Development of a PCR assay to detect plasmids in antibiotic-resistant *E. coli* in the North River

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Introduction

Since the introduction of penicillin to the public in 1942, antibiotics have been renowned as the "miracle drugs," originally being able to relieve patients of their symptoms and their infections with ease [3]. Their original intent and success had come from their ability to kill off the pathogenetic bacteria involved in certain infections [6]. Unfortunately, due to a range of different reasons, the world has plunged into a crisis of antibiotic resistance, creating a world of "superbug" level infections, that are becoming much more challenging to treat.

The crisis-causing origin of antibiotic resistance comes from mutations occurring in bacteria genomes. Humans overuse antibiotics, whether by taking them to treat illnesses that do not need them or using them in the feed of livestock [3]. This extra exposure to antibiotics has led to a bottleneck effect where harmless bacteria are killed off by the antibiotic but bacteria with mutations survive when exposed to the antibiotic, leaving only the stubborn or resistant bacteria [4].

Antibiotic resistance can also be caused by plasmids, or pieces of circular DNA within bacteria's genomes. These plasmids can code for resistance genes and be transferred from one bacterium to the next [4][6]. These resistance genes code for proteins that counteract the mechanisms of the different classes and families of antibiotics currently used in medicine, rendering them useless and allowing the bacterial infection to continue to spread [6].

With the many farms in the Rockingham County area, there are large populations of antibiotic-treated livestock. *Escherichia coli* in the digestive tract of the livestock may be resistant to these antibiotics. Run-offs from these farms can end up with these resistant *E. coli* strains in the North River. In order to assess the severity of this risk, water samples were taken from the North River and analyzed for *E. coli* resistant to the antibiotics penicillin, ciprofloxacin, and tetracycline. DNA was extracted from *E. coli*-resistant bacteria and analyzed by PCR to determine if they carry antibiotic resistant genes.

Identification of Antibiotic-Resistant *E. coli*

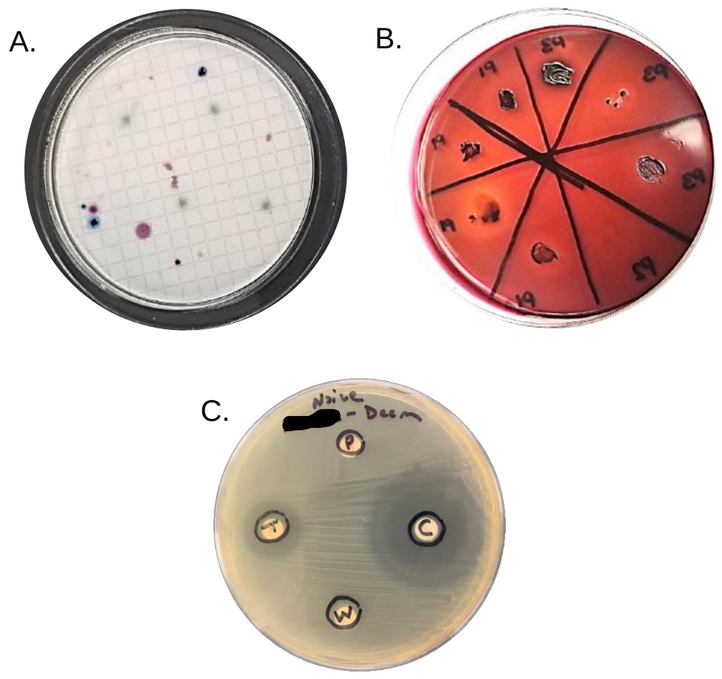

Figure 1. Identification of antibiotic-resistant *E. coli* by A) coliscan medium, B). EMB medium, and C) growth in the presence of antibiotics. A). Representative image of coliscan plate. Navy blue colonies indicates *E. coli*. B). *E. coli* from the coliscan plate was plated on EMB to confirm its identity. Dark colonies with green sheens indicate *E. coli.* C). Representative image of E. coli grown in the presence of three antibiotics. Zones of inhibition indicate bacteria death.

Quantification of *E. coli* Resistance

Table 1. Kirby-Bauer Antibiotic Sensitivity

Antibiotic	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Ciprofloxacin	≤15	16-20	>20
Tetracycline	≤14	15-18	>18
Penicillin	≤14	-	≥15

Notes: Kirby-Bauer has been a widely used and accepted method of determining bacterial resistance to antibiotics.

Table 2. *E. coli* from the North River resistant to tetracycline and penicillin

Sample	Ciprofloxacin	Tetracycline	Penicillin	Interpretation
Naïve	33.5	21	4	Resistant to Pen
1	22	0	0	Resistant to Tet and Pen
2	26	11	0	Resistant to Tet and Pen
3	20.5	0	0	Resistant to Tet and Pen
4	20.5	0	0	Resistant to Tet and Pen
5	27	10.5	0	Resistant to Tet and Pen
6	26	10.5	3.5	Resistant to Tet and Pen
7	28	3.0	0	Resistant to Tet and Pen

PCR of *tet*(B) and *tet*(M) genes on resistant bacteria DNA

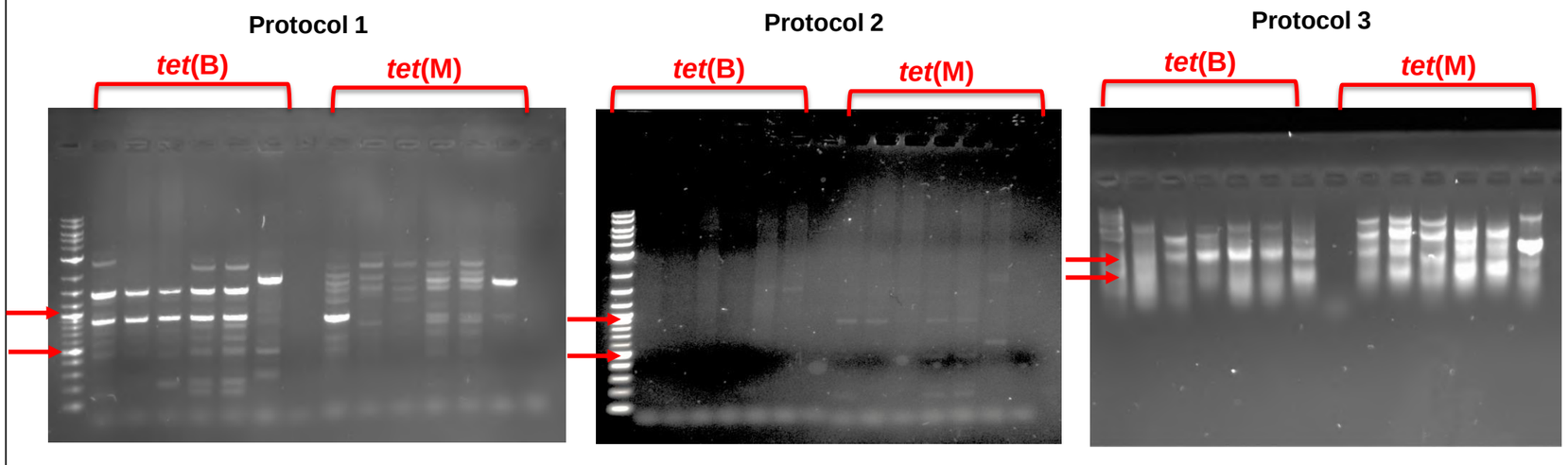

Figure 2.The DNA was extracted from the tetracycline-resistant *E. coli.* Using PCR, we tested the extracted DNA for two common tetracycline-resistant genes: *tet*(B) and *tet*(M). Three different protocols were used for PCR. Protocol 1 was conducted with an initial denaturation of 94ºC for five minutes, followed by 25 rounds of 94ºC for five seconds, 61ºC for thirty seconds, and 61ºC for thirty seconds, with a final extension time of seven minutes at 61ºC [1]. Protocol 2 was conducted with an initial denaturation of 98ºC for thirty seconds, followed by thiry rounds of 98ºC for ten seconds, 65ºC for thirty seconds, and 72ºC for thirty seconds, with a final extension time of two minutes at 72ºC. Protocol 3 was conducted with an initial denaturation of 94ºC for five minutes, followed by 30 rounds of 56ºC for one minute, 72ºC for one minute, 94ºC for one minute, and 45ºC for one minute, with a final extension time of five minutes at 72ºC [5].

Summary

- Of the 7 *E. coli* identified, 100% were resistant to penicillin and 86% were resistant to tetracycline.
- Penicillin resistance is likely due to the *E. coli* structure and not antibiotic resistance genes since naïve strains were also resistant.
- PCR results were inconclusive for determining the presence of *tet*(B) and *tet*(M) resistance genes.

Future Work

- Attempt to modify existing PCR protocols to eliminate non-specific bands by adjusting the annealing temperatures and extension times
- Attempt to PCR other tetracycline-resistant genes to determine if there are other resistance genes in the North River

References

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