

Characterization of pathogenic bacteria cultures and antibiotic-resistant genes in crow feces and their environment



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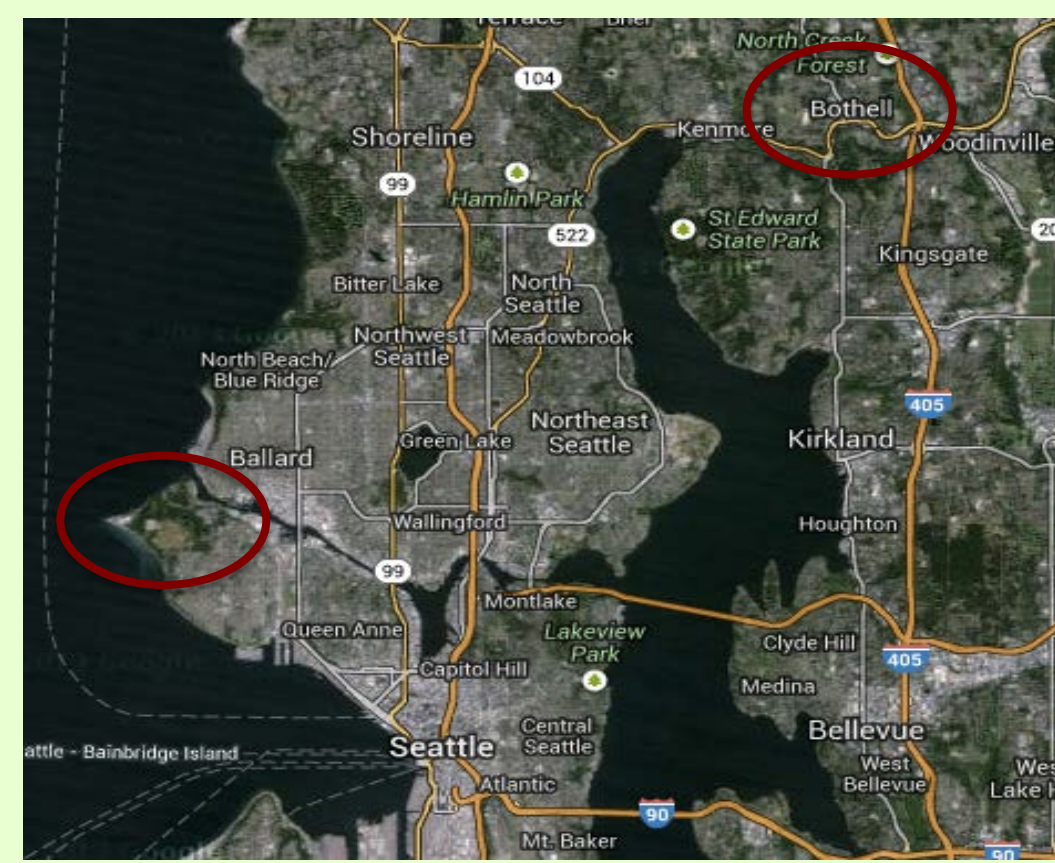
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Introduction

The goal of this study is to discover the relationship between antibiotic resistance genes and bacteria found in crows and their respective environments. Crow feces were collected under UW IACUC approval at UW Bothell. Following DNA extraction PCR was performed to amplify 6 genes attributed to antibiotic resistance from 26 samples (21 crow feces (n=6 crows 2 waste water, 2 cow manure, 1 stream wetland UW Bothell). Cultures for Gram-negative bacteria resistant to ampicillin (Amp), chloramphenicol (Cm), tetracycline (Tc), vancomycin-resistant *Enterococcus* (VRE), *Campylobacter* spp., and *Salmonella* spp. were included. The cow manure was negative for all the bacteria cultures, the stream was positive for Cm^r, Tc^r Gram-negative bacteria and VRE, the waste water was positive for all cultured bacteria, while 6-28% of the crows were positive for each cultured bacteria. The wastewater and cow manure were positive for all antibiotic resistance genes tested by PCR. The stream was positive for the *mef(A)* and *tet(M/O/S)* complex, while 1/3 of the birds were positive for the *erm(B)* and *mef(A)* genes. The crow samples obtained at the waste water treatment plant were much more likely to carry all the cultured bacteria and antibiotic resistance genes examined than crow samples isolated from the farm but this may be due to the low sample numbers. The presence of the *tet(B)* and *tet(M/O/S)* genes in the crows were found in similar percentages from all three environments. The *mef(A)* gene was lower in the farm and UW Bothell site while the *erm(B)* gene was lower in the farm. To determine if some of these bacteria and genes are more common in birds from an environment that also carries them this will require a larger number of samples.

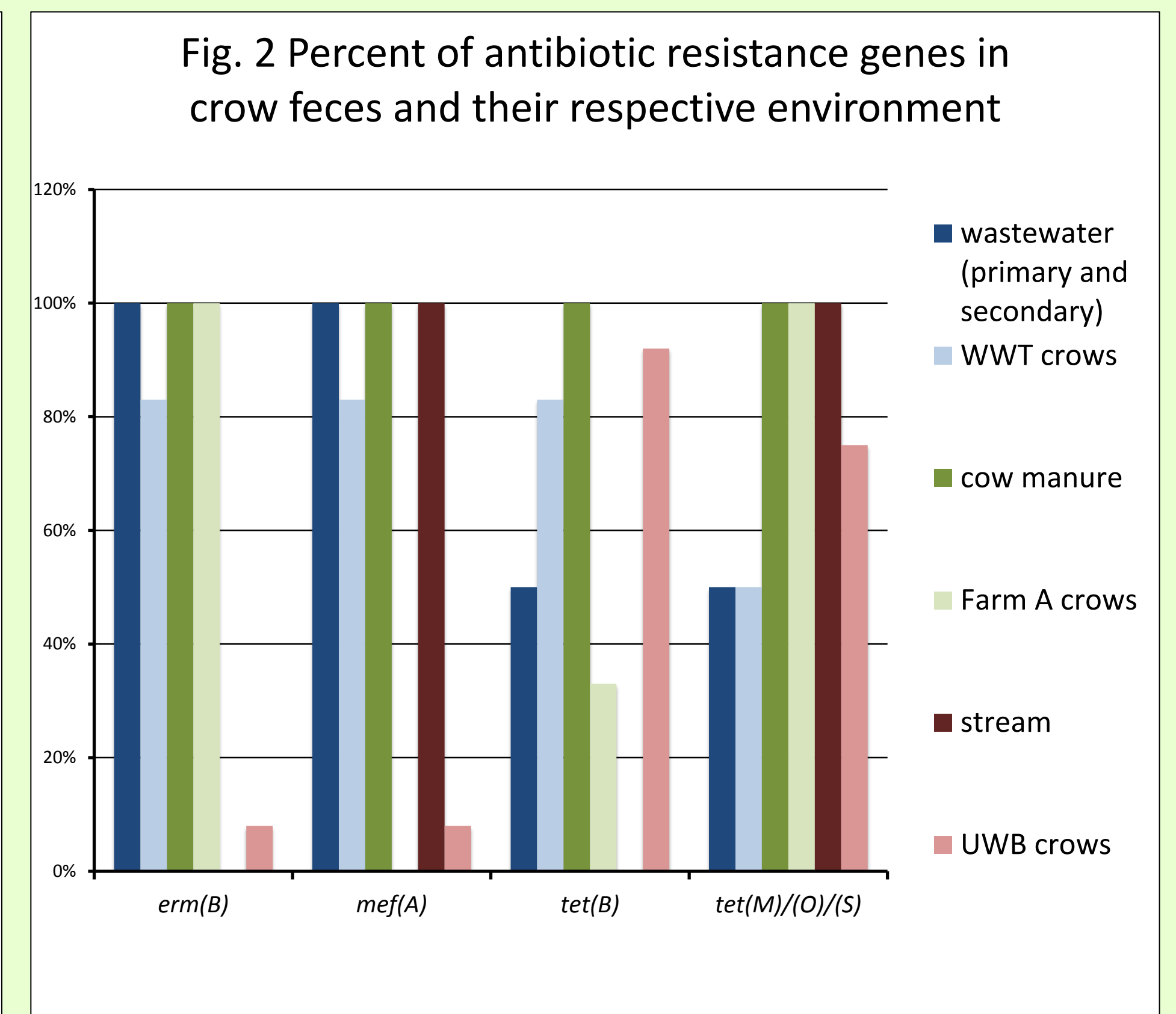
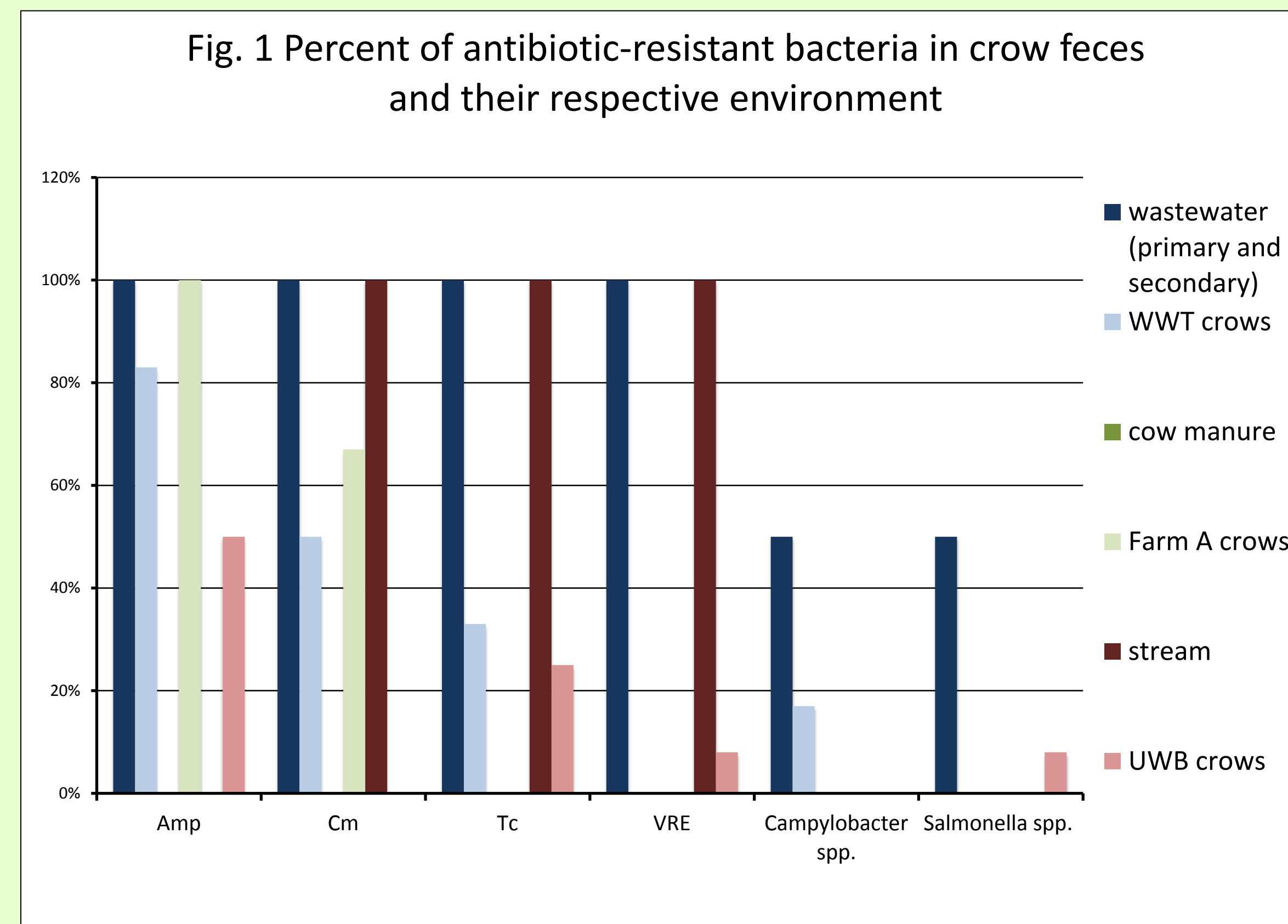
Results

Isolated Culture	Discovery Park WWT 6/15/13			Farm A 6/7/13		UW Bothell 6/4/13		Total n=26
	Wastewater n=2		Crows n=6	Cow Manure n=2	Crows n=3	Stream n=1	Crows n=12	
	Primary n=1	Secondary n=1						
Ampicillin-resistant gram-negative	1	1	5 [83%]	0	3 [100%]	0	6 [50%]	16 [62%]
Chloramphenicol-resistant gram-negative	1	1	3 [50%]	0	2 [67%]	1	0	8 [31%]
Tetracycline-resistant gram-negative	1	1	2 [33%]	0	0	1	3 [25%]	4 [15%]
Vancomycin-resistant <i>Enterococcus</i>	1	1	0	0	0	1	1 [8%]	4 [15%]
<i>Campylobacter</i> spp.	1	0	1 [17%]	0	0	0	0	2 [8%]
<i>Salmonella</i> spp.	1	0	0	0	0	0	1 [8%]	2 [8%]
PCR Gene								
<i>erm(B)</i>	1	1	5 [83%]	2	3 [100%]	0	1 [8%]	13 [50%]
<i>mef(A)</i>	1	1	5 [83%]	2	0	1	1 [8%]	11 [42%]
<i>tet(B)</i>	1	0	5 [83%]	2	1 [33%]	0	11 [92%]	20 [77%]
<i>tet(M)/(O)/(S)</i>	1	0	3 [50%]	2	3 [100%]	1	9 [75%]	19 [73%]



Methods

- Samples analyzed either through classical culture isolation or DNA extraction
 - Cultures incubated overnight
 - Proteinase K treatment
 - Cultures cryogenically frozen in milk
- DNA from samples put through PCR to test for specific gene presence
 - Thermal cycler
- PCR assays tested with gel electrophoresis for positive or negative result
- Crow feces samples compared with results from environmental samples (primary and secondary wastewater, cow manure at farm, local stream)
 - Cultures present
 - Antibiotic resistance genes



Conclusions

- As expected, the wastewater had all cultured bacteria and all of the antibiotic resistant genes tested
- Cow manure expressed all resistant genes but did not yield any cultured bacteria
- The stream had Amp^r and Cm^r bacteria as well as resistance genes *mef(A)*, *tet(M)/(O)/(S)*
- Crows carried cultured bacteria and resistance genes not found in the a few of the environmental samples analyzed
- Crows samples had both cultured bacteria and resistance genes which varied by location but was not statistically significant because of the low number of samples

Prospective Work

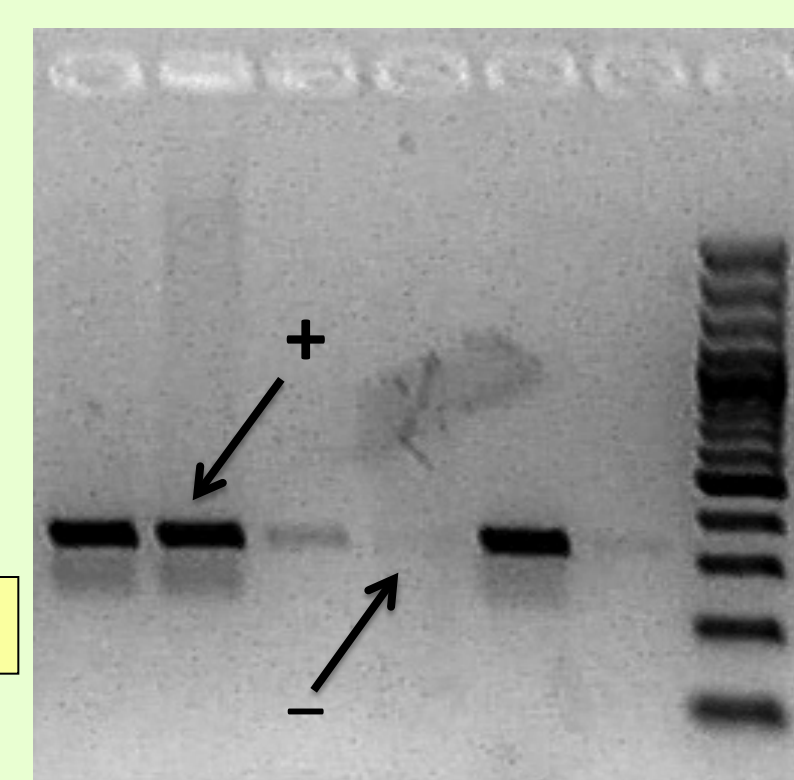
- Collect more samples for further study of the crow/environment relationship to allow statistical analysis to be done

Acknowledgement

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Thermal Cycler



Electrophoresis Gel