Antimicrobial Resistance Report: *Animal Health*

This report presents antimicrobial resistance (AMR) data from diagnostic submissions to the British Columbia (BC) Ministry of Agriculture's Animal Health Centre (AHC) between January 1, 2007 and December 31, 2015, with a specific focus on bacteria with clinical relevance in food-producing animal species. Inclusion of specific animal-production sectors was based on the size of the sector in BC and the AHC case submission volume for the sector. Inclusion of specific bacteria-antimicrobial combinations was restricted by laboratory procedures, in that for each animal-production type, AHC tests for resistance to a limited number of licensed and clinically relevant antimicrobials. Final selection of bacteria and antimicrobials for inclusion in this report was guided by consultation with veterinarians in clinical practice in each animal-production sector. This report summarizes AHC data for florfenicol, oxytetracycline and sulfa-trimethoprim resistance in *Aeromonas salmonicida (A. salmonicida)*, *Yersinia ruckeri (Y. ruckeri)* and *Vibrio anguillarium (V. anguillarium)* isolated from Atlantic Salmon; multiple antibiotic resistance in *Streptococcus dysgalactiae (Strep. dysgalactiae)*, *Streptococcus uberis (Strep. uberis)*, *Staphylococcus aureus (S. aureus)*, coagulase-negative staphylococci and *Escherichia coli* (*E. coli*) isolated from bovine milk samples; and penicillin resistance in *Staphylococcal* species (spp.) isolated from meat-type chickens.

All data was generated from bacterial isolates from passively-acquired submissions to the AHC. Classification of bacterial isolates as resistant or susceptible to antimicrobials was made in accordance with Clinical Laboratory Standards Institute (CLSI) protocols using Kirby-Bauer disk diffusion (CLSI, 2012). Isolate sources included animals submitted for post mortem examination, fecal samples, environmental samples from animal-rearing facilities, and swabs collected by veterinarians in clinical practice. Some important data limitations include that samples are likely to originate from individuals that are sick or unthrifty, and for domestic animals, might be more likely to have received antimicrobial treatment; and that AHC submission numbers are comparatively small relative to total number of animals in BC. As such, inferences about antimicrobial resistance in the source population of animals in BC must be made cautiously and apparent trends will require further verification.

Atlantic salmon

Background

Atlantic salmon are a major aquaculture species in BC. Consultation with aquaculture veterinarians indicated that *A. salmonicida*, *Y. ruckeri* and *V. anguillarium* were bacteria for which antimicrobial sensitivity data would be of interest due to the importance of these pathogens to fish health in BC. The limited selection of approved antimicrobials for use in aquaculture in BC, and reported AMR in aquaculture elsewhere further supported this report's inclusion of AMR of Atlantic salmon pathogens. *Y. ruckeri* is the causative agent of enteric redmouth disease (yersiniosis), a disease with acute and chronic impacts that can lead to significant morbidity and economic losses. *A. salmonicida* is the causative agent of salmon furunculosis, a disease that can cause a variety of symptoms including acute septicemia and chronic poor growth and ulcerative skin disease. *V. anguillarium* is the causative agent of vibriosis, a disease that causes haemorrhagic septicemia in salmon.

There are four antimicrobials licensed for use in aquaculture species in Canada. The AHC Fish panel tests isolates for sensitivity to three of these (florfenicol, oxytetracycline and trimethoprim-sulfadiazine). The fourth licensed antimicrobial, sulfadimethoxine and ormetroprim (trade name Romet 30), is in the sulfonamide group along with trimethoprim sulfa, and is reported by veterinarians to be infrequently used in Atlantic salmon in BC.

In this section of the report, results are presented as tables showing resistance in *A. salmonicida* and *Y. ruckeri* isolates from Atlantic salmon to florfenicol, oxytetracycline and sulfa-trimethoprim. For *A. salmonicida*, this is followed by a graph of proportion of resistant isolates (with 95% confidence intervals) by year. For *Y. ruckeri*, sample sizes were too low to support graphical presentation of data.

Results

V. anguillarium was not isolated from any Atlantic salmon submissions to the AHC between January 1, 2007 and December 31, 2015. There were 37 isolates of *Y. ruckeri* from 25 separate cases, and 130 isolates of *A. salmonicida* from 56 separate cases. There were no submissions that yielded isolates of both *A. salmonicida* and *Y. ruckeri.* Information about the type of tissue or specimen that yielded the bacterial isolates was not available for Atlantic salmon.

Thirty-six of 37 isolates of *Y. ruckeri* were susceptible to all three antimicrobials tested, while one was resistant to oxytetracycline.

Seventy percent (91/130) isolates of *A. salmonicida* were susceptible to the three antimicrobials tested, while 25 percent (32/130) were resistant to all three. Four (3%) were resistant to oxytetracycline and sulfa-trimethoprim, but susceptible to florfenicol. Three (2%) were resistant only to sulfa-trimethoprim. Eighteen cases yielded more than one isolate of *A. salmonicida* (median 4 isolates, range 2-16 isolates/case). Of these, six (33%) cases had differing resistance patterns between isolates.

Antimicrobial resistance in *Yersinia ruckeri* isolates from Atlantic salmon

Table 1: Resistance to florfenicol, oxytetracycline and sulfa-trimethoprim in *Yersinia ruckeri* isolated from Atlantic salmon submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 3 antibiotics	Proportion resistant to:		
			Florfenicol	Oxytetracycline	Sulfa-trimethoprim
All years	37	.97	0	.03	0
2007	3	1.0	0	0	0
2008	5	1.0	0	0	0
2009	3	.66	0	.33	0
2010	2	1.0	0	0	0
2011	4	1.0	0	0	0
2012	3	1.0	0	0	0
2013	0	-	-	-	-
2014	7	1.0	0	0	0
2015	10	1.0	0	0	0

Antimicrobial resistance in *Aeromonas salmonicida* isolates from Atlantic salmon

Table 2: Resistance to florfenicol, oxytetracycline and sulfa-trimethoprim in *Aeromonas salmonicida* isolated from Atlantic salmon submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 3 antibiotics	Proportion resistant to:		
			Florfenicol	Oxytetracycline	Sulfa-trimethoprim
All years	130	.70	.25	.28	.30
2007	2	.50	0	0	.50
2008	0	-	-	-	-
2009	11	1.0	0	0	.19
2010	32	.81	.19	.19	0
2011	13	1.0	0	0	.53
2012	34	.47	.41	.53	.39
2013	31	.61	.39	.39	.50
2014	4	.50	0	0	0
2015	3	1.0	0	0	.30

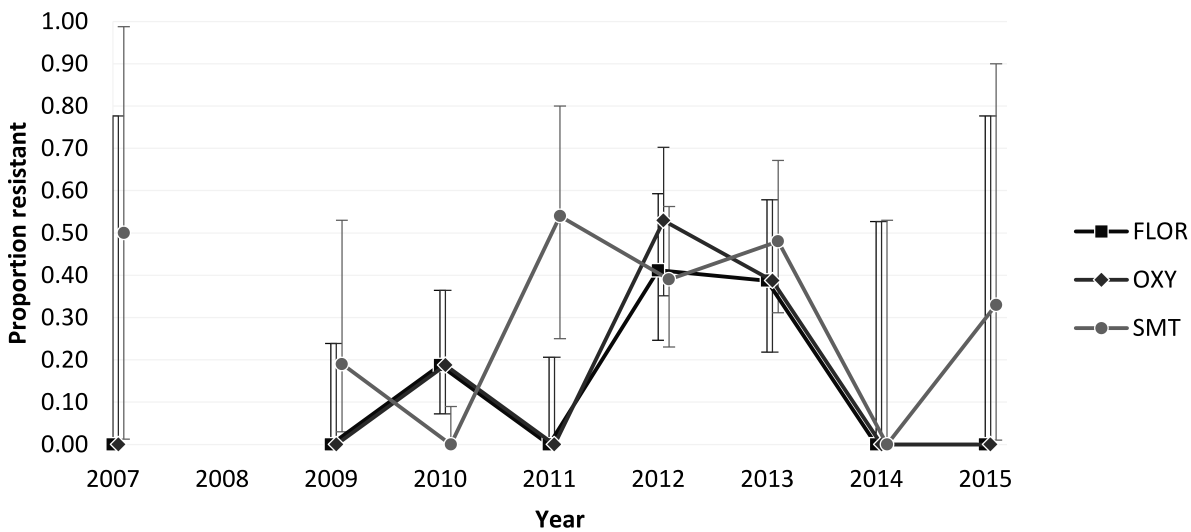

Figure 1: Proportion of *A. salmonicida* isolated from Atlantic salmon submissions to the Animal Health Centre resistant to florfenicol (FLOR), oxytetracycline (OXY) and sulfa-trimethoprim (SMT) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method**.**

Commentary

Antimicrobial resistance in *Y. ruckeri* was very uncommon, with only one isolate showing resistance to one antimicrobial. For *A. salmonicida,* there were no trends of increasing or decreasing resistance to any antimicrobial over time, however isolates that showed resistance frequently appeared to be resistant to multiple antibiotics.

Bovine milk

Background

Dairy cattle are an important food-producing livestock sector in BC, and mastitis is an important health concern facing dairy producers. The AHC performs milk-cultures to help veterinarians optimize mastitis therapy. AHC reports on antimicrobial resistance of bacterial isolates from milk for each six month period in the Animal Health Monitor (http://www.agf.gov.bc.ca/ahc/AHMonitor.pdf).

For this report, data was analyzed over a multi-year period. Consultations with dairy veterinarians indicated that *Strep. dysgalactiae*, *Strep. uberis*, *S. aureus*, coagulase-negative staphylococci and *E. coli* were bacteria for which antimicrobial sensitivity data from milk cultures would be of highest clinical interest. Veterinarians indicated that ceftiofur, cloxacillin, penicillin G, cephapirin and pirlimycin were likely to be used as treatments for gram-positive bacteria. Four of these (ceftiofur, cloxacillin, penicillin G and pirlimycin) are included in the AHC milk antimicrobial panel. Veterinarians indicated that ceftiofur, sulfa-trimethoprim and tetracycline are likely to be used as treatments for gram-negative bacteria. All of these are included on the AHC Milk AMR panel. Therefore, sensitivity of selected gram-positive organisms to ceftiofur, cloxacillin, penicillin and pirlimycin and of *E. coli* to ceftiofur sulfa-trimethoprim and tetracycline are reported.

In this section of the report, results are presented as tables showing resistance from milk submissions for *Strep. dysgalactiae*, *Strep. uberis*, *S. aureus*, and coagulase-negative staphylococci isolates to ceftiofur, cloxacillin, penicillin and pirlimycin; and for *E. coli* to ceftiofur, tetracycline and sulfa-trimethoprim. This is followed by a graphs of proportions of resistant isolates (with 95% confidence intervals) by year.

Results

Of the five bacterial types included in this report, coagulase-negative staphylococci were the most commonly isolated from milk samples, followed by *S. aureus* and *E.coli*, then *Strep. dysgalactiae* and *Strep. uberis* (Table 3).

Table 3: Summary of resistance to ceftiofur (CEF), cloxacillin (CLOX), penicillin (PEN) and pirlimycin (PIR) in selected gram-positive bacterial species isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Bacteria	Total Cases / Isolates	Mean cases / year (min.-max.)	Susceptible to all[1]	Proportion resistant to:				
				CEF	CLOX	PEN	PIR	β lact + PIR[2]
Strep. dysgalactiae	111 / 137	14 (7-24)	.77	.01	.03	0	.22	.03
Strep. uberis	84 / 104	10 (3-17)	.24	.04	.68	.04	.40	.34
S. aureus	182 / 235	25 (16-35)	.72	0	.01	.22	.07	.01
Coag-neg. staphylococci[3]	322 / 506	52 (18-89)	.58	.02	.13	.23	.23	.11

[1] proportion susceptible to all antimicrobials tested (ceftiofur, cloxacillin, penicillin and pirlimycin)
[2] resistant to one or more β lactams and resistant to pirlimycin
[3] coagulase-negative staphylococci

Table 4: Summary of resistance to ceftiofur (CEF), sulfa-trimethoprim (SMT) and tetracycline (TET) in *E. coli* isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Bacteria	Total Cases / Isolates	Mean cases / year (min.-max.)	Susceptible to all[1]	Proportion resistant to: CEF	SMT	TET	All 3 antimicrobials
E. coli	194/ 244	25 (11-56)	.65	.08	.10	.30	.02

[1] proportion susceptible to all antimicrobials tested

Antimicrobial resistance in *Streptococcus dysgalactiae* isolates from milk

Table 5: Resistance to ceftiofur, cloxacillin, penicillin and pirlimycin in *Streptococcus dysgalactiae* isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 4 antibiotics	Proportion resistant to: Ceftiofur	Cloxacillin	Penicillin	Pirlimycin
All years	137	.77	.01	.03	0	.22
2007	11	.64	.09	0	0	.27
2008	16	.56	0	.13	0	.44
2009	8	.38	0	.25	0	.63
2010	10	.90	0	0	0	.10
2011	7	.71	0	0	0	.29
2012	11	1.0	0	0	0	0
2013	24	.92	0	0	0	.08
2014	19	.79	0	0	0	.21
2015	31	.79	0	0	0	.23

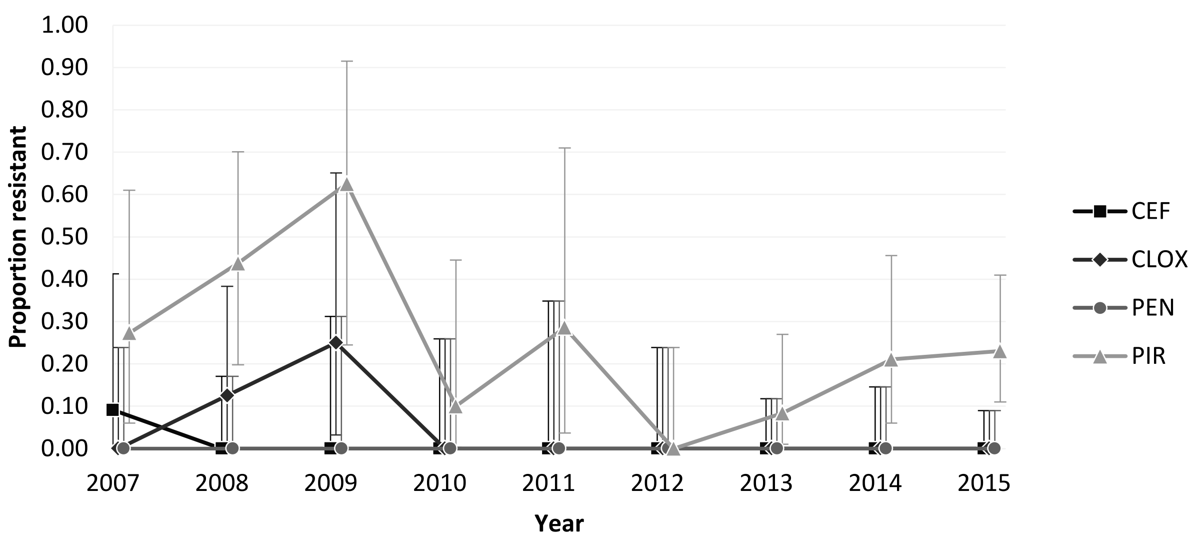

Figure 2: Proportion of *Strep. dysgalactiae* isolates from milk submissions to the Animal Health Centre resistant to ceftiofur (CEF), cloxacillin (CLOX), penicillin (PEN) and pirlimycin (PIR) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method.

Antimicrobial resistance in *Streptococcus uberis* isolates from milk

Table 6: Resistance to ceftiofur, cloxacillin, penicillin and pirlimycin in *Streptococcus uberis* isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 4 antibiotics	Proportion resistant to: Ceftiofur	Cloxacillin	Penicillin	Pirlimycin
All years	104	.24	.03	.68	.03	.39
2007	13	.33	0	.42	.08	.33
2008	9	.33	.11	.44	.11	.56
2009	14	.14	.07	.86	0	.50
2010	6	.50	0	.50	.17	.17
2011	8	.25	0	.75	0	.50
2012	3	1	0	1.0	0	.67
2013	14	.13	0	.80	0	.20
2014	17	.18	0	.75	0	.29
2015	20	.38	.10	.70	.10	.55

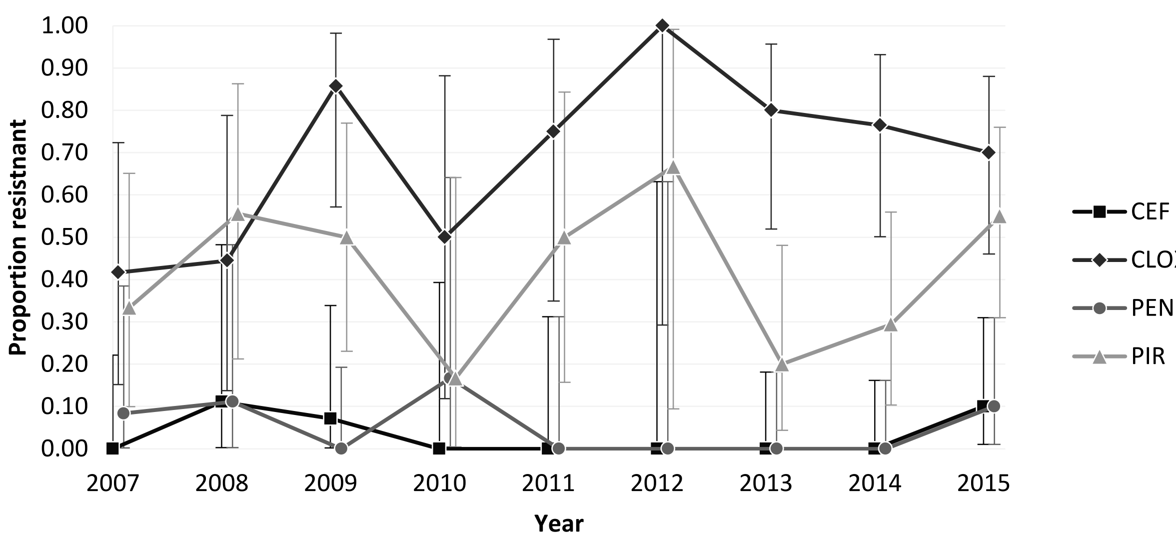

Figure 3: Proportion of *Strep. uberis* isolates from milk submissions to the Animal Health Centre resistant to ceftiofur (CEF), cloxacillin (CLOX), penicillin (PEN) and pirlimycin (PIR) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method.

Antimicrobial resistance in *Staphylococcus aureus* isolates from milk

Table 7: Resistance to ceftiofur, cloxacillin, penicillin and pirlimycin in *Staphylococcus aureus* isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 4 antibiotics	Proportion resistant to: Ceftiofur	Cloxacillin	Penicillin	Pirlimycin
All years	235	.70	0.0	.01	.24	.07
2007	32	.31	0.0	0	.66	.03
2008	18	.56	0.0	.06	.28	.17
2009	21	.76	0.0	0	.24	0
2010	21	.81	0.0	0	.10	.10
2011	28	.79	0.0	.04	.18	.04
2012	26	.62	0.0	0	.31	.08
2013	35	.80	0.0	.03	.14	.09
2014	28	.93	0.0	0	.04	.04
2015	26	.81	0.0	0	.08	.04

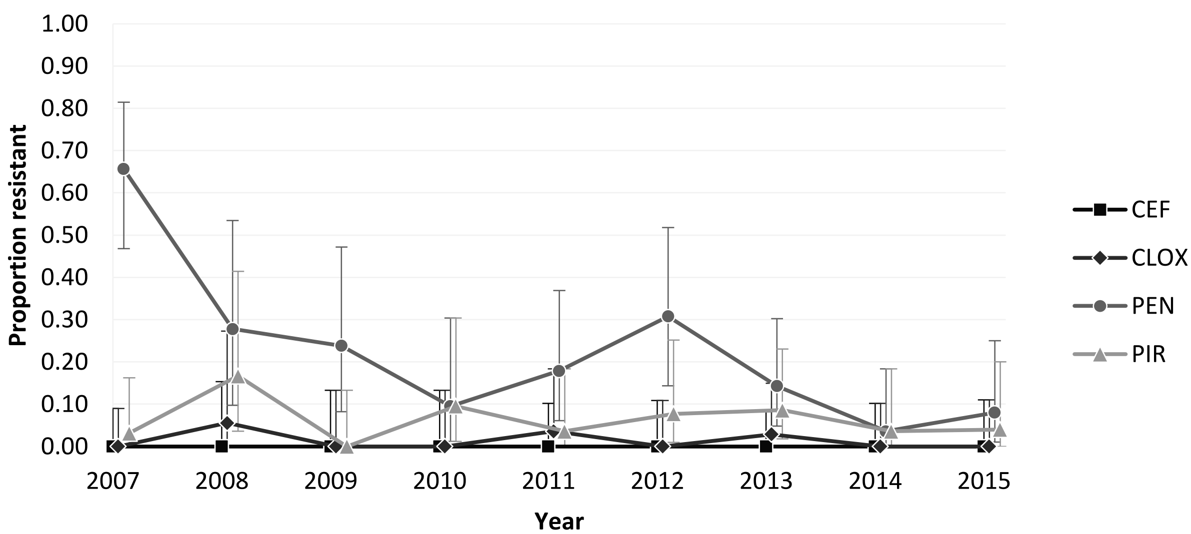

Figure 4: Proportion of *S. aureus* isolates from milk submissions to the Animal Health Centre resistant to ceftiofur (CEF), cloxacillin (CLOX), penicillin (PEN) and pirlimycin (PIR) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method.

Antimicrobial resistance in coagulase-negative staphylococci isolates from milk

Table 8: Resistance to ceftiofur, cloxacillin, penicillin and pirlimycin in coagulase-negative staphylococci isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 4 antibiotics	Proportion resistant to: Ceftiofur	Cloxacillin	Penicillin	Pirlimycin
All years	506	.58	.02	.13	.23	.23
2007	41	.41	0	.02	.39	.27
2008	42	.55	.02	.07	.26	.29
2009	31	.48	.03	.13	.23	.29
2010	18	.39	0	.33	.28	.22
2011	81	.62	0	.10	.23	.16
2012	54	.59	.02	.13	.19	.26
2013	69	.62	.07	.22	.22	.23
2014	89	.61	.02	.18	.17	.22
2015	81	.67	.02	.11	.21	.26

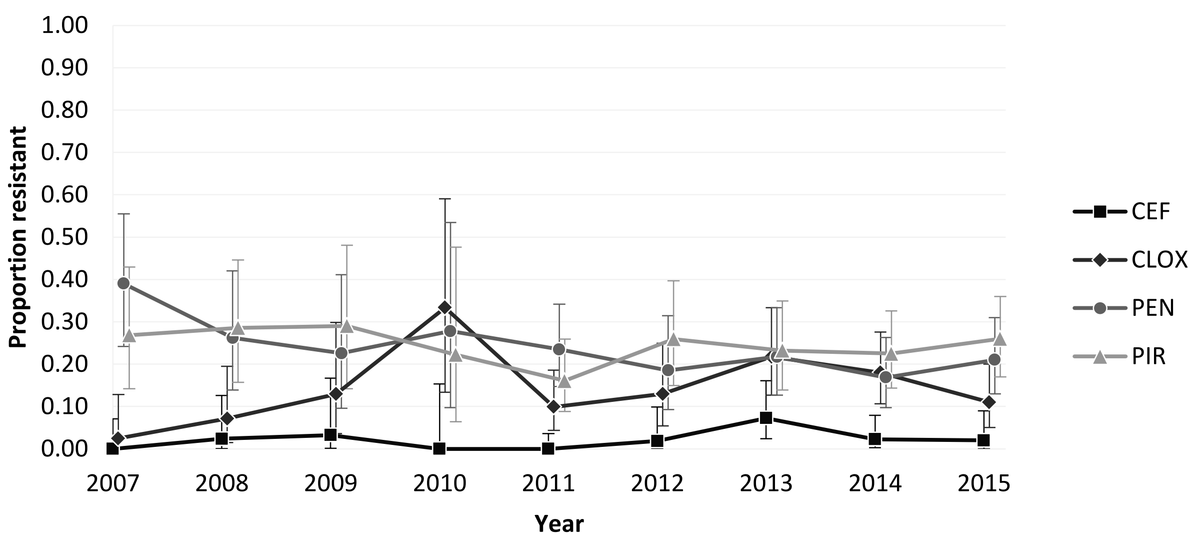

Figure 5: Proportion of coagulase-negative staphylococci isolates from milk submissions to the Animal Health Centre resistant to ceftiofur (CEF), cloxacillin (CLOX), penicillin (PEN) and pirlimycin (PIR) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method.

Antimicrobial resistance in *E. coli* isolates from milk

Table 9: Resistance to ceftiofur, sulfa-trimethoprim and tetracycline in *E. coli* isolated from bovine milk submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion susceptible to all 3 antibiotics	Proportion resistant to: Ceftiofur	Sulfa-trimethoprim	Tetracycline
All years	244	.68	.08	.10	.27
2007	11	.82	0	.09	.18
2008	16	.47	.11	.13	.53
2009	19	.63	.13	.11	.37
2010	17	.63	.10	.06	.31
2011	20	.70	.04	.10	.20
2012	25	.71	.11	.17	.29
2013	45	.70	.11	.09	.20
2014	56	.73	.05	.11	.20
2015	35	.46	.09	.11	.49

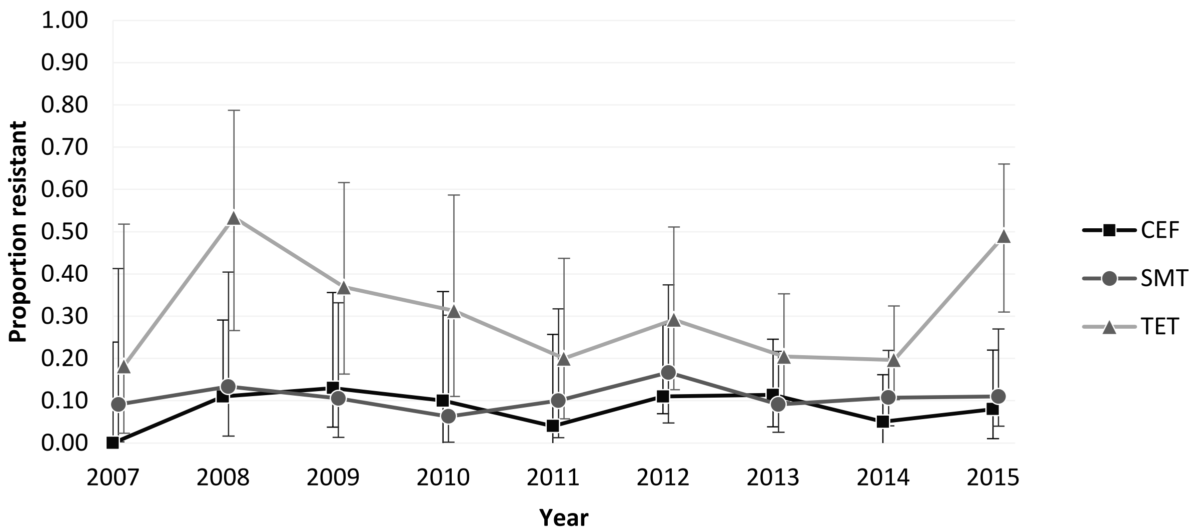

Figure 6: Proportion of *E. coli* isolates from milk submissions to the Animal Health Centre resistant to ceftiofur (CEF), sulfa-trimethoprim (SMT) and tetracycline (TET) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method.

Commentary

There were no trends of increasing or decreasing resistance to any antimicrobial over time for any of the five bacterial types isolated from milk that are included in this report. In general, *Strep. dysgalactiae* showed the highest levels of susceptibility to all antimicrobials, while *Strep. uberis* showed the highest levels of resistance.

Penicillin resistance in *Staphylococcal* species isolates from meat-type poultry

Background

The broiler-chicken sector includes two main farm-types; hatching egg (broiler breeder) and broiler. Broiler breeder farms house adult birds in breeding flocks that produce fertile eggs. After hatching, chicks are distributed to broiler farms where they are reared for sale to market. Consultation with poultry veterinarians indicated concern that resistance of *Staphylococcal* spp. to penicillin might be increasing in the broiler-chicken sector, and as such are included in this report.

Results

Nine hundred and three *Staphylococcal* spp. (including *S. aureus*) isolates from 836 meat-type poultry submissions (553 broilers and 350 broiler breeders) were tested for penicillin resistance between January 1, 2007 and December 31, 2015. Of these, 763 were from animal tissue samples, 3 were from environmental swabs and 137 were from swabs submitted by veterinarians from clinical cases. There was a mean of 100 isolates per year with a minimum of 33 (2007) and a maximum of 145 (2015) isolates per year.

Table 10: Resistance to penicillin in *Staphylococcal* spp. isolated from meat-type chicken submissions to the AHC between January 1, 2007 and December 31, 2015.

Year	Number of isolates	Proportion resistant to penicillin
All years	903	.16
2007	33	.18
2008	62	.27
2009	97	.22
2010	100	.18
2011	95	.13
2012	114	.08
2013	135	.19
2014	122	.16
2015	145	.21

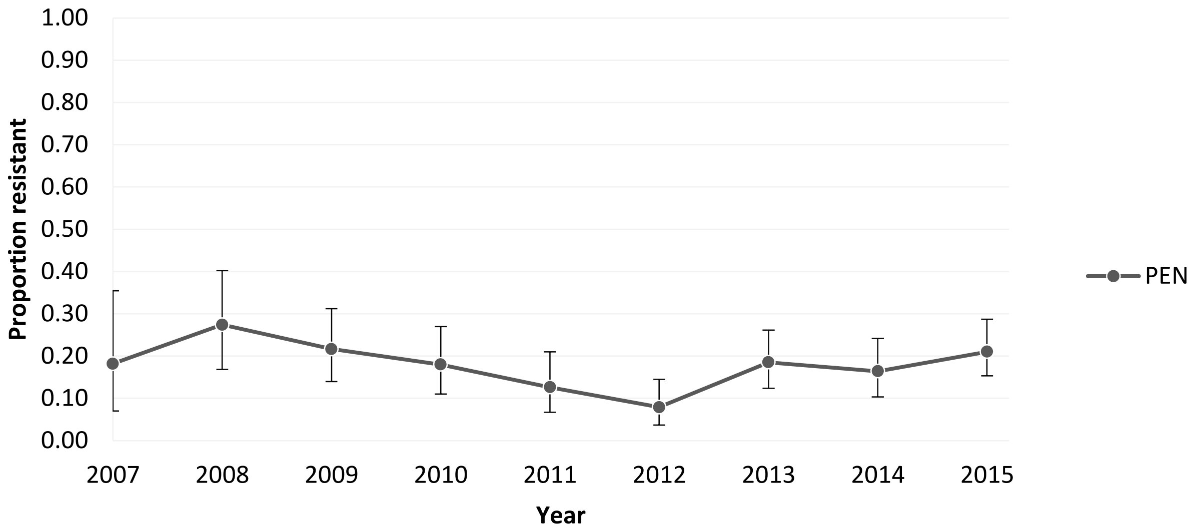

Figure 7: Proportion of *Staphylococcal* spp. isolates from meat-type chickens submissions to the Animal Health Centre resistant to penicillin (PEN) by year. Error bars represent 95% confidence intervals for the proportion calculated by the Clopper-Pearson exact binomial method.

Commentary

There were no trends of increasing or decreasing resistance to any antimicrobial in to penicillin in *Staphylococcal* spp. isolates from meat type poultry over time.

Reference

CLSI. (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard 11th Edition (pp. 1-58). Wayne, PA: Clinical Laboratory Standards Institute.

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