INTRODUCTION

Bacterial antibiotic resistance is a rising problem in medicine, leaving many infections untreatable. The Centers for Disease Control estimates that 25,000 deaths have resulted from this “superbug” (1). Disposing of antibiotics incorrectly, such as through wastewater systems, selects for bacterial mutants that are resistant to antibiotics. A 2015 study in the Regenstrief Laboratories indicated that it is possible that many antibiotic resistance genes originate and are spread through wastewater due to the tailoring of antimicrobial therapy by peripherally and socially

In response to improper drug disposal, Wyoming implemented the Wyoming Medication Donation Program (WMDP). The WMDP is a program that collects medical waste from collection boxes across the state and either refurnishes it or properly disposes of it through incineration as noted by the Wyoming Department of Health (2). The role of the WMDP in preventing incorrect antibiotic disposal is detailed in Figure 1. Not only is this significant for human health, but the WMDP benefits the health of the environment by reducing water pollution.

METHODS

To test our hypotheses, we provided containment to wastewater treatment plants, and they provided samples of influent water from the previously listed counties, Carbon, Sublette, Sheridan, Laramie, and Albany. Our research methods included:

- Dilution and plating on enriched TSA plates to see selectively or differentially cultured growth
- Replica Plating* from TSA agar to MacConkey/MUG aga, TSA+ ampicillin, TISA+ tetracycline and TISA+ gentamicin
- Kirby Bauer with ampicillin with chloramphenicol, amoxicillin, ciprofloxacin, tetracycline, gentamicin, penicillin, oxacillin, and ampicillin

Preliminary identification of isolates has been done through MALDI-TOF (Matrix-Assisted Laser Desorption Ionization - Time Of Flight), to confirm or deny the isolates as f Oral colonies, as well as to assist in analyzing Kirby Bauer Data.

RESULTS

Table 1: The table below shows the results of Kirby Bauer Susceptibility testing.3a it means that the isolate was susceptible, R means that the isolate was resistant to the antibiotic. The columns on the left represent bacterial isolates from each county and which TSA+ antibiotic plates they were included on. Two antibiotics that were included in the Kirby Bauer test to account for the possibility that the isolates would be gram positive, are not included in this table due to the fact that all isolates grown were Gram negative so these antibiotics are not applicable. All isolates with no growth, either in the Kirby Bauer Assay or from initial isolations, have also been removed from the table.

HYPOTHESES

1. Counties in Wyoming that do not participate in the recycling program will have a higher rate of antibiotic resistant bacteria in wastewater.

2. There will be a more extensive antibiotic resistance profile, as determined by Kirby Bauer susceptibility testing, for isolates in counties that do not participate in the Wyoming Medication Donation Program compared to counties that do participate.

DISCUSSION

The counties that did not participate in the WMDP showed significantly higher rates of resistant isolates per person. Niobrara, Carbon, and Sublette Counties all had larger rates of isolated bacteria in wastewater per person by an order of magnitude. The gentamicin resistant colony was only found in Sublette County, which suggest antibiotic resistance in wastewater from the county is due to the WMDP. This result is supported by the counties of Wyoming that do not donate to the recycling program have a higher rate of resistant bacteria in wastewater than counties that do donate antibiotics, which supports our second hypothesis.

Kirby Bauer data showed that the isolates that were resistant to one antibiotic were capable of multi-drug resistance in many cases. Most isolates were completely resistant to the commonly prescribed antibiotic penicillin. This is important because multi-drug resistant isolates in wastewater is a higher threat than single drug resistant strains. We found many challenges growing some of the isolates on MacConkey agar, and data for all of the isolates was not able to be obtained. The results from Kirby Bauer would be better analyzed if we had a complete set of data.

Throughout these experiments, we have learned more about antibiotic resistance in wastewater, and our results align with the conclusions about the spreading of antibiotic resistance in wastewater that is outlined in the literature (3). We determined that the three counties that participated in the WMDP had lower rates of antibiotic resistance when compared to the three counties that do not participate. It should be noted that the extremely high rate of bacterial resistant isolates per person found in Niobrara could be due to outside, contaminating variables. These variables could include that this county is extremely agricultural, and high volumes of antibiotics are used in this industry. This information could be used to spread awareness about medication donation programs and their positive impact, as well as increasing the funding for the Wyoming Medication Donation Program.

CONCLUSION

- The WMDP may play a crucial role in preventing antibiotic resistance in Wyoming wastewater.
- In counties that participate in the WMDP there are less resistant bacterial isolates per person than in counties that do not participate in the program.
- This could have an influence on the future of the WMDP, and may be used to encourage the use of this program throughout the state.
- The future of this research will be to continue examining the extent of resistance in wastewater around the state.
- More samples will be examined through the same processes described in this research, and the experimental software MALDI-TOF will be used to identify each isolate.

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RESOURCES