Detection of *E. coli* 0157:H7 in the Rio Grande Using PCR

E. Penner¹, A. Vazquez¹, E. Holguin², C. Baeza², P. De Los Santos², J. Mendoza¹, M. E. Alvarez¹

El Paso Community College,¹El Paso, Texas, Transmountain Early College High School, El Paso, Texas

Abstract

The proximity of large populations of livestock around the Rio Grande River in El Paso, TX and Juarez, Mexico place the river at risk for contamination with *E. coli* 0157:H7. This toxin-producing strain of *E. coli* has been found in the intestine of cattle and has the potential to cause severe disease in humans. The Rio Grande River is an international boundary between the U.S. and Mexico and is used as a source of drinking water and irrigation, as well as for recreation. Runoff from dairy farms has the potential to contaminate the river and food products grown in this area in personal and commercial gardens. User-friendly, quick and effective ways of monitoring contamination of the river are very important. The MicroScan autoSCAN-4 System by Siemens has been used in our laboratory to identify *E. coli* isolates from the Rio Grande River at a 99.99% probability. The system also provides the minimal inhibitory concentration (MIC) for a variety of antibiotics. The *E. coli* 0157:H7 detection kit from Norgen Biotek Corp. is used in the food industry and has a high sensitivity and specificity for *E. coli* 0157:H7. It can be used with agarose PCR gels or Real-Time PCR. The objective of this study is to determine if *E. coli* 0157:H7 can be isolated from the Rio Grande River. Water samples were collected from the Rio Grande close to the mouth of the Montoya drain in the town of Sunland Park NM. This site is impacted by agriculture runoff, dairy farms, and municipal streams. EPA guide lines in SOP# 2013 procedure were used to collect samples. Thirty five *E. coli* isolates were identified at a 99.99% probability of correct identification using the MicroScan system. Twenty isolates with the highest MICs were selected. Among the highest MICs were Cefotaxime (30µg/ml), Ampicillin (15 µg/ml) and Amoxicillin / K Clavulanate (15 µg/ml). The isolates were then processed with the Norgen Biotek Corp. Kit. Agarose gels were used for the detection of the antigens. Out of the twenty isolates, three tested positive upon visual inspection using the STX2 348 Bp band DNA marker positive control in the kit. Our results show the potential for using these two systems for quick and effective detection of *E. coli* 0157:H7 in river water.

Introduction

The Rio Grande River is used as a source of drinking water and irrigation, as well as for recreation along the region of El Paso, TX and Juarez. Runoff from dairy farms has the potential to contaminate the river water and food products grown in this area in personal and commercial gardens with the strain 0157:H7. This toxin-producing strain of *E. coli* has been found in the intestine of cattle and has the potential to cause severe disease in humans including hemorrhagic diarrhea and hemolytic uremic syndrome. User-friendly, quick and effective ways of monitoring contamination of the river is very important. The objective of this study is to determine if *E. coli* 0157:H7 can be isolated from the Rio Grande River.

Methods and Materials

- **Step 1:** Sample collected (Figure 5.1)
  - Membrane filtration was done through a 0.47 µm cellulose membrane filter.
- **Step 2:** Gram Staining (Figure 5.2)
  - Isolate potential colonies of *E. coli*.
  - Inoculate and scan to confirm *E. coli* isolates.
- **Step 3:** DNA extraction of pure *E. coli*.
- **Step 4:** PCR amplification of gene sequence

Figure 5.1

Figure 5.2

Results

- Thirty five colonies were isolated using differential and selective media from the Rio Grande samples. After testing positive for *E. coli* on Modified M-tec agar by Dilco, the isolates were analyzed using the MicroScan autoSCAN-4 system.
- The 35 isolates were identified at a 99.99% probability of correct Identification as *E. coli* using the MicroScan auto SCAN-4 bacterial identification system.
- Upon further inspection, 20 isolates with the highest MICs were selected to be analyzed for O157:H7 antigens with the Norgen Biotek kit.
- Three of these isolates were positive for the O157:H7 strain of *E. coli* using the Norgen Biotek kit.

Conclusion

Out of twenty *E. coli* isolates, three isolates tested tentatively positive for O157:H7. Further Melt Curve Real Time PCR analysis are needed to verify positive results.

Acknowledgements

- This work was supported in part by the US International Boundary and Water Commission (IBWC). RISE Student Elizabeth Penner and research reported in this publication was supported in part by the National Institute of General Medical Sciences of the National Institutes of Health under RISE Award Number R25GM060424. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Catina Baeza, Eduardo Holguin, and Priscilla De Los Santos, were supported in part by DHS SLA Award #2011-ST062-000043.

Works Cited


