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Recent emergence and rapid dissemination of *bla*_{CTX-M} among dairy calves in Washington State

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INTRODUCTION

The *bla*_{CTX-M} family of extended-spectrum β-lactamase (ESBL) genes have been associated with multidrug resistant (MDR) Enterobacteriaceae worldwide since the late 1980s [1]. Initially associated primarily with hospital pathogens, *bla*_{CTX-M} bacteria have since been identified in environmental and domestic animal sources. Enterobacteriaceae bearing *bla*_{CTX-M} were reported in the United States relatively late compared to other countries and only recently (in 2009) were identified in US dairy cattle [2]. Archived *E. coli* isolates from Washington State dairies suggested that *E. coli* bearing *bla*_{CTX-M} (CTX-M EC) were first prevalent in dairy cattle between 2008 and 2011 (unpublished data). Until very recently, 3rd generation cephalosporin resistance in cattle-associated gram negative bacteria was attributed primarily to plasmid-associated AmpC type enzymes coded by *bla*_{CMY-2} [3-5]. In order to determine risk factors for the occurrence of CTX-M in dairy calves we visited 30 farms to collect samples and administer questionnaires about dairy management factors.

METHODS

During the summer and fall of 2012, we visited 30 farms. Samples collected included individual fecal samples from up to 5 pre-weaned dairy calves per age group (0-6 days, 7-14, 15-21, 22-28, 29-35, 36-42, 43-49, 50-56, and >56 days old) and pooled samples from the lactation pen, the fresh cow pen, the hospital pen, the dairy lagoon and the maternity or close-up pen and one milk filter. The 30 farms were distributed throughout Washington State's dairy regions including south central, central eastern and northwestern Washington State. In the laboratory, the samples were processed as previously published [6] in order to screen for both *bla*_{CTX-M} and *bla*_{CMY-2}-associated phenotypes. Fecal samples were incubated overnight in LB broth with cefotaxime (2 μg/ml), followed by plating onto MacConkey agar supplemented with cefepime (4 μg/ml) or ceftiofur (4 μg/ml). Growth on cefepime and ceftiofur are associated with *bla*_{CTX-M} and *bla*_{CMY-2}, respectively. *E. coli* isolates were confirmed by PCR for *uidA* [7]. The presence of *bla*_{CTX-M} and *bla*_{CMY-2} were determined using published PCR protocols [6, 8]. Questionnaires concerning management factors were administered at each visit. Data were managed in Microsoft Excel and analysed in SAS 9.2. The Wilcoxon test was used to compare prevalences between groups.

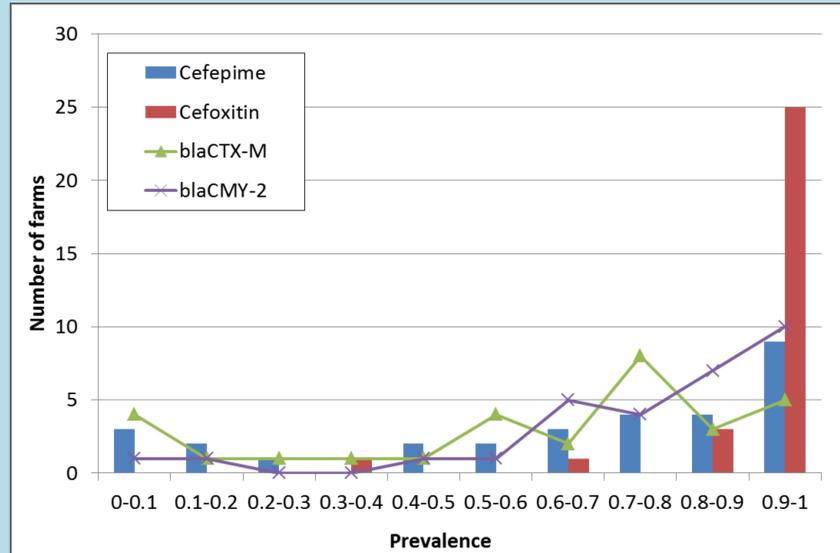


Fig. 1. Frequency of farms according to prevalence of resistance phenotypes and associated genes.

Table 1. Prevalence of *E. coli* bearing *bla*_{CTX-M} or *bla*_{CMY-2} genes on 30 farms.

Factor		N ^d	<i>bla</i> _{CTX-M} ^a	<i>p</i> ^b	<i>bla</i> _{CMY-2} ^c	<i>p</i> ^b
Herd size	< 1,000	11	0.63		0.73	
	≥ 1,000	19	0.73	0.11	0.87	0.04
Region of Washington	Eastern	20	0.75		0.85	
	Western	10	0.42	0.02	0.77	0.45
Ceftiofur used for calf diseases	Yes	16	0.74		0.86	
	No	14	0.67	0.41	0.77	0.07
Animal movements	Any	18	0.72		0.85	
	None	12	0.48	0.16	0.76	0.12
Frequency of adding bedding	Weekly or more often	15	0.63		0.87	
	Less often than weekly	15	0.73	.09	0.76	0.04
Medicated milk replacer	Yes	5	.63		.63	
	No	25	.71	.33	.85	.01
Employees that work at another dairy	Any	5	.71		.96	
	None	25	.67	.43	.80	.03
Number of people on farm per day	< 10	12	0.57		0.70	
	≥ 10	17	0.71	0.12	0.87	0.05

^aMedian prevalence of *bla*_{CTX-M}-positive *E. coli*. ^bWilcoxon test one-sided P-value. ^cMedian prevalence of *bla*_{CMY-2}-positive *E. coli*. ^dNumber of farms.

RESULTS

PCR detection of both β-lactamase genes correlated closely to their resistance phenotype (Fig. 1). The prevalence of CTX-M EC was high and widely distributed among 30 farms but with a lower prevalence in western Washington farms, while *bla*_{CMY-2} *E. coli* (CMY-2 EC) were highly prevalent at 83.3% (25/30) of the farms and was more equally distributed between the 2 regions (Fig. 1, Table 1). The prevalence of CTX-M EC was associated with herd size, animal movements, calf hutch bedding management, and the number of people present on the farm each day, but with only moderate levels of significance which may be due to inadequate sample size. The median prevalence of CMY-2 EC was significantly higher on farms with employees that worked at another dairy but that association was not found for CTX-M EC. Surprisingly, the median prevalence of CMY-2 EC was higher on farms that did not feed medicated milk replacer to calves (Table 1). The results of Spearman's rank order correlations indicated that the prevalence of CTX-M EC was negatively associated with total number of visitors to the farm (veterinarian, nutritionist, hoof trimmer, feed truck) but the prevalence of CMY-2 EC was not. The prevalence of CTX-M EC and CMY-2 EC were not correlated with each other (data not shown).

CONCLUSIONS

To test further the potential associations with human and animal movements among farms, a larger number of herds are needed. The regional difference suggests either a geographic barrier or a difference in management factors between the two regions of Washington State. While average herd size is lower among western Washington herds, the association with herd size was stronger for CMY-2 EC than for CTX-M EC. The lack of correlation between these two *E. coli* populations suggests that different factors influence their success. Characterization of the *bla*_{CTX-M}-associated plasmids as well as chromosomal backbone DNA of the CTX-M EC is underway and will further inform the epidemiology of the rapid dissemination and/or emergence of CTX-M EC.

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