

CRISPR-dCAS9 Suppression of mecA Gene in MRSA

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ABSTRACT

Antimicrobial therapies have seen a decrease in effectiveness as microorganisms have developed a resistance to them. Creating methods to combat pathogens is an increasing area of research, as virulent strains have become more resistance to traditional treatment methods. Through CRISPR (clustered regularly interspaced short palindromic repeats) gene editing technology, it is possible to create sequence-specific targeting mechanisms for virulence genes. The specific gene that was targeted was the *mecA* gene, which causes a strong resistance towards a wide variety of beta-lactam drugs. *Staphylococcus aureus* was targeted with a plasmid that contained a CRISPR/Cas9 system that targeted the gene sequence responsible for the beta-lactam drug resistance and repressed expression. This resulted in a 90% decrease in growth of *Staphylococcus aureus* colonies due to the increased susceptibility to beta-lactam drugs, which were previously ineffective.

The CRISPR/Cas9 plasmid also contained a resistance gene to chloramphenicol. Successful integration of the plasmid was displayed by the organism gaining a resistance to chloramphenicol, a formerly effective antimicrobial. To measure the effect of the CRISPR system, culture growth was measured and CLSI standard antimicrobial susceptibility testing was performed. This study will help open doors in further areas of research in CRISPR/Cas9 therapy as well as possible treatment options for antibiotic resistant bacteria.

INTRODUCTION

Due to the emergence of antimicrobial resistant bacteria, bacterial infections have again become a major threat. At least 2 million people become infected with drug resistant bacteria each year, of which 23,000 die as a direct result of the infection. Methicillin Resistant *Staphylococcus aureus* (MRSA) are strains that have multi-resistance to beta-lactam antibiotics. MRSA possesses a *mecA* gene that produces Penicillin Binding Proteins, these bind to and inhibit beta-lactam rings. CRISPR and CRISPR associated proteins act as an adaptive immune system in prokaryotic organisms, protecting it from phages and viruses. dCas9 is catalytically "dead", both cleavage domains of the Cas9 endonuclease are inactivated. We can exploit the DNA-binding activity of dCas9 for controlled transcription regulation.

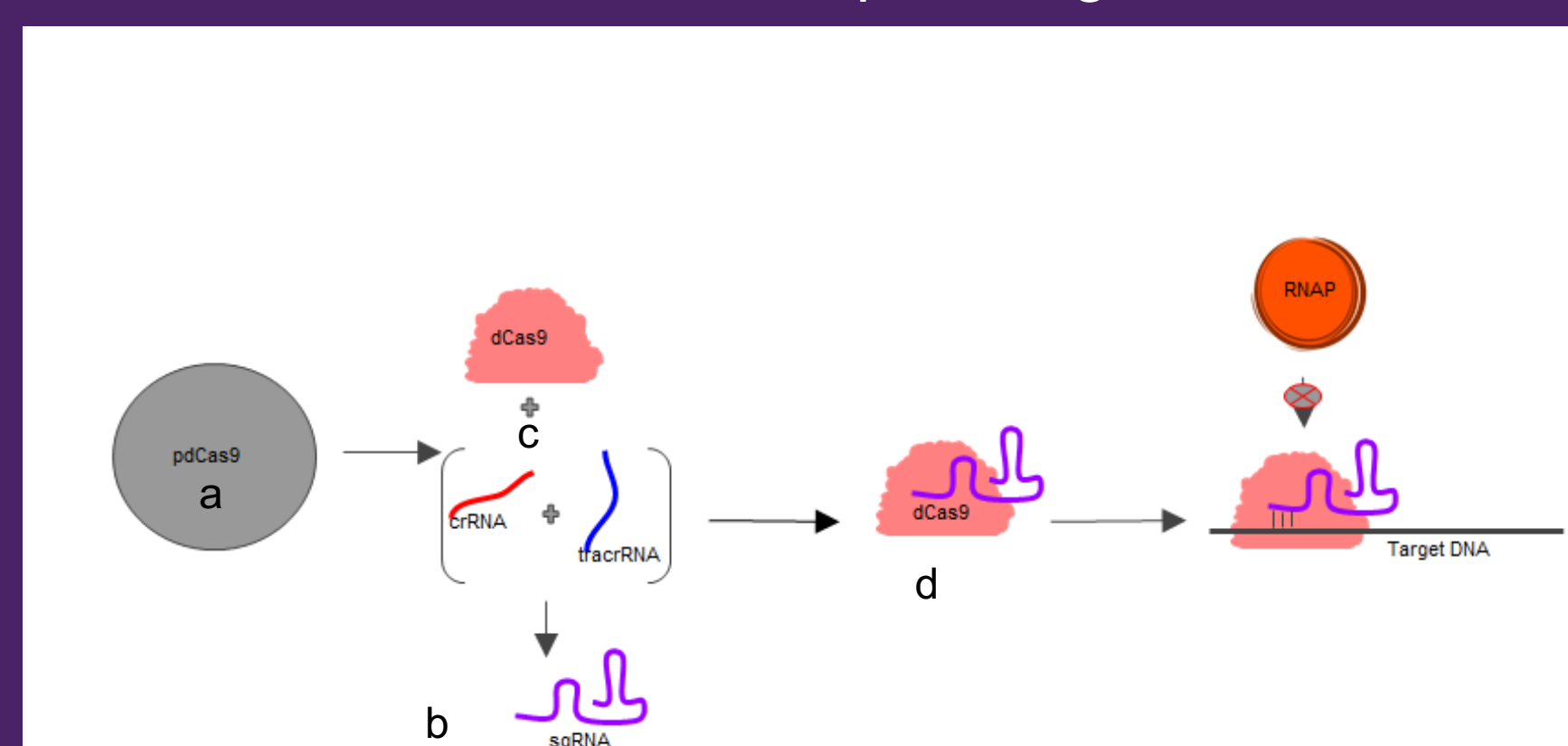


Figure 1: **Plasmid Function**
The pdCas9 plasmid (a) codes for two pieces of RNA (b) and dCas9 endonuclease (c). These form a complex (d) and prevent RNAP from binding to and elongating that section of DNA, stopping transcription

METHODS

The genome sequence for MRSA ATCC 43300 was retrieved from GenBank, at the National Center for Biotechnology Information's website. The sequence was then sent to the University of Utah HSC Cores Department where it was scanned for potential CRISPR-dCas9 binding sites. The two best sites identified were site 43 and site 46, on the coding strand and non-coding strand respectively. Plasmids were then created which included CRISPR-dCas9 systems which target sites 43 and 46. A third plasmid was created which contained a CRISPR-dCas9 that had no programmed target site. The plasmids, named S43, S46, and Pdcas9, also contained a chloramphenicol resistance gene. This portion allowed the organisms to grow in agar and broth containing chloramphenicol. It also caused the plasmid to be expressed when in the presence of chloramphenicol.

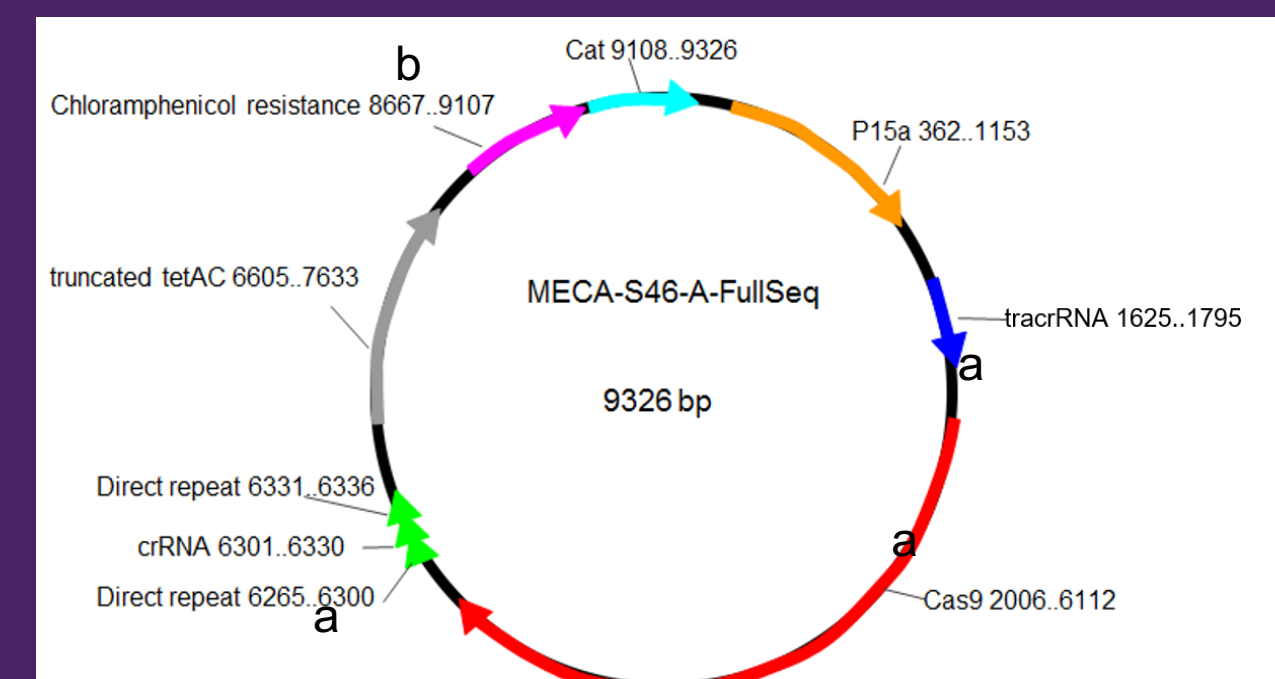


Figure 2: **S46 Plasmid Design**
The various portions of plasmid in color. The CRISPR-dCas9 system (a) is displayed in blue, red, and green. The chloramphenicol resistance (b) is displayed in pink.

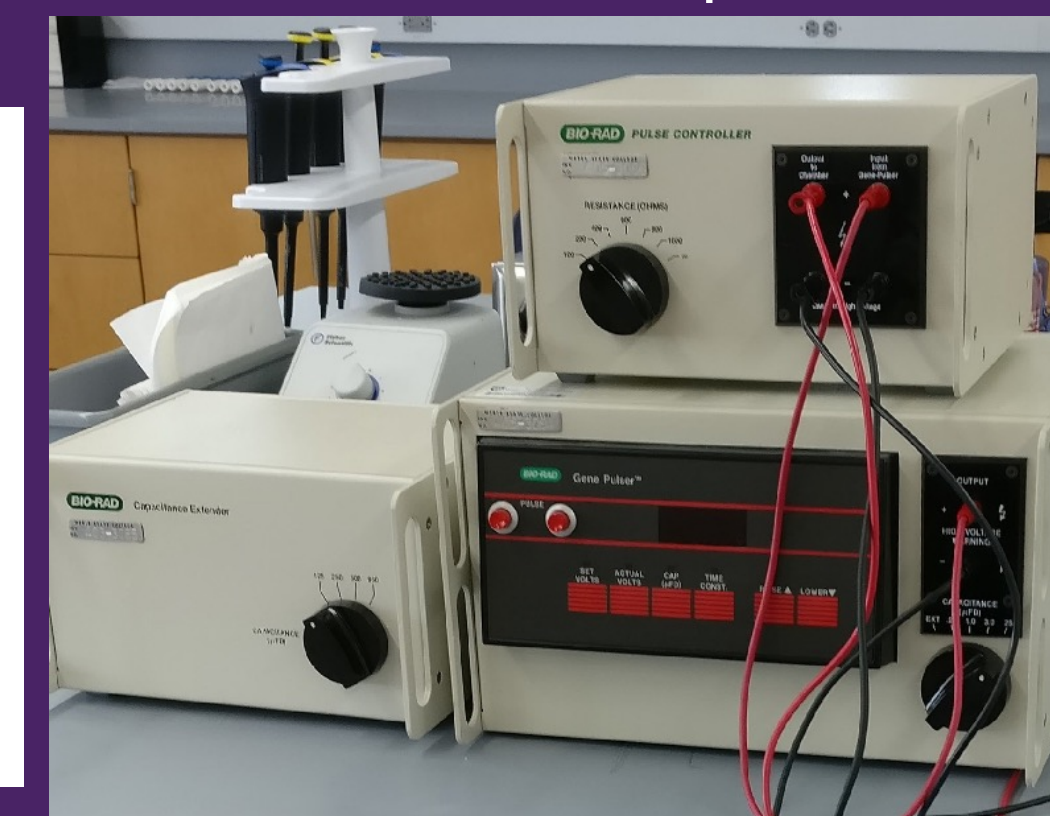


Figure 3: **Gene Pulser Electroporation** system used to electroporate the plasmids into the *E. coli* and MRSA cells.

Electroporation was the method of cell transformation used. This method involves applying an electrical current to the cell membranes, allowing DNA or other foreign objects to enter the cells. In order to survive this process, the cells were made electrocompetent by multiple centrifugations in ice-cold glycerin. Cloning of the plasmids was performed by electroporating them into *Escherichia coli* SAB30 cells. These SAB30 cells do not methylate the foreign DNA they incorporate, which was necessary to keep the plasmids functioning after collection. After cloning the plasmids in the cells on chloramphenicol agar for 24 hours, they were extracted from the SAB30 using a Qiaprep Spin Miniprep Kit. The electroporation and growth process was repeated on the MRSA 43300 strain. Four different MRSA cell populations were created; one with S43, one with S46, one with S43 and S46, and one with Pdcas9. A microtiter plate was set up with dilutions of 16, 8, 4, 2, and 1 µg/mL of Oxacillin and inoculated with 0.5 McFarland Standards of each MRSA population in triplicate. Mueller Hinton agar plates were cultured with each cell population and a cefoxitin disk placed in the center.

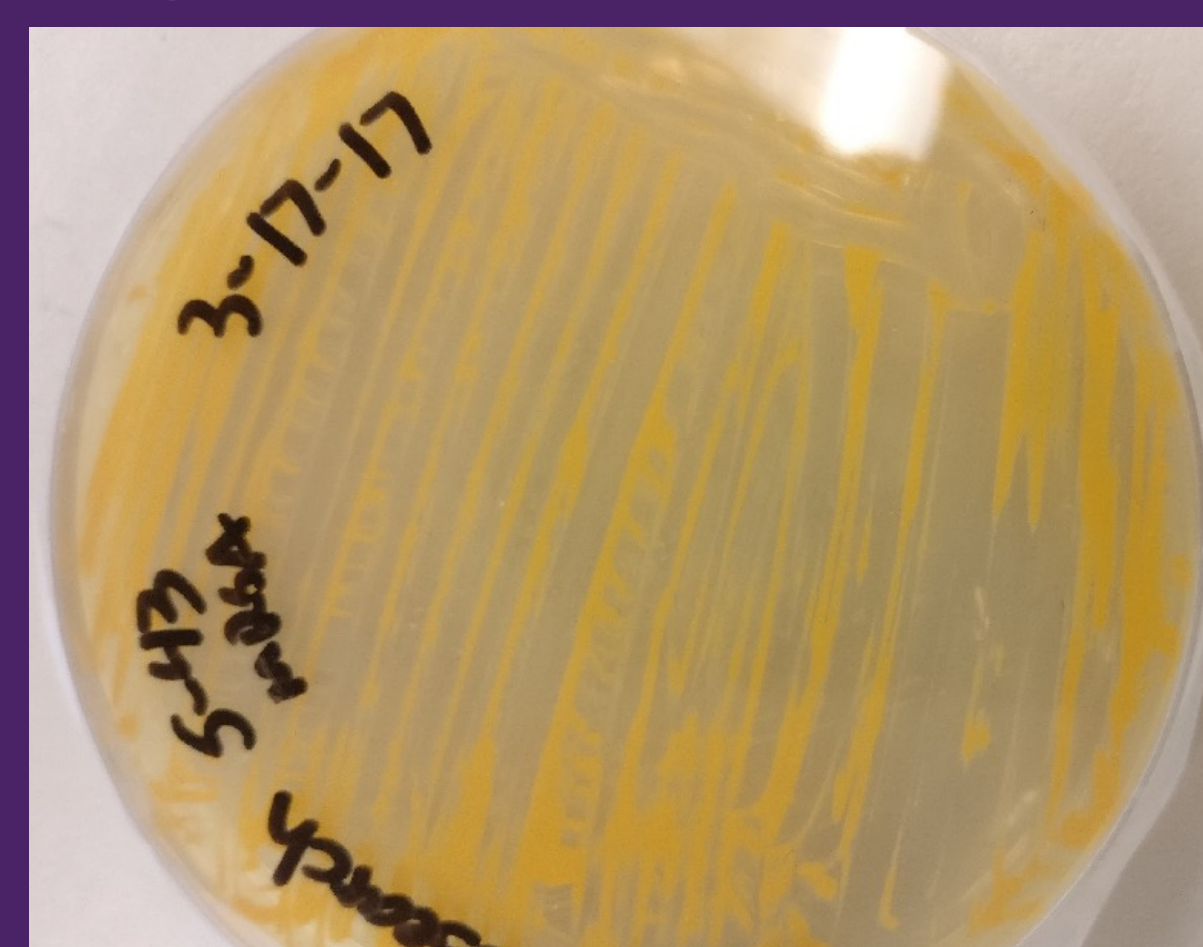


Figure 4: **S43 MRSA culture**
The MRSA ATCC 4300 strain with the S43 plasmid incorporated into its genome. The growth on agar containing chloramphenicol is indicative of successful plasmid incorporation.

RESULTS

The minimum inhibitory concentration (MIC) panel for oxacillin susceptibility was read at 600 nm and the absorbance recorded for each concentration of antimicrobial. The mean absorbance at each concentration was compared to the absorbance of the control well with no antimicrobial for each cell population. CLSI susceptibility testing standards of ≤ 2 µg/mL as susceptible and ≥ 4 µg/mL as resistant were used to evaluate susceptibility (fig. 5).

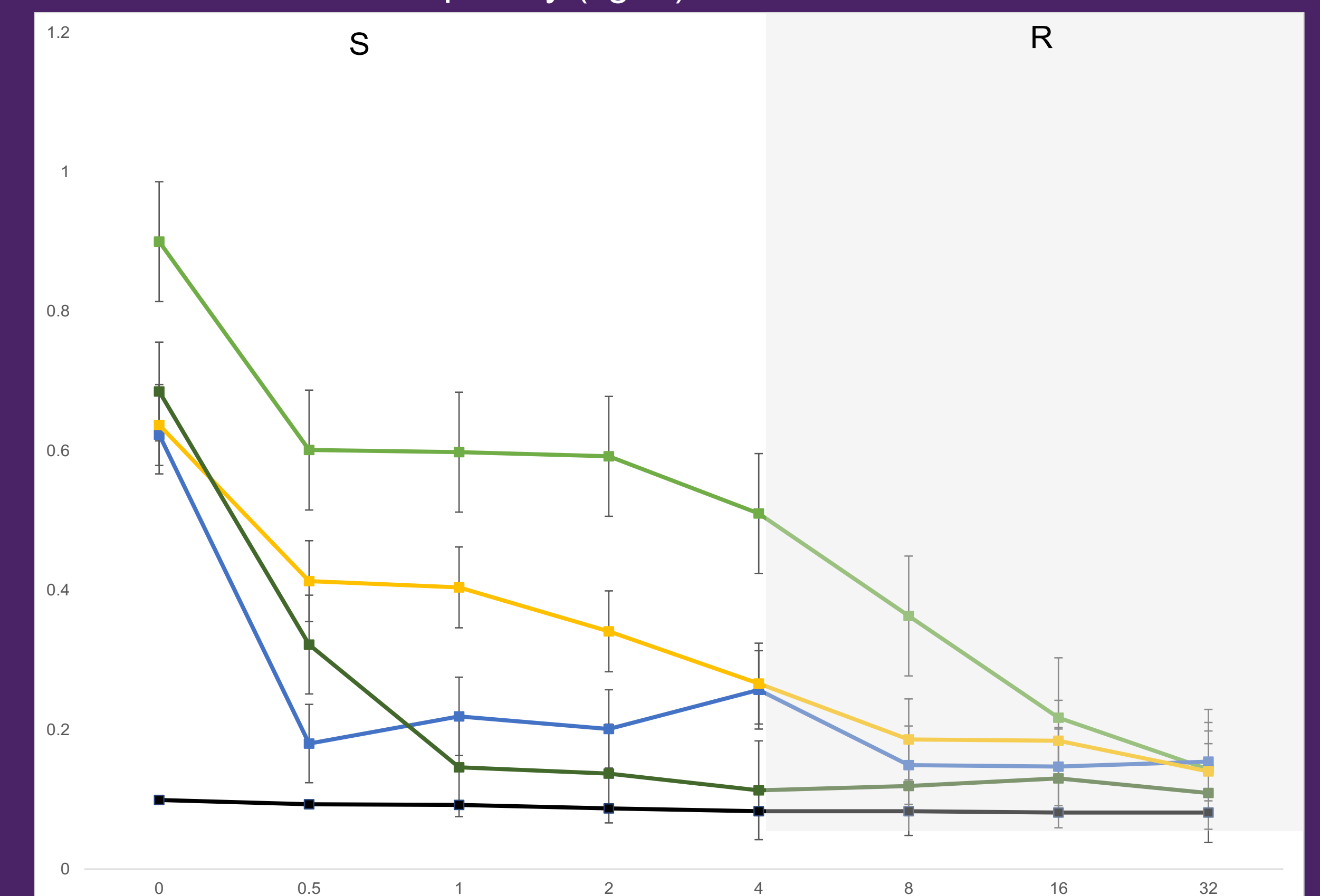


Figure 5: **Oxacillin MIC panel result:** Measurements of Optical Density (OD) of each modified MRSA strain in increasing concentrations of oxacillin (µg/mL). The grey portion of the chart indicates the ≥ 4 µg/mL breakpoint for resistance. Pdcas9 (light green) had no effect on its growth due to the CRISPR having no binding site. S43 (blue) growth dropped dramatically in the presence of oxacillin, indicating increased susceptibility. S46 (yellow) was moderately effective in increasing susceptibility. S43 + S46 (dark green) had the most substantial increase in susceptibility, as growth was significantly reduced at 1µg/mL onward. The black line is a measurement of no growth, where no organism was initially inoculated.

DISCUSSION

Our results showed that the s43 population was more susceptible to oxacillin than the s46 population, which closely resembled the pdcas9 cells. The MRSA that had a combination of s43 and s46 had the greatest susceptibility of the groups tested. This shows that this combination proved to be the most effective at inactivating the *mecA* gene. This was shown by a 90% decrease in growth in the presence of oxacillin at 4 µg/mL, the standard concentration for determining susceptibility of MRSA to oxacillin.

The organisms failed to grow adequately on Mueller Hinton agar, resulting in inconclusive data for cefoxitin disk testing standards set by the CLSI.

Further testing of this system in mammalian hosts, or test systems mimicking in vivo conditions, would be required to show this as an effective alternative to traditional antibiotics. Sequence specific targeting of bacteria with CRISPR-cas9 antimicrobial therapies offer unique strategies for treatments. The bacteria can be targeted for killing or suppression of resistance or virulence genes. Phagemid delivery systems for CRISPR-cas9 antimicrobials have shown promising results in preliminary studies. The specificity of this form of treatment could prove to be a more effective therapy for those bacteria, like MRSA, that are harder to treat with traditional methods.



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