

# Survey of Plasmids Collected from Antibiotic Resistant Enteric Bacteria Found on Lake Michigan Beaches



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## Abstract:

Bacterial antibiotic resistance is becoming increasingly problematic. Antibiotics are being prescribed more and more frequently at doctors' offices, vets, and even in the agricultural industries. The effectiveness of antibiotics is being severely hampered due to increased resistance to these drugs, but increasing the use of these drugs is causing more resistance to be expressed. To break this cycle, more knowledge must be gained about how a bacterium attains its resistance. Since resistance is usually conferred by plasmidal DNA, studying plasmids of resistant bacteria is a good way to understand antibiotic resistance. In this study, 31 strains of tetracycline resistant enteric bacteria were collected, and through a lysing and collection process, the plasmids were removed and run through a gel. Plasmid length was then recorded. This process allows for further testing and analysis. In so doing this, we can further our knowledge of bacterial resistance and plasmidial flow through the environment.

## Introduction:

As bacteria become increasingly problematic to humans, humans strive to find ways to control bacteria. The problem is, the presence of an antimicrobial agent inherently selects for types of bacteria that tend to be more resistant as well as selecting for more resistance in these types of bacteria. Decades of use of antibiotics in the medical, veterinary, and agricultural fields have resulted in multiple strains of resistant pathogenic bacteria, as well as increased amounts of resistant bacteria in the wild. According to McDermott, Walker, and White (2003) there are a few different ways bacteria can acquire resistance: the cell wall permeability can change restricting the chemical agent's target; the bacterium can "learn" to expel the agent; a random mutation can create new chemical and metabolic pathways; the utilization of new enzymatic and metabolic pathways can alter the chemical agent's effectiveness; and the acquisition of new metabolic or enzymatic activities from an outside source can allow the bacterium to avoid the effects of the chemical agent. Such an acquisition could involve transferable extrachromosomal DNA pieces known as plasmids. Plasmids can be obtained through the active transmission of DNA from one bacterium to another known as conjugation, or another process known as transformation where the bacterium absorbs the DNA from its surrounding environment. Depending on the bacterial strain and the condition it's in, a bacterium could survive with or without a plasmid. It is not uncommon to find a single bacterium containing multiple copies of a single plasmid as well as multiple different plasmids. Interestingly, multiple resistance genes can accumulate on a single plasmid. Knowing this, knowing that a bacterium can have more than one plasmid, and knowing that a bacterium can pass its plasmids onto other bacteria, studying plasmids would seem to be a great way of studying antibiotic resistance. A qualitative study has been proposed to characterize the plasmids of 35 resistant enteric bacterial strains collected from the shores of Lake Michigan to facilitate more quantitative studies to take place in the future.

## Materials and Methods:

### Clones Isolated

- Water samples were collected along four Milwaukee Lake Michigan beaches and plated onto tetracycline embedded LB plates.
- A total of 35 strains grew up on the tet plates, indicating their resistance.
- Strains were named and stored in a -80°C freezer.

### Miniprep

- To characterize the plasmids, they must first be removed.
- Freezer isolates were plated onto tetracycline embedded plates.
- Single colonies of bacteria were cultured in tubes with tetracycline overnight to attain appropriate amounts of bacteria, but more importantly, appropriate amounts of plasmids.
- The bacteria were centrifuged, reconstituted, and then lysed to release their plasmids.
- Plasmids were then filtered out and stored in a microcentrifuge tube in a freezer.

### Gel Electrophoresis

- Plasmids were run through gel electrophoresis to characterize their relative sizes.

### Transformation

To see whether or not the plasmid confers resistance, the plasmid has to be observed to protect bacteria with known antibiotic sensitivity. Competent *E. coli* is known to not withstand the presence of antibiotics.

- Competent *E. coli* cells along with previously extracted plasmids were mixed together in ideal conditions to allow for the most effective uptake of the plasmid by the bacteria.

- Bacteria was then plated on antibiotic infused plates so that only the bacteria that took up the plasmid, and only under the condition that the plasmid actually confers resistance, will then yield bacterial colonies.

### Entry Baner

- A test of the newly transformed bacteria was performed to determine what other, if any, antibiotics the plasmid(s) confer resistance to.

100µl  
50µl  
25µl  
12.5µl  
6.25µl  
3.125µl  
1.5625µl



Strain	Size(s) (in kilobases)
BB 624 Mac A	237, 41, 4.8, 2.4, 1.0, 1.1
BB 621 A	507, 15
BB 621 B	507, 15, 4.8, 2.2, 1.1, 0.8
BB 621 C	15, 8.5, 4.8, 4.1, 2.2, 1.8
BB 621 A	11, 8.5, 2.5, 1.4, 1.1
BB 623	No visible plasmids
BB 621 Mac B	
BB 611 A	
BB 611 C	
BB 611 D	
BB 610 A	
BB 610 B	
BB 611 A	
BB 611 D	

## Isolates Tested

BB 611 A	SS 621 D
BB 611 B	SS 624 A mac
BB 611 C	SS 624 B mac
BB 611 D	SS 624 D
BB 611 A	BN 624 A
BB 611 D	BN 624 B
SS 610 A	SS 625 mac A
SS 610 B	SS 625 mac B
BB 610 C	SS 625 mac C
BB 610 A	SS 625 mac D
SS 610 A mac	SS 625 A
BB 610 A mac	SS 625 B
BN 615 A	SS 625 C
BN 615 A	M 621 A
BB 621 A	M 621 A
BB 621 A	BB 621 A