0.5	6.1	10.1	0.8	10.5
CA-MRSA	0.9	2.8	7	3	7.5
	0.5	6.5	11	7.9	12.9
MSSA	0.9	4.9	3.8	6.1	6.3
	0.5	9.8	8.3	10.2	11.2

probability of survival at 4.9 days. Probability of survival for worms treated with MSSA+siRNA^{agrA} was 3.8 days, and MSSA+siRNA^{sarA} or MSSA+siRNA$^{agrA\ and\ sarA}$, 6.3 days (Table 4). The TDmean of worms fed MSSA was 13.4 ± 0.7 days, and this was increased to 17.8 ± 1.1 days when worms were fed MSSA+siRNA^{sarA}. Differences between TDmeans of worms fed untreated vs treated MSSA strains were not significant ($p = 0.0229$).

Figure 4. Kaplan Meier probability plots of killing of *C. elegans* fed HA-MRSA, CA-MRSA and MSSA treated with siRNA targeted to agrA and/or sarA.



Finally, using the paired t-test, only TDmeans of worms fed HA-MRSA strains were significant when compoared to worms fed MSSA ($p = 0.0163$); for HA-MRSA *vs*. CA-MRSA, $p = 0.1817$, and for CA-MRSA *vs.* MSSA, $p = 0.6042$.

Discussion

This study sought to show two things. First, that the use of small interferring RNAs in different strains of *S. aureus* was capable of contributing to decreased transcriptional response in the genes affected by the *agr* and *sarA* circuits. Second, that perturbation of the *agrA* and *sarA* regulatory circuits using siRNAs could lead to attenuated pathogenesis in the strains in the *C. elegans* model.

The growth kinetics of strains exposed to siRNA treatment showed shorter lag phases and longer exponential phases. Repression of the *agr* quorum sensing circuit would lead to the cells remaining in a quiescent stage where metabolic activity is geared toward surviving until cell density reaches critical level to sustain infection. Without this communication taking place, cell division occurs unchecked in relation to cell density and cells enter the exponential phase quicker. Once the cells have entered exponential phase, doubling time is rapid because siRNA repression of the *agr* and/or *sarA* regulon causes a lowered metabolic burden of the cells owing to decreased transcription of virulence genes. While the absence of this confirmation by virulence gene expression profiling is a clear limitation of this study, any increase in growth rate of bacteria treated with siRNA is of concern since elevation of bacterial titres during the infection process may lead to an exacerbation of disease symptoms.

All strains except for MSSA showed decreased transcriptional response when treated with siRNA^{agrA}, in keeping with the repression of a circuit that regulates virulence. Other studies have shown that *agr* mutants

have attenuated virulence [27,28]. Based on our data that showed a decrease in gene expression and not a cessation of the transcriptional response, we postulate that perturbation of the *agr* regulation leads to lowered gene expression from the *agr* locus and could translate into decreased pathogenicity. Added to that, while *agr* activity is arguably the primary regulator of *S. aureus* virulence, other regulatory systems can modulate its activity and that of its effector, *RNAIII* [29] and multiple regulatory RNAs and proteins affect transcription of *RNAII* and *RNAIII* [30]. It is therefore important to view the results of this study juxtaposed with the complex regulatory framework present in *S. aureus* that controls the expression of virulence determinants, taking into consideration the fact that *agr* is not the only locus of control for virulence gene expression. Notwithstanding, the decreased transcriptional response seen in the current study indicates that key virulence determinants are being negatively affected which could lead to decreased pathogenicity and places *agr* silencing as a viable option in developing avirulence or reduced virulence in strains.

Strains exposed to siRNA targeting both loci were expected to have lowered gene expression when compared to strains that were treated with a single siRNA, since *sarA* exerts transcriptional control on the *agr* operon by activating P2 [31]. This was found in HA-MRSA but not in CA-MRSA or MSSA. This increase in the latter could be a result of off-target effects, due to base pairing of siRNA with other non-specific mRNA targets in the cell [32].

Biofilm formation is an important microbiological process, since organisms associated with them often exhibit increased resistance to antimicrobial agents [33], and also because of the potential of these organisms to cause disease in patients with indwelling devices. In the current study, biofilm formation in strains treated with siRNA decreased in all treatment groups except for MSSA+siRNA$^{agrA\ and\ sarA}$. This suggests that the regulatory circuits *sarA* and *agr* have an impact on the expression of genes involved in biofilm formation. The presence of siRNA targeted towards *agrA* and *sarA* had a negative impact on biofilm formation. *RNAIII*, the regulatory RNA transcribed upon continued activation of the *agr* locus, modulates gene expression in *S. aureus* to promote the switch from the expression of genes that are necessary for biofilm formation to secreted toxins. In the current study, gene expression of *RNAIII* decreased in all strains treated with siRNA directed to *agrA* and *sarA*. Further, *agrA* gene expression was attenuated under all siRNA treatments in the hospital and community MRSA strains, while in the MSSA strain, gene expression decreased only in the presence of siRNA directed to *agrA* and *sarA*.

Infectivity and survival analyses in *C. elegans* were conducted to ascertain the impact of treatment with siRNA^{agrA}, siRNA^{sarA} and siRNA$^{agrA\ and\ sarA}$ on *S. aureus* strains. The probability of 90% survival of worms was greater for strains treated with siRNAs, indicating that treatment improved the chances of worm survival in the early stages of gene silencing. This difference in early survival probability is significant since gene silencing lasts between 3-7 days in cells with short generation times such as *S. aureus* [34]. Additionally, after the maximum time of having a 90% survival (7 days), sharp decreases in survival or worms were seen, which is directly due to the operation of siRNAs in the cell. Though the nematode model has its limitations, particularly in relation to the incubation temperature at which experiments are conducted, it had been consistently validated as a suitable model host for examining pathogenicity.

Conclusions

The results of this study place emphasis on the proof of principle regarding the use of siRNAs and position it as an important adjunct to conventional therapeutic options for *S. aureus* infections. Notwithstanding, the use of mammalian models to corroborate the current findings and extend the scope of modelling to different pathophysiologies and disease states, and the examination of global changes in gene expression patterns upon siRNA treatment, remain important future work.

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