Investigating Mobile Genetic Element Induction Dynamics in MW2

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Abstract

Mobile genetic elements (MGEs) such as prophages and *Staphylococcus aureus* pathogenicity islands (SAPIs) play a key role in the virulence and spread of antibiotic resistance genes in *Staphylococcus aureus* (*S. aureus*). This study investigated induction dynamics of MGEs in the community-associated methicillin-resistant *S. aureus* strain MW2. Reporter plasmids were constructed containing regulatory regions from prophages ϕSa2MW2, ϕSa3MW2, and SaPIMW2, fused to a β-lactamase reporter gene (blaZ). These constructs were introduced into both MW2 and the laboratory strain RN4220. Mitomycin C was added to stimulate the SOS response, and the induction of promoter regions for MGEs was monitored through a β-lactamase reporter assay. Results showed that ϕSa2MW2 was strongly induced in both strains, confirming its dominant role in MW2 shown in previous literature. ϕSa3MW2 was only induced in RN4220. SaPIMW2 was not significantly induced in either strain, despite the presence of its helper phage ϕSa2MW2. These findings suggest induction is strongly influenced by the host genetic background and emphasise the relations between co-resident MGEs.

1. Background

Staphylococcus aureus (*S.* aureus) is a common bacterial pathogen that colonises the skin and mucosa of healthy adults (1). It is present in both healthcare and community settings, where it can cause a wide range of infections (2). Methicillin-resistant *S. aureus* (MRSA) strains are harder to treat as they resist many first-line antibiotics (3). MW2 is an example of a community-associated MRSA (CA-MRSA) strain.

The pathogenicity of *S. aureus* is due to the broad spectrum of virulence factors present within its genome - cellular structures that enable microbial pathogens to colonize, evade or suppress the immune response, obtain nutrients from the host, and sense environmental changes (4). In *S. aureus* many of these virulence factors are mobile genetic elements (MGEs) (5).

Hypothesis: MGEs are induced differently in clinical and laboratory MW2 strains.

Aims:

1. To monitor the following MGEs: ϕSa2MW2, ϕSa3MW2, SaPIMW2
2. Determine the induction response of each MGE in the *S. aureus* lab strain RN4220
3. Determine the induction response to mitomycin C (MMC) for each MGE in their native *S. aureus* clinical host strain MW2

1.1 Basics of bacterial genetics

Bacterial DNA is stored in a singular chromosome (6). Along with proteins and RNA, this forms the nucleoid. In addition to the chromosome, bacteria often contain plasmids – small, non-essential, circular, double-stranded DNA molecules (7). These contain advantageous genes to the bacterium, such as those for antimicrobial resistance (8).

Plasmids are also widely used as vectors in genetic engineering to transfer desired genes into host bacterial cells (9) through *in vivo* cloning. Bacteria exchange plasmids through horizontal gene transfer – the movement of genetic information between organisms (10). This involves three well-understood genetic mechanisms (Fig. 1):

1. Transformation: Bacteria take up DNA from their environment
2. Conjugation: Bacteria directly transfer genes to another cell
3. Transduction: Bacteriophages move genes from one cell to another

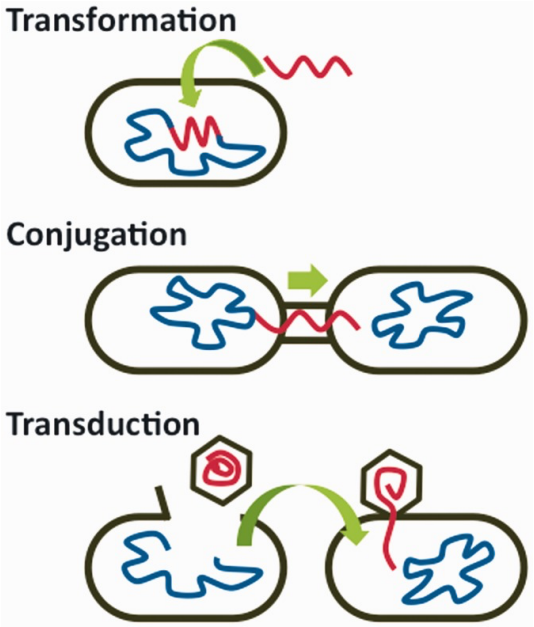

Figure 1. Mechanisms of bacterial horizontal gene transfer (10). During transformation, bacteria take up DNA directly from the environment. During conjugation bacteria directly transfer genes to another cell through pilus formation. Finally, bacteriophages move genes from one cell to another. All genes can be horizontally transferred and proliferate by natural selection (11). Once transferred, the genes and pathogens continue to evolve, often resulting in bacteria with greater resistance (12).

1.2 Bacteriophages

Bacteriophages (phages) are a type of MGE. They are viruses that infect and replicate only in bacterial cells, often carrying genes for toxins or immune evasion (13) Temperate phages insert its genome into the bacterial chromosome or cytoplasm (14).

Temperate phages have a lytic and lysogenic cycle (15). The lytic cycle results in the rapid reproduction of phages and the immediate destruction of the host cell, releasing the phage. In contrast, the lysogenic cycle involves the phage's DNA integrating into the

host's chromosome to become a prophage, which then replicates along with the host DNA in a dormant state.

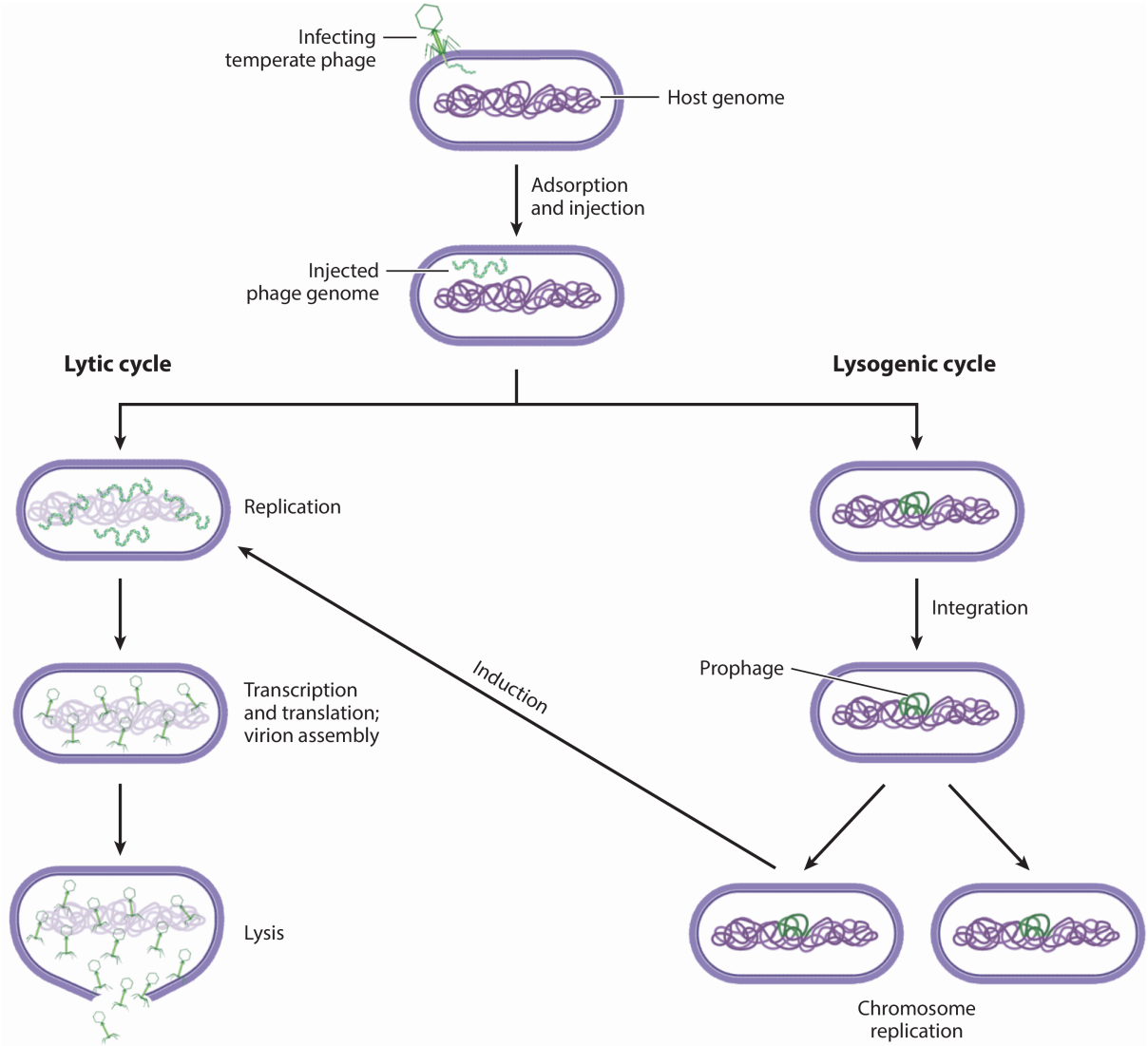

Figure 2. Diagram showing differences between the lytic and lysogenic lifecycles of temperate phages (16). Temperate phages inject their DNA into the bacterial cell after binding to specific host cell receptors. The phage genome then undergoes either the lytic or lysogenic cycle. During the lytic cycle, the phage genome replicates using host cell machinery, producing phage proteins to assemble viral particles which are then released through lysis. These can infect other bacterial cells. During lysogeny, the phage genome is integrated into the host chromosome as a prophage, which is replicated passively as the bacterium divides. Prophage induction can occur following

environmental stresses, where the phage genome is excised from the host chromosome and enters the lytic cycle. An example of an environmental stressor is treatment with mitomycin C.

The SOS response is a conserved pathway in bacteria that is activated under certain stress conditions that compromise cell survival (17). It is involved in restarting stalled and collapsed replication forks, regulation of cell division, and DNA repair (18, 19). The pathway is regulated by proteins LexA and RecA. LexA is an autoregulatory repressor protein.

During normal cellular growth, LexA dimers bind to two sequences in the promoter region of genes involved in the SOS response. This affects RNA polymerase association, preventing transcription (20, 21).

Under DNA damage, for example under treatment with MMC, the SOS response is activated: RecA is activated, stimulating the autocleavage of LexA. This promotes expression of genes contained in the SOS regulon as LexA can no longer bind efficiently (22). This causes the expression of genes required to repair bacterial DNA damage until normal cellular replication can begin. This causes most prophages to switch to the lytic cycle, although the full nature of interaction of the phages studied in the SOS response is unknown.

1. 3 *Staphylococcus aureus* pathogenicity islands (SAPIs)

SaPIs are another type of MGE. They are phage parasites that hijack phage replication (23). Stl is the repressor of SaPIs (24), which blocks transcription of SaPIMW2 genes until derepressed by phage protein DUF3113 produced by helper phage ϕSa2MW2 (25). SaPIs can produce molecules such as proteins and RNA that interfere with phage replication. This interference usually reduces the phage titre and allows the SaPI to be predominantly packaged into phage particles.

1.4 Genetic background of MW2 and RN4220

MW2 carries multiple native MGEs: two phages (ϕSa2MW2 and ϕSa3MW2) and one SaPI (SaPIMW2). In MW2, the endogenous phage ϕSa2MW2 has been shown to derepress SaPIMW2 by producing the DUF3113 protein, which interacts with Stl and initiates the SaPI lifecycle (25).

In contrast, RN4220 is a restriction-defective derivative of RN450 (26). It lacks native MGEs. Induction of MGEs was assessed in RN4220, before completing the same experiment in MW2. Through confirming whether MGE inductions are the same, we can investigate interactions between native MGEs.

Information about strains used can be found in supplementary table S1. All strains were used and made in Haag Labs by my supervisor Dr Andreas Haag.

1.5 Plasmid design

pAH0198 was used as the plasmid reporter backbone for cloning (Figure 3). It carries a replication module (rep and origin) so the plasmid is maintained during cell division, a chloramphenicol acetyltransferase gene *cat*, and a β-lactamase reporter gene *blaZ* (27). *cat* selected for plasmid-containing cells by providing resistance to the antibiotic chloramphenicol (28). It also carries a multiple cloning site, where regulatory regions of interest were inserted upstream of *blaZ*. pAH0198 acted as the negative control as no promoter region is present.

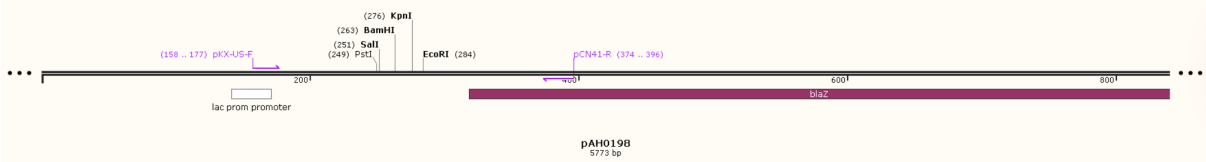

Figure 3. Linear plasmid map of pAH0198 backbone.

The *immAR – cro'* regulatory region of ϕSa2MW2 was inserted into the pAH0198 backbone upstream of *blaZ*, producing pAH0874 reporter plasmid (Figure 4). ϕSa2MW2 is the phage that derepresses SaPIMW2 (25).

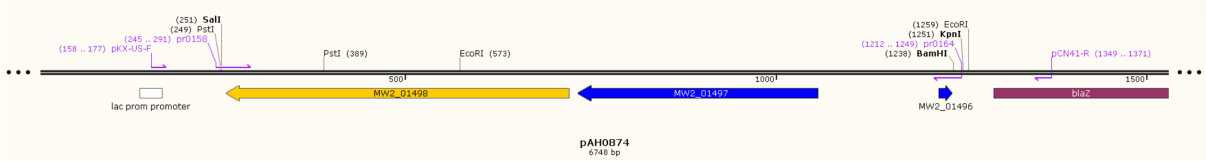

Figure 4. pAH0874 linear plasmid map (pAH0198 with immAR -cro')

The *cI – cro' (29)* regulatory region of ϕSa3MW2 was inserted into the pAH0198 backbone upstream of *blaZ*, producing pAH0875 reporter plasmid (Figure 5). The *cI – cro* region is controlled by *CI*, ϕSa3MW22's repressor.

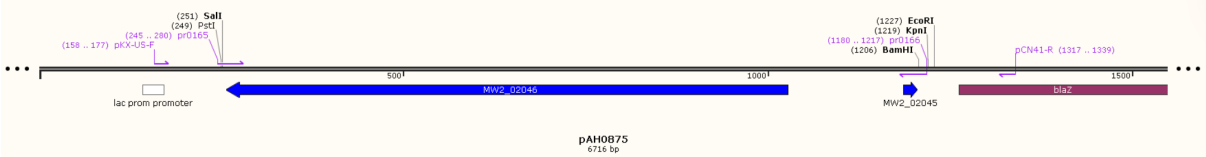

Figure 5. pAH0875 linear plasmid map (pAH0198 with cI – cro)

The *stI – cro* regulatory region of SaPIMW2 was inserted into the pAH0198 backbone upstream of *blaZ*, producing pAH0876 reporter plasmid (Figure 6).

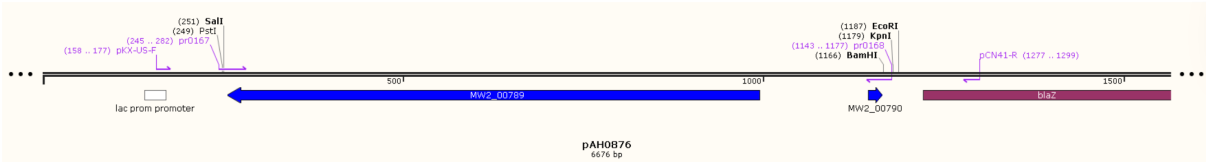

Figure 6. pAH0876 circular plasmid map.

2. Methods

We followed the steps below to collect results:

1. General preparation
2. Construct reporter systems – blaZ
3. Introduce constructs into RN4220 and MW2 clinical isolate
4. Induce MGE activation through β-lactamase assay
5. Data analysis

2.1 Bacterial growth and culture conditions (30)

The bacterial strains used in this study are detailed in Supplementary Table 1. *S. aureus* strains were grown in Tryptic Soy Broth (TSB) or on Tryptic Soy Agar (TSA) plates and *E. coli* strains were grown in Luria-Bertani broth (LB) on LB agar plates. Antibiotic selection was used where indicated: erythromycin (10 µg / ml) or chloramphenicol (20 µg / ml) as needed. *E. coli* was grown in LB supplemented with ampicillin (100 µg / ml) as appropriate.

2.2 Construction of reporter systems

2.2.1 PCR

PCR for promoter amplification was performed using PrimeStar GXL polymerase (Takara) according to the manufacturer's instructions using genomic DNA as template and primers indicated in supplementary table 2.

Sigma-Aldrich GenElute™ PCR Clean-UP Kit and associated protocol(31) was used to purify products for downstream applications.

2.2.2 Restriction Enzyme Digest (32)

Performed double digest of pAH0198 using restriction enzymes BamHI-HF (R3138S, New England Biolabs) and SalI-HF (R3136S, New England Biolabs). rCutSmart™ Buffer (10x) was used. Reaction mixture was incubated overnight at 37°C

2.2.3 Dephosphorylation (33)

rSAP (NEB #M0371) added to double digest reaction mixture. Successful digest confirmed by gel electrophoresis and imaging. Purify digest products using PCR cleanup kit.

2.2.4 Ligation (34)

Used 3:1 plasmid to insert ratio using T4 DNA ligase. Subsequent products then proceeded to transformation.

2.3 Introduction of constructs into RN4220 and MW2 clinical isolate

2.3.1 E. coli competent cell preparation

A 5ml overnight culture of *E. coli* was prepared in LB. A 1/50 dilution of overnight culture and LB broth was prepared. Cells were grown to an OD_{600} of 0.3 – 0.5 in a shaking incubator at 37°C, 200 rpm. Cells were chilled on ice for 10 minutes and then harvested by centrifugation and pelleted. The pellet was resuspended in 5ml of ice-cold 0.1 M $CaCl_2$ and supplemented with 15% (v/v) glycerol. Competent cells were aliquoted for storage at -70°C.

2.3.2 E. coli competent cell transformation

10 µl of ligation or 100 ng of circular plasmid was added to competent cells. The mixture was heat shocked at 42°C and kept on ice for 5 minutes before adding 1 ml of LB broth. The tube was incubated for 1 hour with shaking at 37°C. Cells were spun down on a tabletop centrifuge for 5 minutes at 8000 x *g*. 800 µl of the supernatant was taken off, and the pellet was resuspended in the remaining medium and plated on selective antibiotic plates. DNA sequencing using Eurofins Mix2Seq kit was used to confirm correct plasmid had successfully been inserted. Resulting sequence compared to expected sequence in Benchling (35).

2.3.3 Colony PCR (36)

Colony picked from plate with a pipette and kept in 25µl of LB and ampicillin, using sterile conditions. PCR mixtures were prepared with DreamTaq Buffer (10x), dNTP, forward primer, reverse primer and DreamTaq Polymerase and water. Primers used are detailed in supplementary table 2. LB, ampicillin and colony mixture was added to PCR mixture. 5 µl of LB and ampicillin was added to the control. Presence of insert was confirmed through gel electrophoresis and imaging.

2.3.4 Preparation of Competent S. Aureus cells (37)

A single colony of MW2 *S. aureus* strain was inoculated. Overnight culture was diluted 1:100 in TSB and incubated at 37°C and 250 rpm until OD_{600} was approximately 0.5. Culture was pelleted and washed with sterile 0.5 M sucrose and incubated for 30 minutes on ice. The culture was pelleted by a second centrifuge spin. The cell pellet was re-suspended in sterile 0.5 M sucrose. Cells were stored at -70°C.

2.3.5 Transformation of competent S. Aureus cells (37)

2 µg of plasmid was added to 100 µl of competent *S. aureus* cells and the mix was transferred to a pre-chilled electroporation cuvette. The cuvette was placed into the electroporator and pulsed once using the following settings: 21 kV cm-1, 100 Ω, 25 µF. 1ml of TSB was immediately added and the suspension was incubated at 37°C for 1 – 2 hours. The suspension was pelleted by centrifugation before plating on selective agar plates.

2.4 β-Lactamase Assay

2.4.1 Treatment with Mitomycin C (MMC)

Overnight cultures were grown in TSB supplemented with chloramphenicol for RN4220 and erythromycin for MW2. Cells were grown until $OD_{600} \approx 0.25$.

OD_{600} was normalised to 0.25 for all samples using the following formula using volume in microlitres:

Equation 1

$$\left(\frac{OD_{have}}{OD_{want}} \times Volume\right) - Volume = \text{volume of TSB to be added}$$

The initial OD_{600} was recorded. The appropriate volume of TSB was added and samples were treated with 16 µl MMC. Samples were left in a shaking incubator at 30°C, 80 rpm.

OD_{600} was measured and recorded at 30 (t1), 60 (t2), 90 (t3) and 120 minutes (t4). At each of these timepoints 100 µl of the sample was stored at -70°C for use in the nitrocefin β-lactamase activity assay.

2.4.2 Nitrocefin β-lactamase activity assay (38)

Nitrocefin stock solution was prepared using dimethyl sulfoxide. 50 µl of nitrocefin stock solution was added to 10 ml of 50 mM KPO_4 buffer with sodium azide to form a nitrocefin working solution.

Wells were filled with 200 µl of sample, 800 µl of buffer and 50 µl of nitrocefin.

CLARIOStar Plus microplate reader was set to incubate at 37°C and read absorbance at 490 nm. The β-lactamase assay was run for 20 minutes at 20 second intervals. Samples were automixed for 2 seconds before each measurement.

2.5 Data Analysis

2.2.5.1 Analysis of nitrocefin β-lactamase activity assay results

The following equation was used to calculate relative β-lactamase activity:

Equation 2

$$X = \frac{dA_{490}}{dt\ (h)} \frac{1}{OD_{540} * DF * V}$$

The dilution factor used was 0.2. Promoter activity was calculated for plasmids at each timepoint, with and without MMC treatment across all 4 repeats.

Data was filtered manually to remove plateaus in OD at the end of the dataset as these influenced the slope, creating a false lower gradient. In some instances there was an initial increase followed by a sharp decrease in OD at the beginning of the dataset. This pattern was removed to the lowest OD reached and assumed to be due to the presence of bubbles in the microplate.

For each plasmid, mean promoter activity was calculated at every timepoint for both treatment conditions, using data from four replicates. At this stage we noted that values in pAH0874 repeat 1 and pAH0874 repeat 2, which were both on microplate repeat 3, were internally inconsistent with the other plates and expected MMC responses. We corrected an apparent label inversion: for pAH0874 repeat 1, MMC+ and MMC- were swapped at t3 and t4; for pAH0874 repeat 2 MC+ and MC- were swapped at t3. All subsequent analyses used the corrected dataset; the uncorrected values are retained in the raw data archive.

Welch's two-tailed t-test was used to assess differences in promoter activity between MMC+ and MMC− samples at each timepoint. This test was chosen as it is robust to unequal variances and sample sizes between groups. The t-test assumes approximate normality of the data distribution; visual inspection suggested this was appropriate. A significance threshold of $\alpha = 0.05$ was applied, with results reported as $p < 0.05$ (*), $p < 0.01$ (**), and not significant (NS).

Clustered bar charts were plotted comparing mean promoter activity and standard deviation (s.d.) at each timepoint using GraphPad Prism version 10 for macOS, www.graphpad.com.

3. Results

The experiment was performed to investigate whether induction dynamics of MGEs are different between clinical and laboratory MW2 strains. We explored the effect of native MGEs on induction dynamics.

First we designed and prepared reporter plasmids through genetic engineering. We used Polymerase Chain Reaction (PCR) to insert desired promoter regions into template MW2 strands (39). Gel electrophoresis was used to confirm that the promoter fragments had been successfully inserted into the recipient MW2 strands. A restriction enzyme digest, dephosphorylation and ligation were used to form reporter constructs.

Competent IM01B *E. coli* cells were produced through chemical treatment with $CaCl_2$ and reporter plasmids were transformed into these cells. Transformed *E. coli* was grown on selective antibiotics to confirm that the plasmid had been successfully inserted. Colonies of *E. coli* cells that produced growth were picked for colony PCR (36), to amplify the region inserted into the plasmid and the plasmid backbone. Gel electrophoresis was used to verify the identity of these two distinct DNA fragments, confirming the correct plasmids had been inserted. Samples were also sent for DNA sequencing (40) and Benchling was used to align sequences (35).

After this confirmation competent *S. aureus* cells were produced through treatment with sucrose (37) and then transformed. Cells were plated on antibiotic selective media to confirm if reporter plasmids had been successfully inserted.

The phage mechanism was utilised to insert reporter constructs into the recipient clinical MW2 strain through transduction following infection of RN4220.

Bacterial cultures were grown and then treated with MMC. Samples were taken at 30, 60, 90 and 120 minutes after MMC exposure. This was repeated 4 times, collecting samples at the same time intervals from cultures treated with and without MMC.

β-lactamase activity was measured using a nitrocefin assay. If *blaZ* was expressed, β-lactamase hydrolyses the nitrocefin β-lactam ring, causing a colour change. This was measured by a microplate reader and used to calculate promoter activity, and thus induction of MGEs.

3.1 RN4220 Lab Strain

To assess if and how each MGE was induced individually after MC exposure the reporter plasmids were introduced into the lab strain RN4220, which is devoid of any MGEs. Exponential cultures were treated with or without MMC and induction of each reporter assessed at various timepoints after exposure.

No significant promoter activity was observed with pAH0198 control or pAH0876 reporter construct (Figure 7a, d; Table S3). In contrast, pAH0874 exhibited significant induction at later timepoints (t3-t4), and pAH0875 was induced from t2 - t4 (Figure 7b, c;

Table S3). Significant promoter activity was observed with pAH0874 reporter at t3 and t4.

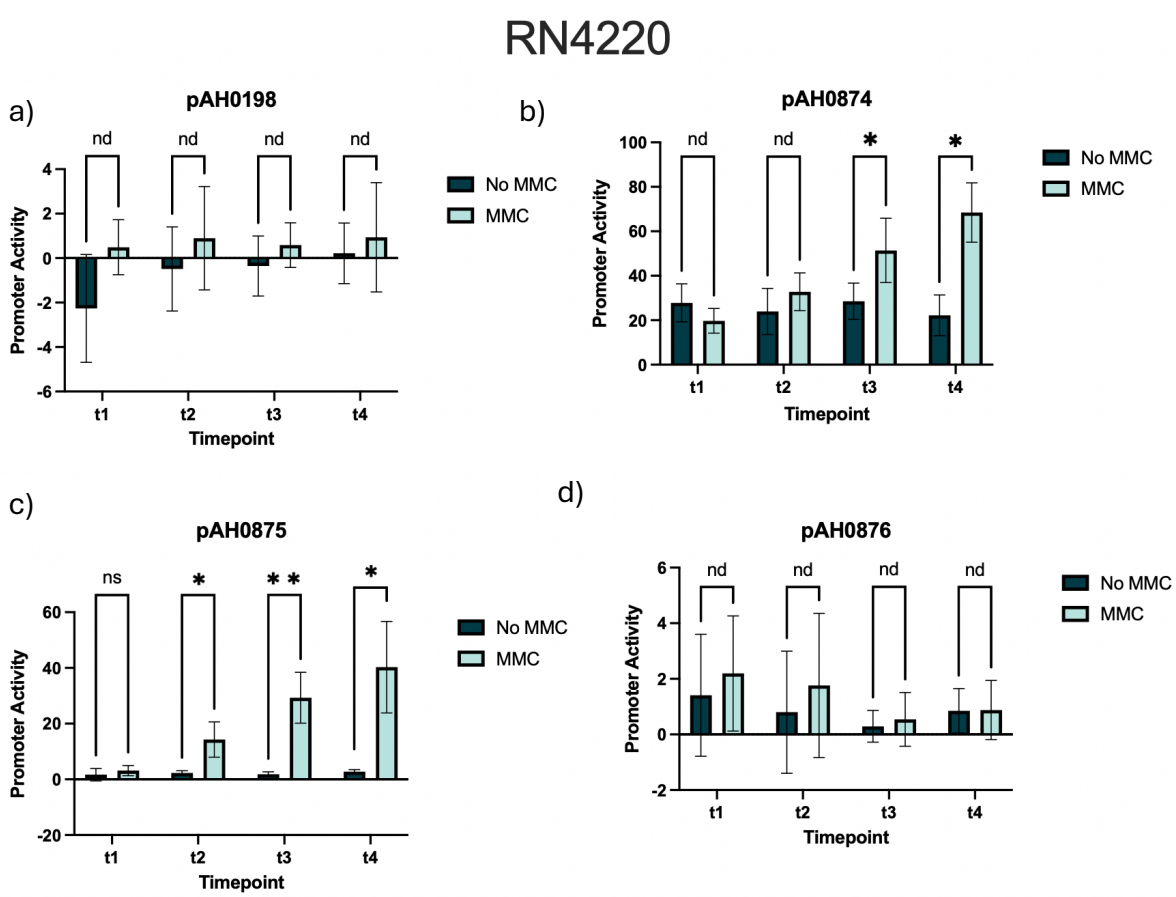

*Figure 7. Graphs showing mean promoter activity at each time point for RN4220 bacterial cultures carrying a reporter plasmid. β-lactamase activity was measured using nitrocefin assay and used to calculate promoter activity. Welch's two-tailed t-test was used to calculate significance, where *P<0.05, **P<0.01; NS, not significant. Error bars indicate ±s.d., n=4 replicates. (a) pAH0198 control. (b) pAH0874 reporter (c) pAH0875 reporter (d) pAH0876 reporter.*

3.2 Clinical MW2 Strain

To investigate interactions between co-resident native MGEs, reporter plasmids were introduced into the clinical MW2 strain, which contains 3 native MGEs. Exponential

cultures were treated with or without MMC and induction of each reporter assessed at various timepoints after exposure.

Only pAH0874 showed significant induction, with significant promoter activity at all timepoints (Figure 8b; Table S4). Neither pAH0198, pAH0875, nor pAH0876 showed significant promoter activity (Figure 8a, c, d; Table S4).

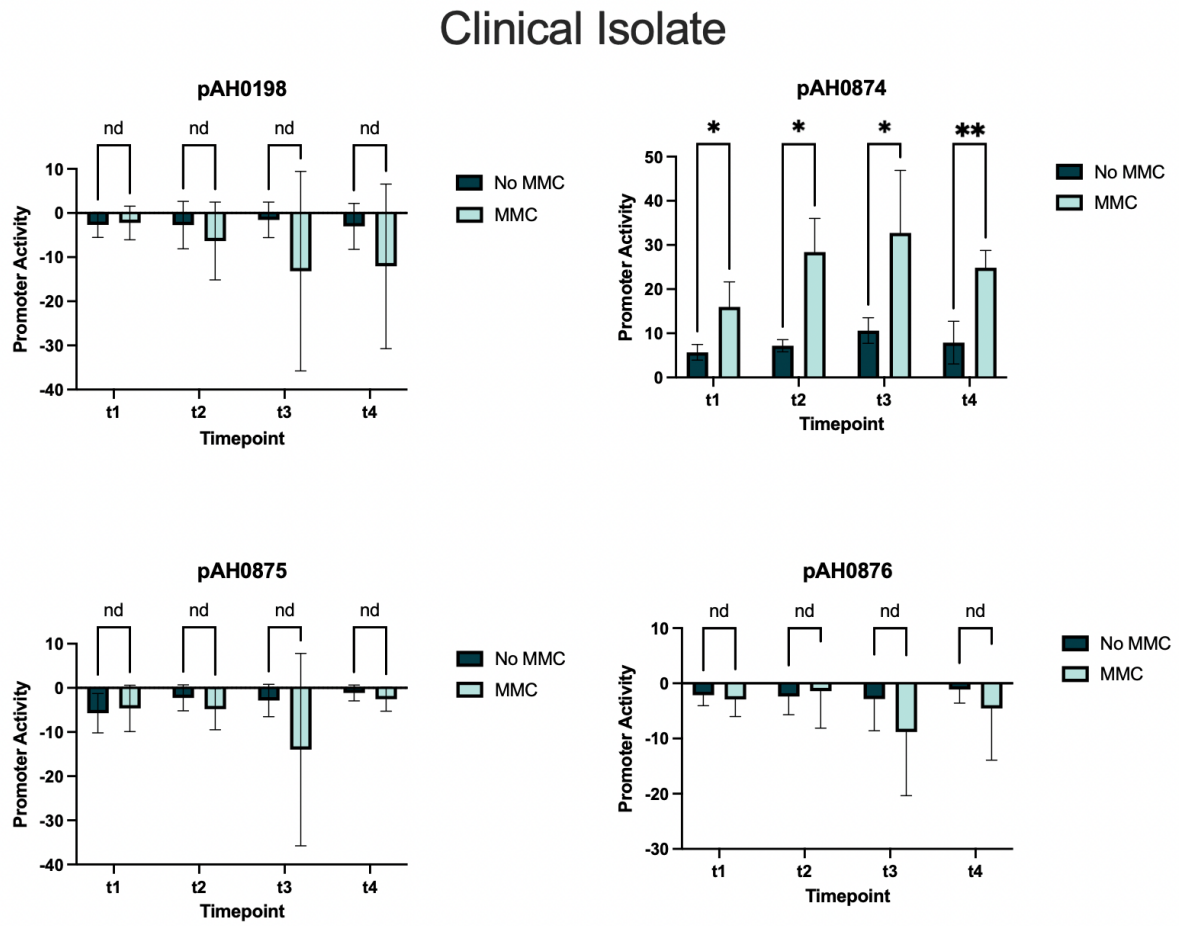

*Figure 8. Graphs showing mean promoter activity at each time point for MW2 clinical bacterial cultures carrying a reporter plasmid. β-lactamase activity was measured using nitrocefin assay and used to calculate promoter activity. Welch's two-tailed t-test was used to calculate significance, where *P<0.05, **P<0.01; NS, not significant. Error bars indicate ±s.d., n=4 replicates. (a) pAH0198 control. (b) pAH0874. (c) pAH0875 reporter. (d) pAH0876 reporter.*

3.3 Summary Table

Only pAH0874 was induced in both strains, while pAH0875 was induced only in RN4220. Neither the control reporter pAH0198 nor pAH0876 showed significant induction in any strain.

Table 1. Summary of promoter induction outcomes in RN4220 and MW2 strains. Induction status was determined from Welch's two-tailed t-test analysis of reporter activity in the presence of MMC.

Construct	Clinical MW2	Lab RN4220
pAH0198 (control)	Not induced	Not induced
pAH0874 (Sa2)	**Induced**	**Induced**
pAH0875 (Sa3)	Not induced	**Induced**
pAH0876 (SaPIMW2)	Not induced	Not induced

4. Discussion

We hypothesised that MGEs are induced differently in clinical and laboratory MW2 strains.

Results showed that in RN4220 both ϕSa2MW2 and ϕSa3MW2 were significantly induced during the SOS response due to MMC damage. However, only ϕSa2MW2 was induced in the MW2 clinical strain. Neither the pAH0198 control nor SaPIMW2 were significantly induced in either strain. This suggests that induction is strain-specific and affected by the genetic background of the host.

As expected, the negative pAH0198 control was not significantly induced in either strain at any timepoint. pAH0198 did not include a promoter region, meaning *blaZ* gene could not be expressed. This confirms that any expression of *blaZ* is because of the promoter region cloned into the plasmid.

In agreement with Cervera-Alamar et al. (25), our reporter assays showed that ϕSa2MW2 is the dominant inducible element in MW2, displaying significant promoter activity at all timepoints.

In contrast, ϕSa3MW2 was only induced in RN4220. In MW2, the ϕSa3MW2 reporter failed to show induction, despite previous RNA-seq evidence of some transcriptional activation. Previous studies have found that co-resident MGEs can interfere with phage replication and packaging (41). This suggests that in MW2 ϕSa3MW2 is either

outcompeted at a post-transcriptional stage or induction of native ϕSa2MW2 is dominant, preventing induction of ϕSa3MW2 within the plasmid.

SaPIMW2 was not significantly induced at any timepoint in either strain. In RN4220 this was expected, as no ϕSa2MW2 was present natively or in the reporter construct. Previous literature has outlined that ϕSa2MW2 acts as a helper phage for SaPIMW2, resulting in production of DUF3113 which derepresses SaPIMW2 through interaction with Stl(25). Thus without the presence of the ϕSa2MW2, the stl – *cro'* regulatory region cannot be expressed and *blaZ* gene cannot be transcribed. This acted as an internal control, further confirming that promoter activity was not caused by MMC itself or any phage-independent effect, as well as further supporting Cervera-Alamar's findings.

However, we would expect SaPIMW2 to be significantly induced in MW2. ϕSa2MW2 is present natively and was expected to be induced by the SOS response, facilitating production of DUF3113 and derepression of SaPIMW2. The lack of induction could be due to competition from co-resident MGEs. Alternatively, SaPIMW2 may have excised or replicated after MMC treatment, but downstream processes such as replication and packaging were blocked, so transcription did not successfully occur. Weak activity at the *cro'* promoter may have gone undetected even if the SaPI lifecycle was initiated. Further studies would explore this possibility.

In every reporter construct that was significantly induced, statistical significance of induction increased from t1 to t4. This temporal pattern indicates a sustained amplifying response rather than transient induction. As time proceeds, sustained *blaZ*

expression causes sustained production of β-lactamase and sustained hydrolysis of nitrocefin's β-lactam ring. This accumulates over time, resulting in an increased colour change.

Limitations

Due to discrepancy in pAH0874 promoter activity significance results were interchanged for pAH0874 in three instances, as outlined in the methodology. This was believed to be due to error when pipetting the samples. Whilst all possible routes of error during the statistical analysis have been checked, there is still a possibility that these results were correct. This compromises the validity of the experiment, which would need to be completed again before to confirm observations in pAH0874 before any future applications.

High s.d. was seen in the absorbance readings from the microplate reader. This was removed through filtering but indicates that the microplate reader may provide inaccurate readings and increase error in calculations.

Similarly, a pattern of an initial increase in OD followed by a subsequent rapid decrease in OD microplate readings was observed consistently across multiple plasmids and repeats. This was removed during filtering due to the effect on the slope and assumed to be due to the presence of bubbles in the microplate. However, this has not been confirmed and could be due to other factors. Its repetitive nature suggested that this

could be due to an issue in the methodology, which should be considered in future repeats.

Significant induction of pAH0875 occurred later in the β-lactamase assay compared to pAH0874. This meant that a plateau in absorbance and thus promoter activity was not reached before the end of the experiment timeframe. Thus the slope calculated may not be representative of the full increase in OD, resulting in a lower promoter activity than would be recorded if the assay had been run for a longer timeframe.

Future Studies

Future studies could investigate MGE induction earlier and later in the growth cycle of bacteria, inducing the SOS response in samples taken before 30 minutes and after 2 hours. Different concentrations of MMC could be used to explore whether strength of induction is dependent on level of DNA damage. Similarly, different methods of inducing the SOS response could be tested to see if the same effect on MGE dynamics is produced. This could include UV light (42), oxidative stress (43) or antibiotics (44) that trigger SOS.

Since MW2 did not show SaPIMW2 induction, a plasmid reporter could be used to add DUF3113 artificially into MW2, to test whether the lack of promoter activity is due to insufficient derepression.

5. Conclusion

This study provides new insights into MGE induction dynamics in the CA-MRSA strain, MW2. Our study demonstrates that while both ϕSa2MW2 and ϕSa3MW2 can be induced through the SOS response in RN4220, only ϕSa2MW2 is strongly induced in MW2. Despite being present with its known helper phage, SaPIMW2 showed no significant promoter activation under the conditions tested.

The hypothesis that MGEs are induced differently in clinical and laboratory MW2 strains was confirmed through the difference in induction of ϕSa3MW2.

Understanding MGE induction dynamics is important for public health. MGEs are central to the spread of antimicrobial resistance genes, and clarifying their behaviours in clinical MRSA strains may further the development of strategies to limit bacterial evolution and improve treatment outcomes.

6. Acknowledgments

I would like to thank my supervisor, Dr Andreas Haag, for his patience and guidance throughout the Laidlaw application process and my research project. I am grateful for the opportunity to learn about microbiology to such depth and experience a wet lab environment.

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Bibliography

1. Williams REO. Healthy carriage of Staphylococcus aureus: its prevalence and importance. Bacteriol Rev. 1963;27:56-71.
2. Wertheim HFL, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. The Lancet Infectious Diseases. 2005;5(12):751-62.
3. Abebe AA, Birhanu AG. Methicillin Resistant Staphylococcus aureus: Molecular Mechanisms Underlying Drug Resistance Development and Novel Strategies to Combat. Infect Drug Resist. 2023;16:7641-62.
4. Casadevall A, Pirofski L-a. Virulence factors and their mechanisms of action: the view from a damage–response framework. Journal of water and health. 2009;7(S1):S2-S18.
5. Ruzin A, Lindsay J, Novick RP. Molecular genetics of SaPI1 – a mobile pathogenicity island in Staphylococcus aureus. Molecular Microbiology. 2001;41(2):365-77.
6. Thanbichler M, Wang SC, Shapiro L. The Bacterial Nucleoid: A Highly Organized and Dynamic Structure. Journal of Cellular Biochemistry 2005;96(3):506-21.
7. Lloyd GS, Thomas CM. Microbial Primer: The logic of bacterial plasmids. Microbiology. 2023;169(7):169:001336.
8. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol. 2008;153 Suppl 1(Suppl 1):S347-57.
9. Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Heyneker HL, Boyer HW, et al. Construction and characterization of new cloning vehicle. II. A multipurpose cloning system. Gene. 1977;2(2):95-113.
10. Burmeister AR. Horizontal Gene Transfer. Evol Med Public Health. 2015;2015(1):193-4.
11. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database. J Clin Microbiol. 2003;41(11):5113-20.
12. Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, et al. Evolution of MRSA during hospital transmission and intercontinental spread. Science. 2010;327(5964):469-74.
13. Wendling CC. Prophage mediated control of higher order interactions - Insights from multi-level approaches. Current Opinions in System Biology. 2023;35.
14. Kasman L, Porter L. Bacteriophages National Library for Medicine: StatPearls Publishing; 2022 [Available from: https://www.ncbi.nlm.nih.gov/books/NBK493185/?utm_source=chatgpt.com.
15. Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB. Lysogeny in nature: mechanisms, impact and ecology of temperate phages. The ISME Journal. 2017;11(7):1511-20.
16. Brady A, Felipe-Ruiz A, Gallego del Sol F, Marina A, Quiles-Puchalt N, Penadés JR. Molecular Basis of Lysis–Lysogeny Decisions in Gram-Positive Phages. Annual Review of Microbiology. 2021;75(Volume 75, 2021):563-81.

17. Maslowska KH, Makiela‐Dzbenska K, Fijalkowska IJ. The SOS system: A complex and tightly regulated response to DNA damage. Environmental and molecular mutagenesis. 2019;60(4):368-84.
18. Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ. The importance of repairing stalled replication forks. Nature. 2000;404(6773):37-41.
19. Schlacher K, Goodman MF. Lessons from 50 years of SOS DNA-damage-induced mutagenesis. Nature Reviews Molecular Cell Biology. 2007;8(7):587-94.
20. Brent R, Ptashne M. Mechanism of action of the lexA gene product. Proceedings of the National Academy of Sciences. 1981;78(7):4204-8.
21. Little JW, Mount DW, Yanisch-Perron CR. Purified lexA protein is a repressor of the recA and lexA genes. Proceedings of the National Academy of Sciences. 1981;78(7):4199-203.
22. Little JW, Mount DW. The SOS regulatory system of Escherichia coli. Cell. 1982;29(1):11-22.
23. Novick RP, Ram G. Staphylococcal pathogenicity islands – movers and shakers in the genomic firmament. Curr Opin Microbiol 2017;38:197-204.
24. Ubeda C, Maiques E, Barry P, Matthews A, Tormo MA, Lasa I, et al. SaPI mutations affecting replication and transfer and enabling autonomous replication in the absence of helper phage. Mol Microbiol. 2008;67(3):493-503.
25. Cervera-Alamar M, Guzmán-Markevitch K, Žiemytė M, Ortí L, Bernabé-Quispe P, Pineda-Lucena A, et al. Mobilisation Mechanism of Pathogenicity Islands by Endogenous Phages in Staphylococcus aureus clinical strains. Scientific Reports. 2018;8(1):16742.
26. Tormo MÁ, Ferrer MD, Maiques E, Úbeda C, Selva L, Lasa Í, et al. Staphylococcus aureus Pathogenicity Island DNA Is Packaged in Particles Composed of Phage Proteins. Journal of Bacteriology. 2008;190(7):2434-40.
27. Rocha GD, Nogueira JF, Gomes dos Santos MV, Boaventura JA, Nunes Soares RA, José de Simoni Gouveia J, et al. Impact of polymorphisms in blaZ, blaR1 and blaI genes and their relationship with β-lactam resistance in S. aureus strains isolated from bovine mastitis. Microbial Pathogenesis. 2022;165:105453.
28. Shaw WV, Brodsky RF. Characterization of Chloramphenicol Acetyltransferase from Chloramphenicol-resistant Staphylococcus aureus. Journal of Bacteriology. 1968;95(1):28-36.
29. Lee S, Lewis DEA, Adhya S. The Developmental Switch in Bacteriophage λ: A Critical Role of the Cro Protein. Journal of Molecular Biology. 2018;430(1):58-68.
30. Haag AF, Podkowik M, Ibarra-Chávez R, Gallego del Sol F, Ram G, Chen J, et al. A regulatory cascade controls Staphylococcus aureus pathogenicity island activation. Nature Microbiology. 2021;6(10):1300-8.
31. Sigma-Aldrich. GenElute™ PCR Clean-Up Procedure: Sigma-Aldrich; [Available from: https://www.sigmaaldrich.com/GB/en/technical-documents/protocol/genomics/dna-and-rna-purification/na1020.
32. Biolabs NE. Optimizing Restriction Endonuclease Reactions: New England Biolabs; [cited 2025 Aug 11]. Available from: https://www.neb.com/en-gb/tools-and-resources/usage-guidelines/optimizing-restriction-endonuclease-reactions.
33. Biolabs NE. Protocol for Dephosphorylation of 5´-ends of DNA using rSAP (NEB #M0371) [Protocol]. New England Biolabs; [cited 2025 Aug 11]. Available from: https://www.neb.com/en-gb/protocols/2013/06/10/protocol-for-dephosphorylation-of-

5-ends-of-dna-neb-m0371?srsltid=AfmBOoo-7GTybwYduHrW3OqeVqWxklvD0J_9VGUhkn3g_YR61RMVGy5_.
34. Biolabs NE. Ligation Protocol with T4 DNA Ligase (M0202): New England Biolabs; [cited 2025 Aug 11]. Available from: https://www.neb.com/en-gb/protocols/0001/01/01/dna-ligation-with-t4-dna-ligase-m0202?srsltid=AfmBOooVFCQmWK7cAOOGiP02-X8xVp6V6piq2s46GuLW_Rib1eAs3p9z.
35. Benchling. Biology Software.
36. Bergkessel M, Guthrie C. Chapter Twenty Five - Colony PCR. In: Lorsch J, editor. Methods in Enzymology. 529: Academic Press; 2013. p. 299-309.
37. Haag A. Preparation and transformation of electro-competent *S. aureus*. 2018.
38. Haag A. Nitrocefin β-lactamase activity assay. 2021.
39. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, et al. Enzymatic Amplification of β-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia. Science. 1985;230(4732):1350-4.
40. Genomics E. Mix2Seq kit instructions. Ebersberg, Germany: Eurofins Genomics.
41. Novick RP, Christie GE, Penadés JR. The phage-related chromosomal islands of Gram-positive bacteria. Nature reviews Microbiology. 2010;8:541-51.
42. Krishna S, Maslov S, Sneppen K. UV-induced mutagenesis in Escherichia coli SOS response: a quantitative model. PLoS Comput Biol. 2007;3(3):e41.
43. Seixas AF, Quendera AP, Sousa JP, Silva AFQ, Arraiano CM, Andrade JM. Bacterial Response to Oxidative Stress and RNA Oxidation. Front Genet. 2021;12:821535.
44. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics. Cell. 2007;130(5):797-810.

Appendix

Table S1. Strains used in this study

Strain number	Species	Derivative of	Plasmid	Antibiotic resistance	Notes
AH3466	*E. coli*	IM01B	pAH0874	100 µg/mL ampicillin	MW2 Sa2MW2 immAR to cro' with blaZ reporter
AH3467	*E. coli*	IM01B	pAH0875	100 µg/mL ampicillin	MW2 Sa3MW2 cI to cro with blaZ reporter
AH3468	*E. coli*	IM01B	pAH0876	100 µg/mL ampicillin	MW2 SaPIMW2 reporter immAR to cro' with blaZ reporter

AH3477	*S. aureus*	RN4220	pAH0198	10µg/mL chloramphenicol	blaZ reporter control
AH3481	*S. aureus*	RN4220	pAH0874	10µg/mL chloramphenicol	MW2 Sa2MW2 immAR to cro' with blaZ reporter
AH3482	*S. aureus*	RN4220	pAH0875	10µg/mL chloramphenicol	MW2 Sa3MW2 cl to cro with blaZ reporter
AH3483	*S. aureus*	RN4220	pAH0876	10µg/mL chloramphenicol	MW2 SaPIMW2 reporter immAR to cro' with blaZ reporter

Table S2. Oligonucleotides used in this study

Plasmid sequencing	Oligo name	Sequence (5' – 3')
pAH0874	pr0158	CTGCAGGTCGACGTTTATATTTCCTTATATTTAAAAACTCTCAACGG
pAH0874	pr0168	ACCCGGGGATCCGACATTGAACTCATACATTTGCTCAC
pAH0875	pr0165	CTGCAGGTCGACTCACAATACAACTTTGCCCATTAC
pAH0875	pr0166	ACCCGGGGATCCCGTGAGTAGTCGTAACACATAAAAGC
pAH0876	pr0167	CTGCAGGTCGACTCATTTCTTGTTTTTATGAGTAAGGG
pAH0876	pr0168	ACCCGGGGATCCGGATAAGCTAGTTTTGTCATGCG
pAH0874, pAH0875, pAH0876	pKX-US-F	CCGGCTCGTATGTTGTGTGG
pAH0874, pAH0875, pAH0876	pCN41-R	CTCTTTGGCATGTGAACTGTTTG

Table S3. Raw p values from Welch's two-tailed t-test comparing results in RN4220, corresponding to Figure 7

Plasmid	Timepoint	P Value	Significant?
pAH0198	t1	0.1061	No
pAH0198	t2	0.3936	No
pAH0198	t3	0.3084	No
pAH0198	t4	0.6310	No
pAH0874	t1	0.1714	No
pAH0874	t2	0.2365	No
pAH0874	t3	0.0429	**Yes**
pAH0874	t4	0.0019	**Yes**
pAH0875	t1	0.3482	No
pAH0875	t2	0.0312	**Yes**
pAH0875	t3	0.0089	**Yes**
pAH0875	t4	0.0196	**Yes**
pAH0876	t1	0.6208	No
pAH0876	t2	0.5922	No
pAH0876	t3	0.6755	No
pAH0876	t4	0.9663	No

Table S4. Raw p values from Welch's two-tailed t-test comparing results in clinical MW2, corresponding to Figure 8

Plasmid	Timepoint	P Value	Significant?
pAH0198	t1	0.8650	No
pAH0198	t2	0.5157	No
pAH0198	t3	0.3821	No
pAH0198	t4	0.4097	No
pAH0874	t1	0.0305	**Yes**
pAH0874	t2	0.0103	**Yes**
pAH0874	t3	0.0495	**Yes**
pAH0874	t4	0.0018	**Yes**
pAH0875	t1	0.7646	No
pAH0875	t2	0.3959	No
pAH0875	t3	0.3843	No
pAH0875	t4	0.4299	No
pAH0876	t1	0.6993	No
pAH0876	t2	0.8065	No
pAH0876	t3	0.4021	No
pAH0876	t4	0.5234	No