

## Role of Genes in Reversing Antibiotic Resistance

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### Abstract

Bacteria have evolved to overcome a wide range of antibiotics and resistance mechanisms against most of the conventional antibiotics have been identified in some bacteria. Pathogen resistance to antibiotics is a rapidly growing problem and development of new antibiotics faces numerous obstacles. To reverse the antibiotic resistance phages are used as a genetic tool to increase bacterial susceptibility to antibiotics. Genes are also used to reverse antibiotic resistance in certain pathogens. In this review article the uses temperate phages to introduce, by lysogenization, the genes *rpsL* and *gyrA* conferring sensitivity in a dominant fashion to two antibiotics, streptomycin and nalidixic acid, respectively. The use of phages with genes restores antibiotic efficiency by reversing pathogen resistance.

**Keywords:** Antibiotic resistance; Genes; Phages; Streptomycin; Nalidixic acid

### Introduction

Antibiotics have always been considered one of the wonder discoveries of the 20th century. This is true, but the real wonder is the rise of antibiotic resistance in hospitals, communities, and the environment concomitant with their use. Antibiotic resistance is a natural phenomenon; the widespread use of antibiotics has contributed to the increase of antibiotic resistance in bacteria, including those causing infections in both humans and animals [1]. Bacteria have evolved to overcome a wide range of antibiotics, and resistance mechanisms against most of the conventional antibiotics have been identified in some bacteria. Pathogen resistance to antibiotics is a rapidly growing problem, leading to an urgent need for novel antimicrobial agents [2]. To reverse the antibiotic resistance phages are used as a genetic tool to increase bacterial susceptibility to antibiotics. Genes are also used to reverse antibiotic resistance in certain pathogens. In this review article we discuss about the phages and genes that are used in resistant bacteria to reverse antibiotic resistance and increase bacterial susceptibility to antibiotics.

### Streptomycin-resistant bacteria lysogenized with phage $\lambda$ encoding *rpsL* become streptomycin sensitive

A model phage  $\lambda$ , that has been extensively studied, as a gene delivery tool. This phage can infect its *E. coli* host and proceed to the lytic or lysogenic cycle. A common phage mutant ( $\lambda$ gt11) can be used that is directed to a specific cycle type according to the ambient temperature and has a deletion (*nin5*) designed to allow stable insertion of up to 5 kb of foreign DNA. This phage mutant was engineered to contain wt *rpsL*, *rpsL*-sil, or a mock *rpsL*, each linked to the tellurite resistance genes and designated, respectively,  $\lambda$ -RpsL-wt-tell,  $\lambda$ -RpsL-sil-tell, and  $\lambda$ -RpsL $\Delta$ 4-tell. One of the streptomycin-resistant strains, Sm13, was lysogenized with the recombinant phages and selected on agar plates supplemented with 1.5  $\mu$ g/ml tellurite at 32°C, a temperature at which it favors the lysogenic cycle. The lysogenized bacteria were propagated, and their streptomycin MICs were determined. Lysogenization of Sm13 by the phages resulted in sensitization of the resistant mutants. The MIC value for the  $\lambda$ -RpsL $\Delta$ 4-tell lysogen was 200  $\mu$ g/ml, compared to 25  $\mu$ g/ml and 50  $\mu$ g/ml for  $\lambda$ -RpsL-wt-tell and  $\lambda$ -RpsL-sil-tell, respectively. The decreased sensitization observed for lysogenization relative to plasmid transformation is due to a lower number of *rpsL* gene copies introduced by the  $\lambda$  phage. To test this and improve the sensitization, the two different *rpsL* alleles cloned (wt *rpsL*

and *rpsL*-sil) into the  $\lambda$  phage, designated  $\lambda$ -2xRpsL-tell, and used it to lysogenize the resistant strain Sm13. Introduction of two gene copies dramatically enhanced the sensitization efficiency of the lysogenized strains, resulting in a significant decrease of the MIC from 200  $\mu$ g/ml to 1.56  $\mu$ g/ml, comparable to the MIC observed for the sensitive strain. These results constitute a proof of principle for restoration of sensitivity to streptomycin using a phage that carries sufficient copies of *rpsL* at the “genetic cost” of a resistance marker to a toxic compound [3].

### Nalidixic acid-resistant bacteria lysogenized with phage $\lambda$ carrying *gyrA* show restored nalidixic acid sensitivity

The quinolone drug family targets the enzyme gyrase, encoded by *gyrA*, resulting in DNA replication arrest. Mutations in *gyrA* are observed in a specific region termed the “quinolone resistance-determining region” (QRDR). The wt *gyrA* allele is dominant sensitive and may therefore reverse resistance. Nalidixic acid, the first of the synthetic quinolone family antibiotics, was used here as a representative of the quinolone family [4]. Then next introduced the wt *gyrA* expressed from its endogenous promoter or a control construct, both linked to tellurite resistance genes, into  $\lambda$  phages, designated  $\lambda$ -GyrA-tell and  $\lambda$ -Ctrl-tell, respectively. These phages are used to lysogenize a nalidixic acid-resistant strain, Na2, harboring an S83L substitution in GyrA. The lysogens were selected on 4  $\mu$ g/ml tellurite and tested for sensitization by measuring MICs using nalidixic acid. The *gyrA* construct significantly reversed the mutant's resistance. The MIC of the resistant mutants decreased 2-fold when lysogenized by a *gyrA*-bearing phage compared to the control phage. The significance of this sensitization was confirmed by experiments in which *gyrA*-bearing plasmids transformed into nalidixic acid-resistant mutants and observed a decrease by 3 orders of magnitude in the number of CFU on 50  $\mu$ g/ml nalidixic acid compared to resistant cells transformed with a mock plasmid [4].

### Conclusion

Pathogen resistance to antibiotics is a rapidly growing problem,

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leading to an urgent need for novel antimicrobial agents. Unfortunately, development of new antibiotics faces numerous obstacles, and a method that resensitizes pathogens to approved antibiotics therefore holds key advantages. This system uses temperate phages to introduce, by lysogenization, the genes *rpsL* and *gyrA* conferring sensitivity in a dominant fashion to two antibiotics, streptomycin and nalidixic acid, respectively. Unique selective pressure is generated to enrich for bacteria that harbor the phages carrying the sensitizing constructs. This selection pressure is based on a toxic compound, tellurite, and therefore does not forfeit any antibiotic for the sensitization procedure. This system restores antibiotic efficiency by reversing pathogen resistance.

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