Ofloxacin Resistance Conferred by *qnrS* Gene Encoded on Bacterial Plasmids

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ABSTRACT

For several years, plasmid-mediated antibiotic resistance has been detected in bacterial species from water environments in Tahlequah, Oklahoma. In this study, we determined the sequences of plasmids separated from *Escherichia coli* that demonstrated moderate levels of resistance to the antibiotic, ofloxacin, a member of the common antibiotic class of fluoroquinolones. After determining the plasmid sequences, we created a map of the genes present on each plasmid using the National Center for Biotechnology Information database. We identified one gene which encoded a specific allele of the quinolone resistance protein gene family, *qnrS*, that was likely contributing to the observed ofloxacin resistance in each bacterial isolate. Several mechanisms of antibiotic resistance exist and *qnr*S is one of many plasmid-encoded genes that is known to contribute to ofloxacin resistance. The *qnr* gene family is known to code for proteins that can bind to DNA gyrase and topoisomerase IV within bacterial cells to protect them from fluoroquinolone targeting. Importantly, *qnrS* is a gene that has been identified on plasmids in bacterial isolates separated from Tahlequah streams in previous studies. The identification of *qnrS* in this study indicates that the plasmid-encoded *qnrS* gene is likely a common fluoroquinolone resistance mechanism utilized by bacteria in Tahlequah aquatic environments. Future studies should continue to identify and monitor the antibiotic resistance mechanisms of bacteria present in Tahlequah waters.

INTRODUCTION

The ability to treat simple bacterial infections is currently under threat as antibiotic-resistant strains of bacteria continue to evolve. Due to the global overuse and misuse of antibiotics, along with minimal development of new drugs, many pathogenic bacteria are becoming major public health threats. Although this crisis may seem most prevalent in clinical settings, various strains of resistant bacteria have been characterized throughout the environment.

Fluoroquinolones are one of the most common antibiotic classes, but the rapid development of resistance in several bacterial species is jeopardizing the drug's effectiveness. Fluoroquinolones target bacteria by increasing DNA gyrase and topoisomerase IV activity within cells. To undergo cell division, DNA gyrase and topoisomerase IV are important enzymes involved in the generation of double-stranded breaks in the bacterial chromosome. This increased enzyme activity leads to greater chromosome fragmentation and concomitantly, DNA damage.

Various mechanisms of antibiotic resistance have been observed in bacterial species. Previous studies have indicated that quinolone resistance proteins (qnr) are common plasmid-encoded elements that can result in fluoroquinolone resistance. Although plasmid-encoded resistance genes do not tend to confer a high enough level of resistance to be considered in clinical laboratory resistance screens, they do contribute to increased pathogenicity and thus require extensive research to better understand bacterial antibiotic resistance.

Qnr genes are commonly integrated into plasmids of fluoroquinolone-resistant bacteria. The *qnr* genes are significant in that they code for proteins of the pentapeptide repeat family that generally bind to DNA gyrase and topoisomerase IV to provide protection from fluoroquinolone targeting.

Based on previous studies conducted at Northeastern State University, our research project was focused on identifying bacterial resistance to the specific fluoroquinolone, ofloxacin, conferred by qnrS proteins encoded on transferrable plasmids. We identified the presence of the *qnrS* gene in three plasmids extracted from *E. coli* cells. This study provided further data to the monitoring of antibiotic resistance mechanisms of bacteria in Tahlequah waters. It is important to identify patterns of antibiotic resistance throughout the environment to contribute to a better understanding of the development of resistance.

RESULTS

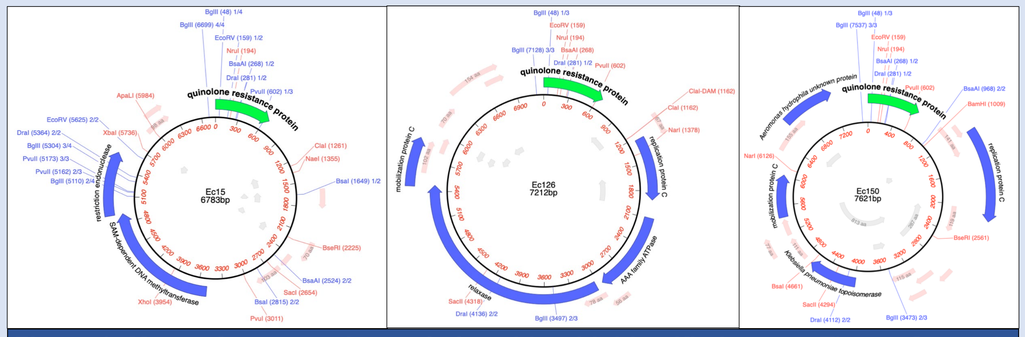

Figure 1: Maps of our experimental plasmids with identified genes. The *qnrS* gene is indicated in green.

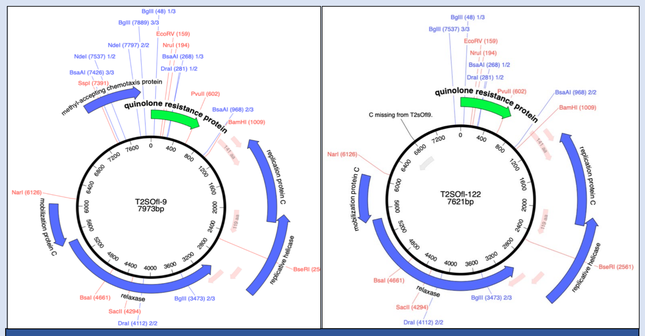

Figure 2: Maps of our reference plasmids with identified genes. The *qnrS* gene is indicated in green.

BLAST searches were performed to determine the possible gene identity of each open reading frame by comparing its amino acid sequence to reference sequences within the NCBI protein database. Several genes appeared to be conserved between the three experimental plasmids and the two reference plasmids (Table 1). The significant identification was the gene that encodes the quinolone resistance protein, specifically the qnrS2 allele. This gene presumably conferred the low level of ofloxacin resistance observed in the bacteria, and its identification was expected. The presence of other genes conserved between multiple plasmids could be significant for a variety of reasons.

Gene	Experimental Plasmids			Reference Plasmids	
	Ec15	Ec126	Ec150	T2SOfi-9	T2SOfi-122
quinolone resistance protein	**+**	**+**	**+**	**+**	**+**
replication protein C	-	**+**	**+**	**+**	**+**
relaxase	-	**+**	-	**+**	**+**
mobilization protein C	-	**+**	**+**	-	-
replicative helicase	-	-	-	**+**	**+**
methyl-accepting chemotaxis protein	-	-	-	+	-
SAM-dependent DNA methyltransferase	+	-	-	-	-
restriction endonuclease	+	-	-	-	-
AAA family ATPase	-	+	-	-	-
Klebsiella pneumoniae topoisomerase	-	-	+	-	-
Aeromonas hydrophila protein	-	-	+	-	-

Table 1: Presence of genes in each plasmid identified by BLAST searches. A "**+**" indicates presence while a "**-**" indicates absence. Genes that were conserved between at least two plasmids are indicated in bold.

METHODS

All plasmids were isolated from environmental samples using the New England Biolabs Monarch Plasmid Miniprep kit and the Qiagen Plasmid kit according to the manufacturers' instructions. The reference plasmids were isolated directly from environmental strains. Resistant isolates were selected for by treatment with 8 µg/mL ofloxacin. Some environmental isolates contained multiple plasmids. Plasmid mixtures were isolated using the same plasmid isolation kits and these plasmids were transformed into competent *E. coli*. The resistant *E. coli* were selected for by treatment with 0.4 to 0.8 µg/mL ofloxacin. These experimental plasmids were isolated from the *E. coli* with the same kits.

Sequencing was performed at Eurofins Genomics US. Dr. de Banzie chose the first set of primers to begin the sequencing of the plasmids. These primers began at the *qnrS* gene location, which was present on the experimental plasmids as selected for by the 0.4 to 0.8 µg/mL ofloxacin treatment. Blayke Haggard and I used the software, MacVector, to analyze the obtained sequences to verify any uncertainties and delete any unacceptable sequence. We then performed a primer search within MacVector to choose a new set of primers that was near the ends of our given sequences to continue DNA sequencing around the plasmids.

Once sequencing was completed around the entirety of the plasmids, we identified which genes were present in each plasmid using MacVector. First, the software was able to identify the complementary sequences obtained from the extensions of primers on opposite DNA strands to construct circular plasmid sequences. We then searched for all possible open reading frames within each plasmid. For each open reading frame, we translated the nucleotide sequence into an amino acid sequence. We used the Basic Local Alignment Search Tool (BLAST) to search for similar amino acid sequences in the National Center for Biotechnology Information (NCBI) protein database. For any open reading frame that had at least one 100% match to another amino acid sequence within the database, we designated that open reading frame as the matched gene.

DISCUSSION

We expected to identify the *qnrS* allele of the *qnr* gene family in our experimental plasmids. We identified an amino acid sequence in all three plasmids that was 100% identical to a qnrS2 protein from the NCBI protein database, and conserved in our reference plasmids.

It is a generally accepted idea that the presence of a *qnr* gene within a bacterial plasmid does not confer a high level of resistance alone, but combines its actions with other resistance mechanisms (e.g. chromosomal mutations) to create a high level of resistance. Of the several known alleles, *qnrS* appears to be one of the most common. It is difficult to determine the unique actions of the different alleles, but it is known that the *qnr* gene family codes for proteins that bind to DNA gyrase and topoisomerase IV to provide protection from fluoroquinolone targeting. It has been suggested that the presence of a *qnrS* allele within a plasmid may specifically lead to a higher level of bacterial fitness, as compared to other *qnr* alleles.

Fluoroquinolone-resistant bacterial isolates have been identified in Tahlequah water environments for over a decade. We were able to identify a plasmid-encoded gene, *qnrS*, in all three of our experimental plasmids from *E. coli* that has been well characterized in other bacterial species in previous studies at NSU. We thus conclude that bacterial resistance to fluoroquinolones in Tahlequah waters has been maintained, at least in part, by conserved qnrS-encoding plasmids that can be transferred among different species of bacteria. Future studies should continue to monitor the different methods of fluoroquinolone resistance in bacteria from Tahlequah waters, as consistent monitoring is important to understand how bacteria are evolving to counteract antibiotic treatments.

ACKNOWLEDGEMENTS

This research project was supported by the Department of Natural Sciences at Northeastern State University. We would also like to thank our research advisors, Dr. John de Banzie and Dr. Michael Morgan, for their guidance and Dr. Cindy Cisar for the environmental strains.