Antibiotic Resistance of Escherichia Coli in the Lamoille River Basin

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Introduction

Bodies of water become contaminated with fecal coliform bacterium from several sources: human sewage, dysfunctional septic systems, waste water discharge, animal waste (both domestic and wild), agricultural practices, and storm water runoff (Fincher et al. 2009). A major contributor in Lake Champlain, especially in the Burlington area, is dog feces (Lake Champlain Basin Project 2013). Escherichia coli is a fecal coliform found universally in sewage (Wose Kinge et al. 2010). This gram negative bacterium is used as an indicator organism when determining microbial quality of water and sanitation (Talukdar, PK et al. 2013). Its presence in the water indicates sewage contamination and the potential presence of pathogenic microbes (Wose Kinge et al. 2010). This contamination is a concern, as it can lead to human infection. The majority of E. coli infections are waterborne as surface water can be heavily contaminated with this organism (Talukdar, PK et al. 2013).

Antibiotic resistance in this bacterium is of increasing concern throughout the world. Wose Kinge et al. (2010) states “Antibiotic resistance in E. coli has been globally identified in isolates from environmental, animal and human sources”. This antibiotic resistance has shown an increase in recent years, at times, leading to point-break situations where no antibiotic treatment options remain (Talukdar, PK et al. 2013). Antibiotic resistance in nonspecific types of Escherichia coli isolated from streams and rivers in the United States have been documented (Fincher et al. 2009). In one study, Sayah et al. (2005) found that 80.6% of E. coli isolates collected from surface waters located near swine and other livestock facilities were resistant to at least one antibiotic (Sapkota, A et al. 2007).

Materials and Methods

Subsurface water samples were collected from our 19 Lamoille River tributaries. E. coli was isolated from each water sample using standard membrane filtration methods: U.S. Environmental Protection Agency Method 1604 (U.S. EPA 2002). After filtration and incubation, blue/green colonies that fluoresced when exposed to long wave UV light were selected and grown in LB broth tubes on an incubated shaker for 18-24 hours at 35˚. Twelve isolates from each stream site were chosen.

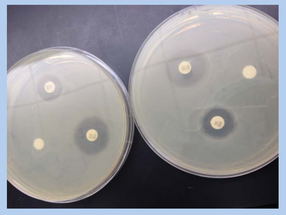
The samples were then streaked on selective MacConkey II agar with MUG and again grown at 35˚ for 18-24 hours. The resulting colonies were selected and picked and again grown in LB broth. Colonies selected were both isolated from others and fluorescent under UV light. This process, LB broth to MacConkey agar was repeated twice to increase the likelihood of the sample containing E. coli.

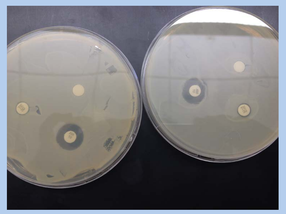
The sample was then streaked onto LB agar and again incubated for the aforementioned time and temperature. A single colony was then selected and was tested with an Enterotube II. Results were read and interpreted according to manufactures specifications.

E. coli positive samples were then selected using a random number generator created in Microsoft Excel. Once selected, samples were tested with antibiotics using the Kirby-Bauer method (Hudzicki) with the following modification: No McFarland standard was utilized. For each sample, the E. coli were swabbed on Mullen-Hinton agar plats. Antibiotic paper disks were then placed on the Mueller-Hinton agar plates, which were then incubated at 35˚ for 24 hours. The following antibiotics were tested: tetracycline 30ug and ampicillin 10ug. After incubation, the zones of inhibition were measured and interpreted according to protocol values. This process was repeated in order to compare results.

Results

Stream Site	Sample Number	Inoculation Trial 1 Diameter (mm)		Inoculation Trial 2 Diameter (mm)		Average Diameter (mm)	
		AM	TE	AM	TE	AM	TE
Brewster River 1048	JSC-EC13-008	15	18	14	19	14.5	18.5
Brewster River 532	JSC-EC13-018	14	7	13	7	13.5	7
Brewster River 533	JSC-EC13-028	15	19	14	18	14.5	18.5
Browns River 355	JSC-EC13-046	14	18	17	19	15.5	18.5
Browns River 535	JSC-EC13-056	15	17	17	19	16	18
Browns River 859	JSC-EC13-071	17	20	17	21	17	20.5
Deer Brook 365	JSC-EC13-077	15	20	16	21	15.5	20.5
Deer Brook 380	JSC-EC13-090	16	21	19	22	17.5	21.5
French Hill 992	JSC-EC13-099	15	20 *	16	18	15.5	18
Gihon River 492	JSC-EC13-114	16	19	18	21	17	20
Mill Brook 430	JSC-EC13-126	14	20	14	18	14	19
Mill Brook 512	JSC-EC13-143	13	17	14	18	13.5	17.5
North Branch 502	JSC-EC13-150	14	17	17	19	15.5	18
North Branch 555	JSC-EC13-162	17	11	18	7	17.5	9
Ryder brook 675	JSC-EC13-172	14	18	16	21	15	19.5
Seymour River 442	JSC-EC13-184	13	18	15	20	14	19
Wild Branch 1212	JSC-EC13-199	18	19	17	19	17.5	19
Wild Branch 721	JSC-EC13-207	16	20	17	20	16.5	20
Wild Branch 759	JSC-EC13-221	15	18	18	19	16.5	18.5
* Multiple ring pattern							
Susceptible							
Intermediate							
Resistant							
Resistant- Outlier							





Possible resistance to tetracycline

Conclusions

The results showed an unexpectedly high number of intermediate and resistant inhibition zones. This prevalence can, most likely, be attributed to the lack of McFarland standard. The antibiotic disks are proven effective against specific concentrations of bacteria. Increased concentration of bacteria will therefor affect the resulting inhibition diameter. The Mullen-Hinton media used in the creation of the plates expired November of 2012. Though the media still supported the growth of the bacteria it is possible that it did not facilitate the diffusion of the antibiotic as effectively as non-expired media. Though the results may not be accurate, some conclusions can still be drawn.

Two samples (018 and 162) proved to be exceptionally resistant to tetracycline while showing little resistance to ampicillin. The bacterial concentration and expired media may have caused this resistance interpretation, however, because these two samples showed such powerful resistance (less than 2mm diameter around disk), they may, in fact, be resistant. The average inhibition diameter was less than 2.5 mm from susceptible level inhibition.

This project was proposed and executed to open the doorway for further research into antibiotic resistance of the E. coli in these 19 stream sites. Though the lack of a concentration standard and expired media may have colored the results, this data can be used as a jumping off point for further research.

Literature Cited

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