Antibiotic Resistance Genes in Las Vegas Valley Bacteria

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Abstract

A recent British study concluded that annually, there are 700,000 deaths world wide that can be attributed to antibiotic resistant bacteria. [1] Mapping the spread of resistance genes in the wild is a necessity as a method to measure both the impact of antibiotic over-use on bacterial populations *in situ* rather than *in vivo*, and to determine the presence of resistance genes in wild bacterial populations that could be selected for in the presence of antibiotics even if resistance has not yet been expressed. The purpose of this study was two fold; to establish procedures that would allow for the detection of resistance genes in local soil and water samples, and to use these methods to assess samples from four discreet locations in the Las Vegas Valley. One sample produced repeatable positive PCR results for two antibiotic resistance genes.

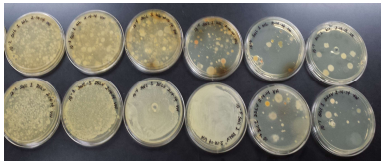
Sample Locations

Clockwise from top Left, Las Vegas Wetlands Park, Lake Harriet Las Vegas, RC Farms North Las Vegas, Red Rock Canyon Conservation Area

Methods

CFU/gm Soil and genomic DNA

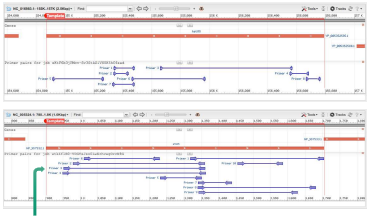
Soil was plated on LB agar with amphotericin B using ten fold dilution scheme from 10^{-3}-10^{-8} to determine CFU/gm of Soil



Location	Soil CFU/gm	Genomic [DNA]
RCP pig farm	2.75×10^7	14.8 ng/µl
Lake Harriet	3.17×10^5	6.5 ng/µl
Red Rock	1.23×10^5	3.3 ng/µl
Clark C. Wetlands	8.2×10^3	2.5 ng/µl

Correlations of baterial CFU/gm soil with concentrations of genomic DNA. Nanodrop 260:280 ratio was 1.68.

Primer Alignments



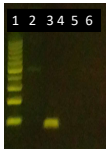
Graphical representation of PCR primers and their binding locations from the NCBI PrimerBLAST online tool. The 409 Bp pair shown as Primer 3 was used to screen for Str-A. The 67 Bp Primer used for TetM is not shown above.

Methods

PCR for Resistance Genes

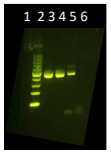
Results for tetM 67 bp product

Lane 1 Mol Wt. Ladder 100 bp.
Lane 2 Wetlands soil (tetM)
Lane 3 Wetlands water (tetM)
Lane 4 Negative control (tetM)
Lane 5 Blank
Lane 6 Unknown Pig Soil (tetM)
Lane 7 Positive control (tetM)
Lane 8 100 bp Mol wt ladder

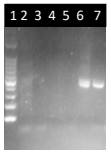


Lane 1. 100 bp Mol Wt. ladder
Lane 2. Harriet lake soil (tetM)
Lane 3. Positive control (tetM)
Lane 4. Negative control (tetM)
Lane 5. Red Rock soil (tetM)
Lane 6. Red Rock water (tetM)

Results for strA 409 bp product



Lane 1: Negative control
Lane 2: 100 bp Ladder
Lane 3: Pig Soil (strA)
Lane 4: Pig water - (strA)
Lane 5: Positive control (strA)
Lane 6: Wetlands soil (strA)



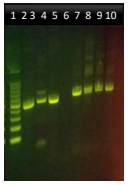
Lane 1: Ladder
Lane 2: Soil Harriet Lake - (strA)
Lane 3: Water Harriet Lake (strA)
Lane 4: Soil Red Rock - (strA)
Lane 5: Negative control - (strA)
Lane 6: Positive control - s (strA)
Lane 7: Positive control - (strA)

Results detected the presence of the strA and tetM genes in pig farm soil and water but not in Red Rock, wetlands, or Lake Harriet soil and water

Methods and Conclusion

16S rRNA gene verification

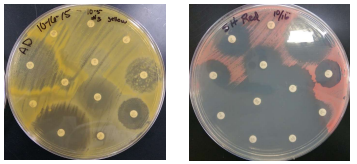
Results for 16S 466 bp product



Lane 1: !00 bp ladder
Lane 2: Soil StrA
Lane 3: Bacterial Water StrA
Lane 4: Colony Bacteria StrA
Lane 5: StrA Positive Control
Lane 6: 16S Positive Control
Lane 7: 16S Soil
Lane 8: 16S Bacteria Water
Lane 9: 16S Colony Bacteria
Lane 10: Negative Control

The Presence of 16S rRNA was used to verify bacterial DNA in all samples including negative results.

Kirby-Bauer Results



Samples from the RC pig farm (left) that produced positive results for StrA and TetM also demonstrated less sensitivity to antibiotics using the Kirby-Baur method

The methods used in this study are effective in evaluating the presence or absence of specific resistance gene sequences in samples taken *in situ*. Although there is not enough data to support a specific correlation between sample location and the presence of resistance genes, it is notable that the only sample to demonstrate both the presence of resistance genes and to express resistance was taken from an agricultural site. This farm does not use antibiotics in feed, but feed from other sources, and the pigs themselves may have detectable resistance genes present. Thus, future studies will be needed to explore the possible correlation between in vivo antibiotic use and the local spread of resistant bacteria.

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