Diffusion of antimicrobial resistance across management niches on dairy farms

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INTRODUCTION

A major challenge to develop strategies that mitigate the global impact of farm origin antibiotic resistance is a failure to describe dissemination patterns of resistance within the farm via animals or environmental flow.

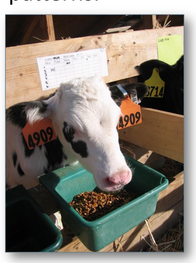
This study focuses on the presence and diversity of phenotypic antibiotic resistance within niches on the whole dairy enterprise. The goals are:

- identify niches where diversity is generated and maintained
- identify niches connected by phenotype similarity suggesting dissemination, and
- identify niches that narrow diversity.

The premise is: diversity is generated in niches with high antibiotic use and disseminate through the dairy system with animal movement or environmental flow.

METHODS

- We sampled from commercial dairy herds that maintain a milking herd and rear replacement animals.
- Farm niches were defined by housing and function. Housing niches are areas with pre-weaned heifers, weaned and bred heifers, early lactation cows, lactating cows, pregnant non-lactating cows, antibiotic treated cows, and cows leaving the herd. Function niches include milk, waste, water, and feeds.
- For each sampling, 72 animal fecal samples, 8 water, 8 feed samples, 1 milk filter, samples from the waste stream, and soil samples are collected.
- We used *Escherichia coli (EC)* as our model bacterium and antimicrobial susceptibility tested 4 isolates from each sample against 13 antimicrobials.
- Latent class analysis (LCA) was used to organize isolate-based resistance patterns.



RESULTS

Twelve farms were enrolled and each visited 3 times.

More than 14,000 EC isolates were tested for antimicrobial susceptibility patterns. Based on LCA, 26 different susceptible patterns were observed. The most common pattern was pan-susceptible (68%) followed by a pattern with tetracycline resistance only (7%). The greatest resistance diversity was observed in pre-weaned and weaned calves followed by treated animals. EC from adult cows were pan-susceptible (approaching 90% of isolates). EC from soil samples were pan-susceptible except for samples in pre-weaned housing areas. There were farm-specific patterns and between farm differences in diversity.

C=	19	8	9	17	2	24	20	6	15	11	3	7	4	25	14	18	5	22	1	23	21	13	12	10	16	S
N=	30	90	242	119	316	26	17	65	16	181	203	232	42	48	140	314	237	10	129	15	329	201	240	81	1229	9911
AM	1.000	1.000	1.000	0.982	0.987	1.000	1.000	0.000	1.000	0.976	0.971	0.376	1.000	0.152	0.515	0.000	1.000	1.000	0.000	0.000	0.013	0.961	0.923	0.124	0.057	0.000
CHL	1.000	1.000	0.945	0.928	0.795	0.271	0.942	0.974	1.000	0.465	0.859	1.000	0.000	0.120	0.000	1.000	0.954	0.103	0.780	0.928	0.231	0.097	0.000	0.000	0.025	0.000
FIS	1.000	1.000	1.000	0.983	0.985	1.000	0.887	1.000	0.332	1.000	1.000	0.998	1.000	0.940	1.000	0.969	1.000	0.000	0.957	0.000	0.798	0.000	0.000	0.153	0.193	0.000
KAN	1.000	1.000	0.872	1.000	1.000	1.000	0.638	1.000	0.081	0.722	0.310	1.000	0.000	1.000	0.000	0.000	0.000	0.853	0.000	0.000	0.229	0.145	0.003	0.1446	0.028	0.000
AN	1.000	0.000	0.000	0.123	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.098	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000
SXT	0.900	1.000	1.000	1.000	0.981	1.000	0.033	1.000	0.153	1.000	0.398	0.325	0.719	1.000	1.000	0.288	0.000	0.892	0.406	0.000	0.013	0.000	0.000	0.017	0.000	0.000
STR	1.000	0.963	0.943	1.000	0.910	0.012	0.000	1.000	0.947	0.661	0.968	0.731	0.000	0.349	0.649	1.000	0.218	0.000	0.242	0.119	0.632	0.449	0.023	0.323	0.000	0.000
TET	1.000	0.990	1.000	0.992	1.000	0.919	0.603	1.000	0.883	0.917	0.955	0.996	1.000	1.000	0.890	1.000	0.995	0.255	0.798	0.857	0.986	1.000	0.019	0.260	0.818	0.000
AMC	0.867	0.549	0.154	0.488	0.990	0.000	0.000	0.000	1.000	0.306	0.729	0.000	0.000	0.000	0.136	0.015	0.000	0.000	0.023	0.000	0.000	0.028	0.476	0.000	0.000	0.000
NAL	1.000	0.985	1.000	0.000	0.188	0.961	1.000	1.000	0.780	0.000	0.000	0.087	0.000	0.079	0.095	0.012	0.000	0.595	0.000	1.000	0.000	0.000	0.000	0.074	0.000	0.000
GM	1.000	0.141	0.647	0.950	0.197	0.000	0.651	0.143	0.000	0.008	0.229	0.108	0.082	0.000	0.017	0.019	0.009	0.180	0.000	0.000	0.000	0.000	0.000	0.039	0.000	0.000
XNL	0.933	0.931	0.262	0.869	0.330	1.000	0.873	0.000	0.540	0.720	0.352	0.000	1.000	0.000	0.000	0.000	0.000	0.112	0.000	0.000	0.002	0.104	0.535	0.050	0.000	0.000
CIP	0.900	1.000	0.685	0.007	0.000	0.851	0.876	0.066	0.177	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000

Figures:
26 Resistant Classes by tested antibiotic

Distribution of classes by production animal niche

Distribution of classes by sampled farm

Type	Niche	19	8	9	17	2	24	20	6	15	11	3	7	4	25	14	18	5	22	1	23	21	13	12	10	16	S	Sum
Enviro	Milk Filter	.	.	.	.	1	.	.	.	.	.	.	.	1	1	.	4	.	.	.	.	1	.	7	.	7	78	100
	Wastewater	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1	1	.	2	5	81	92
Fecal	Bred Older Heifers	.	.	.	1	.	.	.	.	.	.	2	6	.	1	2	1	3	.	3	.	22	12	17	1	96	1111	1278
	Culled Cows	.	.	.	.	4	.	.	.	.	.	2	2	1	.	8	3	9	1	4	.	6	3	5	.	33	600	681
	Dry Cows	.	.	.	.	.	.	.	.	.	2	1	2	1	2	.	2	7	.	3	.	12	5	25	6	74	1092	1234
	Fresh Cows	.	.	.	.	.	.	.	.	4	5	1	2	.	.	5	32	.	1	.	4	9	8	8	92	1078	1249
	Hospital Pen	.	3	4	.	5	1	3	.	.	7	1	2	11	.	5	8	49	.	3	.	13	21	59	3	77	899	1174
	Lactating Cows	.	.	1	.	6	.	.	.	.	1	.	4	.	1	4	.	7	.	1	.	8	11	3	10	91	1096	1244
	Maternity Calving Pen	.	.	.	2	.	.	.	.	.	3	1	1	1	.	1	3	13	.	2	.	19	6	5	1	72	1108	1238
	Pre-weaned Calves #1	6	5	10	21	18	4	.	1	1	11	9	4	.	.	10	2	1	.	.	.	.	7	6	5	10	69	200
	Pre-weaned Calves #2	6	14	27	14	33	1	.	.	2	30	7	3	5	.	1	.	1	.	3	.	.	7	3	1	1	25	184
	Pre-weaned Calves #3	9	15	37	9	25	4	1	3	1	24	3	.	.	.	6	4	.	.	.	.	4	3	.	.	5	30	183
	Pre-weaned Calves #4	1	17	31	7	25	1	4	5	.	8	6	6	1	2	13	9	1	.	.	.	6	.	1	1	6	33	184
	Pre-weaned Calves #5	5	13	23	3	22	1	.	6	3	19	10	2	.	5	9	5	1	.	6	.	5	7	1	.	16	33	195
	Pre-weaned Calves #6	.	5	19	7	19	1	.	16	4	14	11	11	.	4	4	9	11	.	.	.	11	3	4	4	17	26	200
	Pre-weaned Calves #7	.	1	14	8	16	1	1	9	3	5	15	16	7	.	8	6	2	.	1	.	12	1	.	.	24	44	194
	Pre-weaned Calves #8	.	1	13	3	10	7	1	14	1	7	6	11	1	5	5	9	7	.	2	.	12	3	10	.	23	30	181
	Pre-weaned Calves #9	.	5	15	4	23	3	4	6	.	12	9	7	.	1	6	3	1	.	.	2	10	5	1	.	20	22	159
	Pre-weaned Calves #10	.	.	11	10	9	.	.	1	.	3	14	9	.	.	.	6	1	.	3	.	11	4	1	.	25	18	126
	Pre-weaned - Other	.	.	2	2	4	.	.	.	.	.	1	4	.	.	.	2	.	.	3	.	1	.	.	.	8	16	43
	Weaned Calves	.	4	6	10	38	2	.	3	1	11	61	94	.	12	28	178	25	.	61	9	102	41	6	12	255	368	1327

Farm	19	8	9	17	2	24	20	6	15	11	3	7	4	25	14	18	5	22	1	23	21	13	12	10	16	S	Sum
WSU1035	.	2	5	8	4	.	.	.	5	19	6	3	4	47	44	24	.	15	.	33	4	16	5	114	663	1021
WSU1036	.	.	4	.	12	.	.	.	9	4	3	2	.	1	41	56	.	20	.	74	17	13	9	85	968	1318
WSU1147	.	1	3	4	27	.	.	1	16	13	15	.	7	3	23	10	1	5	.	6	11	10	5	73	806	1040
WSU1152	.	36	8	.	16	20	.	.	11	1	10	21	.	4	11	15	.	6	.	16	26	59	3	79	763	1105
WSU1153	.	2	3	.	25	.	.	.	3	14	27	5	1	.	26	8	.	14	.	24	22	11	5	112	858	1160
WSU1180	.	.	.	.	2	.	.	1	5	10	8	.	.	17	47	16	1	11	.	16	18	5	2	77	903	1139
WSU3	.	.	.	4	20	1	2	.	33	21	1	5	.	14	7	16	1	2	.	24	27	42	2	76	730	1028
WSU35	.	1	3	8	32	.	.	2	7	55	8	.	.	3	18	25	.	14	.	8	24	17	4	68	979	1276
WSU39	7	13	45	2	11	1	.	8	.	11	8	38	.	5	7	31	11	.	6	1	33	6	12	3	87	822	1168
WSU39C	13	23	116	32	58	1	.	44	1	33	5	50	.	10	10	16	6	7	7	2	32	6	3	6	75	29	585
WSU46	8	6	16	39	31	1	1	2	.	14	7	9	1	8	7	8	13	.	4	.	31	11	22	14	125	636	1014
WSU51	2	1	4	15	57	.	.	7	.	28	12	48	.	11	18	15	16	.	3	.	13	8	22	20	57	699	1056
WSU60	.	5	35	7	21	2	14	4	11	6	34	9	5	2	9	27	21	.	22	12	19	21	8	3	201	1055	1553
Total	30	90	242	119	316	26	17	65	16	181	203	232	42	48	140	314	237	10	129	15	329	201	240	81	1229	9911	1446

CONCLUSIONS

There are niche specific patterns of resistance that suggest there is little to no dissemination across niches.

Diversity is generated in areas where antibiotic use is greatest, hospital pens and pre-weaned calf areas

Weaned calves also have a high level of diversity which may be a result of transitioning across age groups.

The between-farm patterns also suggest that resistance traits are farm-specific and driven more by local features rather than shared across spatially distinct farms.

Funded by USDA:NIFSI: "Minimizing Antibiotic Resistance Transmission Throughout the Food Chain" and USDA:NIFA: "Integrating Biology, Psychology, and Ecology to Mitigate Antibiotic Resistance in Food Animal Production Systems". The Washington State University Institutional Review Board reviewed the project and gave it exempt status.