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Rakendusuuringu teema: Mikroobide resistentsuse ohjamise ja vähendamise võimalused

Antimicrobial resistance of *Escherichia coli, Enterococcus* spp, *Campylobacter* spp and *Salmonella* spp originated from animal and food in 2010-2018 in Estonia

Raport

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Introduction

Several documents and guidelines have been issued by the European Commission and its institutions confirming the importance of continuous monitoring of AMR in member states and taking appropriate measures to minimise the risk for the development of resistant microbes both in human and veterinary medicine. In June 2017, the European Commission adopted the EU One Health Action Plan against AMR to summarise the ongoing and forthcoming plans and activities regarding AMR (European Commission, 2017). According to the Directive 2003/99/EC EU and Commission decision 2013/652/EL, member states are obliged to monitor and report data on the resistance of *Salmonella* spp, *Campylobacter (C.) jejuni*, *C. coli*, indicator commensal *E. coli*, *E. faecalis* and *E. faecium* isolates from animals and animal products. There is an AMR monitoring programme of the abovementioned bacteria in place in Estonia.

Additionally, national resistance monitoring programmes are in place in many countries. In Estonia, there is no annual AMR monitoring programme regarding other bacteria, which are not covered by EU legislation, e.g., bacteria from diseased animals and commensal bacteria from pet animals. However, monitoring of phenotypic antimicrobial resistance of bacteria originating from animal and food

has been performed by the collaboration of Estonian University of Life Sciences and Estonian Veterinary and Food laboratory in 2006 and the project has been financed by the Estonian Ministry of Rural Affair in years 2006-2017.

The main objective of this report is to give an overview about resistance studies in veterinary medicine in Estonia during 2010-2017.

Antimicrobial resistance of *Escherichia coli* isolated from healthy animals and clinical submissions during 2010-2015

Antimicrobial susceptibility of *E. coli* was tested by using broth microdilution methods. Commercial microdilution plates (VetMIC®) developed by the Swedish Veterinary Institute were used in 2010-2015. From 2017, Sensititre (TREK Diagnostic System Ltd.) broth microdilution system (EUVSEC) was available for antimicrobial susceptibility testing. For interpretation of results for susceptibility testing, epidemiological cut-off values (ECOFF) issued by the EUCAST (http:// www.escmid.org) are used. A multiple logistic regression mixed model with random herd effect to control for clustering was used to study association between resistance and animal species.

Study done by Aasmäe *et al*. 2019 pointed out, that the resistance of *E. coli* originated from faecal samples from clinically healthy swine (n= 120) compared to cattle (n= 171) was significantly higher to ampicillin (OR = 6.5; 95% CI 2.70-15.56; $p < 0.001$), streptomycin (OR = 8.5, 95% CI 4.27-17.03; $p < 0.001$), ciprofloxacin (OR = 10.5; 95% CI 1.27-86.76; $p = 0.029$), tetracycline (OR = 6.4; 95% CI 3.16-12.89; $p < 0.001$) colistin (OR = 5.5; 95% CI 1.7-17.3; $p = 0.004$), sulfamethoxazole (OR = 8.7; 95% CI 3.87-19.70; $p < 0.001$) and trimethoprim (OR = 8.4; 95% CI 3.33-21.04; $p < 0.001$). The resistance against gentamycin was significantly lower (OR = 0.17; 95% CI 0.06-0.47; $p < 0.001$) in swine *E. coli* isolates compared to cattle isolates (Table 2).

Table 2. Resistance of *Escherichia coli* isolates originating from faecal samples of healthy cattle and swine and clinical submissions collected from 2010 to 2015 in Estonia (Aasmäe et al., 2019).

Antimicrobial	**Breakpoints for resistance (mg/l)[1]**	**Healthy animals**				**Diagnostic submissions**			
		Dairy cattle (n = 171)		**Swine (n = 120)**		**Dairy cattle (n = 63)**		**Swine (n = 143)**	
		%	**(95% CI)**	**%**	**(95% CI)**	**%**	**95% CI**	**%**	**95%**
Ampicillin *H	>8	**3.5**	**(0.8-6.3)**	**21.5**	**(14.3-29.1)**	58.7	(46.5-70.9)	53.9	(45.7-62.1)
Cephotaxime	>0.5	1.2	(-0.4-2.8)	2.5	(-0.3-5.3)	7.9	(1.2-14.6)	4.2	(0.9-7.5)
Cephazidime	>0.5	2.9	(0.4-5.4)	3.3	(0.1-6.5)	7.9	(1.2-14.6)	7.7	(3.3-12.1)
Streptomycin *H	>16	**7.0**	**(3.2-10.8)**	**39.2**	**(30.5-40.8)**	63.5	(51.6-6.4)	54.6	(46.4-62.8)
Gentamycin *D	>4	7.0	(3.2-10.8)	12.5	(6.6-18.4)	**20.6**	**(10.6-30.6)**	**5.6**	**(1.8-9.4)**
Kanamycin	>16	8.8	(4.6-13.1)	10.0	(4.6-15.4)	0.0	NA[2]	0.0	NA[2]
Ciprofloxacin *H	>0.06	**0.6**	**(-0.6-1.8)**	**5.8**	**(1.6-10.0)**	38.1	(26.1-50.1)	32.2	(24.5-39.9)
Nalidixic acid *D	>16	0.6	(-0.6-1.8)	3.3	(0.1-6.5)	**17.5**	**(8.1-26.9)**	**32.2**	**(24.5-39.9)**
Tetracycline *H	>8	**7.0**	**(3.2-10.8)**	**32.5**	**(24.1-40.9)**	58.5	(46.3-70.7)	60.2	(52.2-68.3)
Colistin *H	>2	**2.4**	**(0.1-4.7)**	**11.6**	**(5.9-17.3)**	3.2	(-1.6-7.6)	5.6	(1.8-9.4)
Chloramphenicol	>16	2.4	(0.1-4.7)	5.8	(1.6-10.0)	9.5	(2.3-16.7)	18.2	(11.9-24.5)
Florfenicol	>16	0.0	NA[2]	0.8	(-0.8-2.4)	0.0	NA[2]	0.7	(-0.7-2.1)
Trimethoprim *H	>2	**3.5**	**(0.8-6.3)**	**22.4**	**(14.9-29.9)**	55.6	(43.3-67.9)	53.9	(45.7-62.1)
Sulfamethoxazole *	>64	**4.7**	**(1.5-7.9)**	**30.0**	**(21.8-38.2)**	60.3	(48.2-70.4)	68.5	(60.1-76.1)

[1] SWEDRES/SVARM 2015. Solna/Uppsala ISSN 1650-6332, 117, Table 2.17. *H and *D Statistically significant difference ($p < 0.05$) between healthy dairy cattle and swine, and between dairy cattle's and swine's clinical submissions. Corresponding percentages are also presented in bold face.

*

The proportion of multidrug resistant (simultaneous resistance more than three antimicrobial, MDR) isolates from clinical submission was very high both in cattle (n = 42; 66.7%) and swine (n = 93; 65.0%), without statistical differences. The *E. coli* isolates from clinically healthy swine (n = 35; 29.2%) showed significantly higher multidrug resistance (OR = 11.2; 95% CI 4.23-29.22; $p < 0.001$) than the isolates from cattle (n = 6; 3.5%).

The same study done by Aasmäe *et al*. (2019) found ESBL phenotypes in one *E. coli* isolate from clinically healthy cattle and in eight isolates from organ materials both from cattle and swine. The same genotype- $bla_{\text{TEM-52C}}$, was found in three *E. coli* strains. All these strains originated from clinical submission.

In total, four strains representing AmpC phenotypes were found. One plasmid-encoded AmpC type β-lactamases producing *E. coli* from clinically healthy cattle was found to harbour the $bla_{\text{CMY-1}}$ gene, and another from clinically healthy swine carried the $bla_{\text{CMY-2}}$ gene.

Antimicrobial resistance of *Escherichia coli* isolated from healthy animal 2017-2019

In 2017, 68 caecal samples from healthy pigs were collected at slaughterhouses and in total, 91 *E. coli* isolates were included in the resistance study. In total, 42 (46.1%) isolates were fully susceptible and either ESBL or AmpC phenotypes were found in 24 (26.3%) isolates.

In 2018, 85 caecal samples from poultry were collected and 154 *E. coli* isolates were included in the resistance study. In total, 8 (5.2%) of isolates were fully susceptible and 69 (44.8%) isolates were ESBL and/or AmpC positive. There was a higher risk to find resistant isolates in poultry compare to swine (OR= 16,1; $p < 0.001$)

Overall, the highest resistance levels were determined for ampicillin, tetracycline, sulfamethoxazole, and trimethoprim (Table 3). These drug classes are also the most frequently used in veterinary medicine in Estonia. ESBL negative *E. coli* isolates (n = 67) from pig's were most commonly resistant to ampicillin (14.9%), tetracycline (17.9%) and sulfa/trimethoprim (15%). One isolate was resistant to ciprofloxacin/nalidixic acid. In previous years, the resistance to the abovementioned antibiotics was comparable.

Very high resistance proportion for ciprofloxacin (87%) and nalidixc acid (78.8%) was found in ESBL negative *E. coli* isolates from poultry caecal samples (Table 3).

Table 3. Phenotypic resistance of ESBL and/or AmpC negative *E. coli* originated from swine and poultry caecal samples in 2017-2018

Antimicrobial	**Breakpoints for resistance (mg/l)***	**Swine n= 67**		**Poultry n= 85**	
		%	95% CI	%	95% CI
Ampicillin	>8	17.9	8.7-21.3	71.8	62.2-81.3
Azithromycin	>16	0	NA	3.5	-0.3-7.4
Cefotaxime	>0.25	0	NA	0	NA
Ceftazidime	>0,5	0	NA	0	NA
Ciprofloxacin	>0.06	1.5	-1.4-4.4	87.0	74.9-91.4
Nalidixic acid	>16	1.5	-1.4-4.4	78.8	70.1-87.5
Chloramphenicol	>16	4.5	-0.4-9.3	14.1	7.1-22.4
Tetracyclin	>8	19.4	9.9-28.7	20.0	11.5-17.6
Tigecycline	>1	0	NA	0	NA
Meropenem	>0.125	0	NA	0	NA
Gentamycin	>2	0	NA	0	NA
Colistin	>2	0	NA	0	NA
Sulfomethoxazole	>64	14.9	6.3-24.4	32.9	22.9-42.9
Trimetoprim	>2	16.4	7.5-25.3	44.7	34.1-55.2

The MDR was found in eight (11.9%) of isolates. The most common MDR was developed against ampicillin, tetracycline, and trimethoprim.

Among the ESBL and/ or AmpC positive isolates, the ciprofloxacin resistance was 16.7%, chloramphenicol 20.8% and trimethoprim 45.4% (Table 4). The MDR was found in five ESBL and/or AmpC positive isolates, where the most common simultaneous resistance has occurred against chloramphenicol, tetracycline, and trimethoprim. No carbapenemase resistance was found. Overall, quinolone resistance among ESBL/AmpC negative and ESBL/AmpC positive *E. coli* was significantly higher in poultry isolates compare to swine isolates. Out of 136 ESBL/AmpC producing *E. coli* isolates, the majority (70.5%) belonged to the ESBL group. As ESBL or AmpC gene types were not analysed, the possible way of transmission not clear (Table 5).

Table 4. Antibiotic resistance of ESBL, AmpC and carbapenem resistant *E. coli* isolated from caecal samples and meat in swine and poultry in 2017-2018.

Antimicrobial	**Breakpoints for resistance (mg/l)***	**Swine n = 24**		**Poultry n = 69**	
		%	95%CI	%	95%CI
Ampicillin	>8	100	100	100	100
Azithromycin	>16	0	NA	0	NA
Cefotaxime	>0.25	100	100	100	100
Ceftazidime	>0.5	100	100	100	100
Ciprofloxacin	>0.06	16.7	1.8-31.6	31.2	20.8-42.8
Nalidixic acid	>16	12.5	-0.7-25.6	26.1	15.7-36.4
Chloramphenicol	>16	20.8	4.5-37.1	0	NA
Tetracyclin	>8	25.0	7.6-42.1	20.3	10.8-26.7
Tigecycline	>1	0	NA	0	NA
Meropenem	>0.125	0	NA	0	NA
Gentamycin	>2	0	NA	11.5	5.9-21.2
Colistin	>2	0	NA	0	NA
Sulfomethoxazole	>64	29.2	14.9-41.2	18.8	11.3-21.6
Trimetoprim	>2	45.8	25.9-61.7	2.9	-0.1-6.8

Table 5. Number of ESBL, AmpC and/or carbapenem resistant isolates originated from faecal samples and meat in 2018.

Origin of samples	**ESBL**	**AmpC**	**ESBL+ AmpC**	**Carbapenem resistance**
Faecal samples from swine (n = 24)	20	4	0	0
Faecal samples from poultry (n = 69)	53	15	1	0
Pork (n = 3)	2	1	0	0
Poultry meat (n = 38)	21	17	0	2
Total (n = 136)	96	37	1	2

Resistance of *Enterococcus faecalis* and *Enterococcus faecium* in production animals

Enterococci (*E. faecalis*, *E. faecium*) are commensal bacteria in the intestines of humans and domestic animals, but they can also be detected in the environment, from soil, water, wild animals and birds. Both enterococci may cause urinary tract infections, wound infections, bacteraemia and infective endocarditis in humans. Thus, monitoring of resistance of enterococci, especially vancomycin-resistant enterococci (VRE) has a great significance for public health. Vancomycin resistance genes can be found in production animals even the ban of glycopeptides as growth promoters and resistant enterococci may act as reservuars of resistance genes (Haenni *et al.*, 2009; Hammerum, 2012). Avoparcin, a chemically similar antibiotic to vancomycin, was never used in Estonia.

According to Boerlin *et al.*, 2001, the use of avoparcin and tylosin has been associated with a high level of vancomycin-resistant and erythromycin-resistant enterococci in farm animals.
Vancomycin-resistant *Enterococcus* spp. is a major health problem worldwide and livestock has been implicated in constituting a reservoir for the transmission of vancomycin resistance to zoonotic pathogens.
In several national monitoring programs have shown, that enterococci from swine and cattle are commonly resistant to tetracyclines and macrolides/lincosamides (erythromycin, lincomycin) (Jackson *et al.*, 2011; Finres-Vet, 2010-2012; DANMAP, 2015; MARAN, 2015).
In Estonia, resistance of enterococci has been monitored 2010-2015 during national resistance monitoring program. In 2017, according to EU decision 2013/652EL, surveillance of *Enterococcus* spp. has started. Phenotypic resistance of *Enterococcus* spp. was determined in 2010-2015 *in vitro* using the microdilution method (VetMIC®, Sweden) with cut-off values presented in SWEDRES-SVARM 2015 report Table 7.12. Since 2017, broth micordilution method by TREK MIC system (EUVENT 50 µl) is used.

Antimicrobial resistance of *Enterococcus* spp isolated from healthy swine and cattle in 2010-2015 and 2017

In years 2010-2015, faecal samples were collected from healthy swine and cattle and species related association of antibiotic resistance was investigated. In 2010-2015, enterococci from both animal species were mainly resistant to tetracycline (33.3% in cattle; 40.4% in swine) and erythromycin (21.6% in cattle; 26.7% in swine). Enterococci from swine were also resistant to streptomycin (30.0%) and kanamycin (26.7%) (Table 6.)

Table 6. Resistance of *Enterococcus* spp. isolates originating from faecal samples of healthy cattle and swine in 2010-2015 in Estonia. (Aasmäe *et al*., 2019)

Antimicrobial	**Breakpoints for resistance (mg/l)[1]**	**Dairy cattle (n = 51)**		**Swine (n = 60)**	
		%	**(95% CI)**	**%**	**(95% CI)**
Ampicillin	>4	0.0	NA[2]	1.7	(-1.6-5.0)
Erythromycin	>4	21.6	(10.3-21.9)	26.7	(15.5-37.9)
Virginiamycin					
E. faecalis	>32	1.9	(-1.9-5.7)	5.0	(-0.5-10.5)
E. faecium	>4				
Gentamycin	>32	1.9	(-1.9-5.7)	1.7	(-1.6- 5.0)
Streptomycin*					
E. faecalis	**>512**	**11.7**	**(2.9-20.5)**	**35.0**	**(22.9-47.1)**
E. faecium	>128				
Kanamycin*	**>1024**	**3.9**	**(-1.4-9.2)**	**26.7**	**(1.5-37.9)**
Tetracycline	>4	33.3	(20.4-46.2)	40.4	(27.6-52.4)
Chloramphenicol	>32	1.9	(-1.9-5.7)	6.7	(0.8-13.3)
Vancomycin	>4	5.9	(-0.63-9.4)	10.0	(2.4-17.6)
Narasin	>2	3.9	(-1.4-9.2)	3.3	(-1.2-7.8)
Bacitracin	>32	3.9	(-1.4-9.2)	6.6	(0.4-13.3)
Linezolid	>4	0.0	NA[2]	1.7	(-1.6-5.0)

[1]Swedres-Svarm 2015. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden. Solna/Uppsala ISSN 1650-6332, 117, Table 2.17.

[2] Not assessed (NA).

*Statistically significant difference ($p < 0.05$) between resistant *Enterococcus* spp. isolates from healthy dairy cattle and swine. Corresponding percentages are also presented in bold face.

Table 7. Resistance of *Enterococcus faecalis* and *Enterococcus faecium* isolated from caecal content of swine at slaughterhouse in 2017

Antimicrobial	**Breakpoints for resistance (mg/l)[1]**	***E. faecalis* (n = 59)**		***E. faecium* (n = 123)**	
		%	95% CI	%	95% CI
Ampicillin	>4	0.0	NA	0.8	(-0.7-2.4)
Erythromycin	>4	39.8	(26.5- 52.5)	28.5	(20.4-36.4)
Quinupristin/ dalfopristin*	>1	0.0	NA	59.3	(50.6-68.0)
Gentamycin	>32	5.1	(1.7-13.9)	0.8	(-0.7-2.4)
Tetracycline	>4	69.5	(56.8-79.7)	17.9	(11.1-24.6)
Tigecycline	>0,25	0.0	NA	0	NA
Chloramphenicol	>32	16.9	(7.3-26.5)	0	NA
Ciprofloxacin	>4	3.4	(-1.2-8.0)	27.6	(20.5-36.1)
Vancomycin	>4	0.0	NA	0	NA
Daptomycin	>4	0.0	NA	2.4	(-0.2-5.1)
Teicoplanin	>2	0.0	NA	0	NA
Linezolid	>4	0.0	NA	0	NA

*According to EU decision 2013/652EL ECOFF breakpoint < 1 mg/l; EUCAST clinical breakpoint <4mg/l

Compared to 2010-2015, the phenotypic resistance against tetracycline, chloramphenicol and erythromycin has increased, but against vancomycin has decreased in 2017. No resistance was found against antibiotics used only in human medicine (quinupristin/ dalfopristin, tigecycline, daptomycin, teicoplanin).

Antimicrobial resistance of *Campylobacter* spp. of animal, human and food origin in Estonia

Campylobacteriosis is the most commonly reported zoonosis in the European Union with 246,158 confirmed human cases, which represents a notification rate of 64.8 per 100,000 population in 2017 (EFSA, 2018). In Estonia, 411 *Campylobacter* enteritis cases were reported in 2018, with a notification rate of 31.2 per 100,000 inhabitants (Terviseamet, 2019). Since 2012 campylobacteriosis has been the most prevalent bacterial enteric infection in Estonia with increasing trend for notified human cases.

World Health Organization has named *Campylobacter* as one of the 12 bacteria that pose the greatest threat to human health because of resistant *Campylobacter* strains isolated from livestock and clinical samples in several countries.

Fluoroquinolones, especially ciprofloxacin, are the most common antibiotics used for treatment of human and animal *Campylobacter* infection. Over the time this has lead to increased antimicrobial resistance making fluroquinolones less effective (Sproston et al., 2018).

First time in Estonia the resistance of *Campylobacter* isolates was determined in bacteria originating from swine caecal material collected at slaughterhouse level. The highest resistance was found (Table (8) against streptomycin (70.8%), ciprofloxacin (37.5%) and nalidixic acid (29.2%). In many other European countries the resistance of *Campylobacter* spp. isolated from swine caecal/faecal material against ciprofloxacin has been even higher (62.1%; 7 European countries) than presently found in Estonia, but swine *Campylobacter* isolates show higher resistance against fluoroquinolones than those originating from Estonian broiler chicken meat where the resistance was 25.0% (Mäesaar et al., 2018). Recent Polish study found that 89.3% of *C. jejuni* and *C. coli* isolates from pork were resistant against ciprofloxacin (Andrzejewska et al., 2019). At the same study, the resistance of swine origin *Campylobacter* against tetracycline was 64.3%, much higher than 37.5% that was found in Estonia.

Table 8. Resistance of *Campylobacter coli* and *Campylobacter jejuni* isolated from caecal content of swine at Estonian slaugtherhouses

Antimicrobial	**Breakpoints for resistance (mg/l)***	***Campylobacter coli/jejuni* (n = 24)**	
		%	**95% CI**
Erythromycin	>4	0	NA
Ciprofloxacin	>0,5	37.5	18.1-56.8
Nalidix acid	>16	29.2	10.9-47.3
Gentamycin	>2	0	NA
Streptomycin	>4	70.8	52.6-89.2
Tetracycline	>2	37.5	18.1-56.8

* TREK Sensititre EUVSEC2

Most of the Estonian *Campylobacter* spp. related studies performed in Estonia are focused on the fresh broiler chicken meat, because the main reservoir and source for human campylobacteriosis is considered to be poultry, and retail broiler chicken meat is a crucial vehicle for consumer's exposure to *Campylobacter* (Stella et al., 2017).

By the Mäesaar et al. (2016), the antimicrobial resistance profiles of *Campylobacter* spp. isolated from broiler chicken meat of Estonian, Latvian and Lithuanian origin at Estonian retail level and from patients with severe enteric infections in Estonia were studied.

In total, 98 *Campylobacter* isolates obtained from Estonian, Lithuanian and Latvian products were selected for MIC determination. These countries represent the most common origins of the poultry available in the Estonian retail market. From the total of 98 *Campylobacter* isolates 36 (36.7%), 46 (46.9%) and 16 (16.3%) originated from Estonian, Lithuanian and Latvian broiler chicken meat, respectively. Additionally, in collaboration with the Estonian hospitals, 28 *Campylobacter* human isolates were obtained. The MICs were determined for all 126 *Campylobacter* isolates by the broth microdilution VetMICTH method (National Veterinary Institute; Uppsala, Sweden).

There were differences in the resistance rates of *Campylobacter* isolates from the broiler chicken meat originated from different countries (Estonia, Latvia and Lithuania) (Table 9). Antimicrobial resistance to one or more antimicrobials was less frequent (p-value < 0.05) in the *Campylobacter* isolates of Estonian origin than in the isolates of Latvian or Lithuanian origin where the resistances was 87.5% and 89.1%, respectively. *Campylobacter* isolates of Estonian origin were also resistant to ciprofloxacin and nalidixic acid less frequently (p-value < 0.05) than the Latvian and Lithuanian isolates. The resistance to fluoroquinolones among Estonian chicken meat isolates was significantly different (p < 0.05) from the human isolates. Differences were not found in comparison of Estonian human isolates with broiler chicken meat isolates of Latvian (p = 0.28) and Lithuanian (p = 0.14) origin.

Table 9. Number and proportion of antimicrobial resistant *Campylobacter jejuni* and *Campylobacter coli* isolates from broiler chicken meat and humans

Antimicrobial	Broiler chicken meat isolates				Human isolates
	Estonian N (%)	Latvian N (%)	Lithuanian N (%)	All N (%)	N (%)
Erythromycin	0 (0.0)	0 (0.0)	1 (2.2)	1 (1.0)	0 (0.0)
Ciprofloxacin	6 (16.7)	14 (87.5)	39 (84.8)	59 (60.2)	19 (67.9)
Tetracycline	4 (11.1)	1 (6.3)	9 (19.6)	14 (14.3)	12 (42.9)
Streptomycin	1 (2.8)	0 (0.0)	7 (15.2)	8 (8.2)	3 (10.7)
Gentamicin	1 (2.8)	0 (0.0)	1 (2.2)	2 (2.0)	0 (0.0)
Nalidixic acid	7 (19.4)	14 (87.5)	37 (80.4)	58 (59.2)	19 (67.9)
Sensitive to all six	29 (80.6)	2 (12.5)	5 (10.9)	36 (36.7)	8 (28.6)
Resistant to one or more	7 (19.4)	14 (87.5)	41 (89.1)	62 (63.3)	20 (71.4)
Multidrug resistant	1 (2.8)	0 (0.0)	4 (8.7)	5 (5.1)	2 (7.1)
Total No[a]	36	16	46	98	28

[a] Total No of strains doesn't equal the sum of rows, since some strains are multidrug resistant

The antimicrobial resistance phenotypes of all *Campylobacter* isolates are presented in Table 9. One (1.0%) *Campylobacter* isolate of broiler chicken meat origin was resistant to four unrelated antimicrobials: ciprofloxacin/nalidixic acid combination, tetracycline, streptomycin and gentamicin. The main pattern of resistance to three unrelated antimicrobials was resistance to the combination of ciprofloxacin/nalidixic acid, tetracycline and streptomycin, and this pattern was exhibited in 3.1% and 7.0% of the *Campylobacter* isolates of broiler chicken meat and human origin, respectively. The most frequent combination of resistance was ciprofloxacin/nalidixic acid and tetracycline. Broiler chicken meat isolates were resistant to ciprofloxacin (60.2%), nalidixic acid (59.2%), tetracycline (14.3%), streptomycin (8.2%), gentamicin (2.0%) and erythromycin (1.0%). Among 28 Estonian origin human *Campylobacter* isolates, the highest frequencies of resistance were against nalidixic acid and ciprofloxacin (for both antimicrobials 67.9%), followed by tetracycline (42.9%), streptomycin (10.7%).

We found greater levels of resistance to tetracycline in the human isolates (42.9%) than among the animal isolates (14.3%). *Campylobacter* isolates from the broiler chicken meat of Estonian, Latvian and Lithuanian origin were resistant to tetracycline at 11.1%, 6.3% and 19.6% respectively (Table 9). These findings might indicate that some of the human *Campylobacter* infections in Estonia have non-poultry meat sources.

The observed levels of multidrug resistance among the broiler chicken meat and human *C. jejuni* isolates were 5.1% and 7.1%. Among Estonian, Latvian and Lithuanian-origin broiler chicken meat isolates the multidrug resistance was detected at 2.8%, 0% and 8.7%, respectively. Compared to Estonian and Lithuanian products, the proportions of Latvian-origin fresh broiler chicken meat sales in the Estonian retail market are small; therefore, the risks of *Campylobacter* exposure and its related consequences from Latvian products are expected to be smaller than those of Lithuanian broiler chicken meat products, as the latter represents approximately 30% of the total sales of fresh poultry meat in the Estonian retail market.

Table 10. Antimicrobial resistance phenotypes among *Campylobacter jejuni* and *Campylobacter coli* isolates from broiler chicken meat and human clinical samples, Estonia

Antimicrobial resistance phenotype[a]	Broiler chicken meat		Human clinical samples	
	No	Proportion, %	No	Proportion, %
CI/TC/SM/GM/NA	1	1.0	-	-
CI/SM/GM/NA	1	1.0	-	-
CI/TC/SM/NA	3	3.1	2	7.1
CI/TC/NA	8	8.2	8	28.6
CI/SM/NA	2	2.1	1	3.6
EM/CI/NA	1	1.0	-	-
TC/NA	1	1.0	1	3.6
CI/TC	-	-	1	3.6
CI/NA	41	41.8	7	25.0
CI	2	2.1	-	-
TC	1	1.0	-	-
SM	1	1.0	-	-
Sensitive for all antimicrobials	36	36.7	8	28.6
Total	98	100.0	28	100.0

[a] Antimicrobial agents: EM, Erythromycin; CI, Ciprofloxacin; TC, Tetracycline; SM, Streptomycin; GM, Gentamicin; NA, Nalidixic acid

-, Not detected

Despite the lower rates of antimicrobial resistance found in the *Campylobacter* isolates from Estonian broiler chicken meat in this study compared to the results from previous Estonian studies, the prudent use of antimicrobials in Estonian broiler chicken production is needed to minimize the spread of antibiotic-resistant *Campylobacter* into the environment and into the food production. It was concluded that the problems caused by the inappropriate use of antimicrobials are far beyond the country where the food originates; therefore, both domestic and international interventions and agreements are required to implement common policies on antimicrobial usage and to minimize the emergence of *Campylobacter* drug resistance (Mäesaar et al., 2016).Most recent Estonian study (Mäesaar et al., 2018) determined genotypes of *Campylobacter jejuni* in Baltic fresh broiler chicken meat and in Estonian human origin samples by using MLST, and investigated whether resistance to selected antimicrobials differs between certain MLST clonal complexes (CC) and sequence types (ST). The study combined MLST and antimicrobial resistance data of *C. jejuni* from broiler chicken meat samples originating from all three Baltic countries at Estonian retail, and human isolates obtained from patients with severe *Campylobacter* enteric infections in Estonia. Sample consisted of *C. jejuni* strains isolated from Estonian (n=16), Latvian (n=8), Lithuanian (n=13) broiler chicken meat

products at retail level and human isolates (n=11) from clinical hospital laboratories located in northern Estonia.

Table 11. Distribution of clonal complexes, sequence types and antimicrobial resistance among the *C. jejuni* isolates from broiler chicken meat at Estonian retail level and human patients of Estonia.

CC	ST (n)	Country (n)	Source (n)	Resistance (n)
21	21 (1)	EST (1)	B (1)	CIP (1); NAL (1)
	50 (5)	EST (1); LIT (4)	H (1); B (4)	CIP (4); TET (1); STR (2); GEN (1); NAL (4); Susceptible (1)
	883 (1)	EST (1)	H (1)	Susceptible (1)
45	11 (2)	EST (2)	B (2)	Susceptible (2)
	45 (5)	EST (3); LIT (2)	B (5)	Susceptible (5)
	137 (1)	LIT (1)	B (1)	CIP (1); NAL (1)
	583 (1)	LIT (1)	B (1)	CIP (1); NAL (1)
48	429 (2)	EST (1); LIT (1)	B (2)	CIP (2); NAL (2)
61	61 (1)	EST (1)	H (1)	Susceptible (1)
257	257 (1)	EST (1)	H (1)	CIP (1); TET (1); NAL (1)
283	383 (2)	EST (1)	B (2)	Susceptible (2)
353	5 (11)	LAT (8); LIT (3)	B (11)	CIP (11); STR (1); NAL (11)
	353 (3)	EST (3)	H (3)	CIP (3); TET (3); NAL (3)
	356 (1)	EST (1)	H (1)	CIP (1); TET (1); NAL (1)
354	354 (2)	EST (1); LIT (1)	B (2)	CIP (2); TET (2); NAL (2)
443	51 (1)	EST (1)	H (1)	Susceptible (1)
464	8188 (3)	EST (3)	B (3)	Susceptible (3)
658	658 (1)	EST (1)	B (1)	CIP (1); TET (1); NAL (1)
794	677 (1)	EST (1)	B (1)	Susceptible (1)
832	828 (1)	EST (1)	H (1)	CIP (1); TET (1); NAL (1)
2221	n.d. (1)	EST (1)	B (1)	Susceptible (1)
2274	n.d. (1)	EST (1)	H (1)	CIP (1); NAL (1)

n.d., not defined; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetratcycline; STR, streptomycin; GEN, gentamicin; EST, Estonia; LAT, Latvia; LIT, Lithuania; B, broiler chicken meat; H, human; bold is indicating novel sequence type

We found that, the resistance against fluoroquinolones among Estonian broiler meat origin *C. jejuni* isolates was 25.0%. Very high proportions of fluoroquinolone resistance among Latvian (100.0%) and Lithuanian (84.6%) origin broiler chicken meat *C. jejuni* isolates was found in this study which probably indicates the wide use of fluoroquinolone antibiotics in poultry farms in these countries. All studied *Campylobacter* strains were isolated from broiler chicken meat purchased at Estonian retail level.

The overall resistance to fluoroquinolones was very similar for human (63.6%) and broiler meat (62.2%) isolates. Study found greater levels of resistance to tetracycline in the human isolates

(54.6%) than among the broiler chicken meat isolates (10.8%). The low proportion of resistant isolates found in broiler chicken meat likely reflects the limited use of tetracyclines in poultry production. The resistance to one or more antimicrobials was found in 62.5% of the *C. jejuni* isolates, which indicates the public health concern. It was found that Latvian and Lithuanian origin broiler chicken meat and Estonian human *C. jejuni* isolates were highly resistant to fluoroquinolones. Imported broiler chicken meat has higher *Campylobacter* prevalence and contamination with highly resistant *Campylobacter* strains and is most probably the main source of human *Campylobacter* infections in Estonia. This was also evidenced with molecular characterization of broiler chicken meat and human origin *C. jejuni* strains (Mäesaar et al., 2018).

This study showed that the most prevalent *C. jejuni* CC was ST-353 CC, found in Latvian and Lithuanian broiler chicken meat, and Estonian human isolates (Table 11). Another multiple sources related *C. jejuni* CC was ST-21 CC, associated with isolates from Estonian humans and from Estonian and Lithuanian broiler chicken meat. ST-353 CC together with ST-5 were significantly ($p<0.01$) associated with fluoroquinolone resistance in the present study. In the study, ST-45 CC was found in association with Estonian and Lithuanian broiler chicken meat isolates, but not with human *C. jejuni* isolates. It was found that Latvian and Lithuanian origin broiler chicken meat and Estonian human *C. jejuni* isolates were highly resistant to fluoroquinolones.

Antimicrobial resistance of *Salmonella* spp. of animal and food origin in 2017-2018 in Estonia

From a public health perspective, resistance of *Salmonella* spp. from farm animals is of greater concern than resistance in isolates from wild animals or pets. This is because bacteria from animals raised for food production can contaminate carcasses at slaughter and be transmitted to humans through the food chain.

In Estonia, 120 salmonella isolates were detected in animals during 2017-2018 (Table 12). The most common serotype was *S. diarizonae* isolated from sheep. Among all isolates, *S. typhimurium* and monophasic *S. typhimurium* were found in 9 (7.5%) and 8 (6.7%) of cases, respectively. In cattle, 12 isolates out of 22 belonged to serovar *S. dublin*. Additionally, six *S. typhimurium* and two monophasic *S. typhimurium* was detected.

In poultry and other domestic bird (quail, duck), the most prevalent serovar was *S. enteriditis* (10 out of 22). In Denmark, more than 80% of all *Salmonella* serotypes were *S. typhimurium* (DANMAP 2017, 2018). Also in Sweden, *S. tyhpimurium* was most common serovar (48 out of 92) (SVARM 2017). Half of *Salmonella* serovars detected in swine and cattle in Netherland were *S. typhimurium* (51.7%) followed by *S. dublin* (23%) (MARAN 2018). In Estonia, 54.5% of serovars were *S. dublin* and 36.3% *S. typhimurium*. *Salmonella* spp. has not been isolated from companion animal in Estonia, while in Sweden the prevalence was 33.6% and 90% of isolated *Salmonella* serovars were *S. typhimurium (*SVARM 2017).

Table 12. Number of salmonella serovars isolated from animal and food during 2017-2018

Salmonella serovars	Number of *Salmonella* serovars from animal (n = 120)						Number of *Salmonella* serovars from food (n = 36)			
	Swine n= 16	Cattle n = 22	Sheep n = 60	Poultry n = 10	Duck n = 3	Quail n = 9	Pig carcasses, surface samples n = 29	Lamb n = 1	Cattle carcasses (surface samples), Meat cuts n = 4	Quail meat n = 2
S. agona (n = 5)	3						2			
S. anatum (n = 1)						1				
S. arizona (n = 1)			1							
S. cholerasuiz (n = 1)	1									
S. derby (n = 35)	10	1		1			22	1		
S. diarizonae (n = 59)			59							
S. dublin (n = 13)		12							1	
S. enteritidis (n = 11)				7	2	1	1			
S. infantis (n = 2)						2				
S. mbandaka (n = 3)		1							2	
S. seftenberg (n = 1)				1						
S. typhimurium (n = 12)		6		1	1	1	1		1	1
S. typhimurium monophasic (n = 11)	2	2				4	2			1
S. worthington (n = 1)							1			

Table 13. Antimicrobial resistance of *Salmonella* spp. originated from animal and food in 2017-2018

Antimicrobial	Breakpoints for resistance (mg/l)*	Animal n = 118		Food n = 36	
		%	95%CI	%	95%CI
Ampicillin	>8	6 (5.1)	0.2-11.5	7 (19.4)	6.5-32.3
Azithromycin	>16	0	NA	0	NA
Cefotaxime	>0.25	0	NA	0	NA
Ceftazidime	>0,5	0	NA	0	NA
Ciprofloxacin	>0.06	5 (4.2)	0.6-7.8	1 (2.8)	-2.5-8.1
Nalidixic acid	>16	4 (3.4)	0.1-6.6	1 (2.8)	-2.5-8.1
Chloramphenicol	>16	1 (0.8)	-0.8-2.6	2 (5.6)	-1.9-13.4
Tetracyclin	>8	10 (8.4)	3.4-13.5	3 (8.3)	-0.7-17.6
Tigecycline	>1	0	NA	0	NA
Meropenem	>0.125	0	NA	0	NA
Gentamycin	>2	0	NA	0	NA
Colistin	>2	12 (10.2)	4.7-15.6	0	NA
Sulfomethoxazole	>64	9 (7.6)	4.1-13.8	6 (16.7)	7.8-31.8
Trimetoprim	>2	2 (1.7)	-0.6-4.1	2 (5.6)	-1.9-13.4
Multi-drug resistance		6 (5.1)	0.2-11.5	3 (8.3)	-0.7-17.6

For the monitoring of *Salmonella* spp., three antibiotic compounds (azithromycin, meropenem and tigecycline) used in human medicine, but not in veterinary practice, have been added to the susceptibility panel and three antimicrobials of less importance for treatment of human infections (florfenicol, kanamycin and streptomycin) have been deleted since the implementation of TREK system. Tigecycline is structurally related to tetracyclines, but has a broader spectrum of activity. Azithromycin is a potent macrolide and in human medicine often used instead of erythromycin for treatment of infections caused by gram-positive bacteria, due to the effectiveness of a once-daily administration during a few days. Given its activity against *Enterobacteriaceae* and its favourable pharmacokinetics, it is also used for typhoidal *Salmonella* cases for which *in vivo* efficacy has been demonstrated. Meropenem belongs to the carbapenems, which are last resort antimicrobials that are used to treat infections with multi-drug resistant bacteria.

The MDR was detected among six isolates (two *S. typhimurium* and two *S. derby* isolates). In Sweden, MDR was not detected in 2018. In Denmark MRD has increased, especially Amp/Tet/Sulfa resistance both in Danish pig and pork. Quinolone resistance was found in 4% among the *Salmonella* isolates in Estonia. No quinolone resistance was detected in Netherland and Sweden in cattle and pig isolates during 2017-2018 (MARAN 2018, SWERRES/SWARM 2018). In Denmark one isolate carried plasmid-mediated quinolone resistance (DANMAP, 2017)

Colistin has been used widespread in veterinary medicine for prevention and treatment of diarrhoeal diseases in livestock. In human medicine, colistin can be used for treatment of human infections with multidrug-resistant carbapenemase producing bacteria. In Estonia, 8 out of 12 colistin resistant isolates were *S. dublin*, which were isolated from cattle. Three *S. enteritidis* from poultry and one *S. derby* form swine showed MIC value above 4 mg/l. As there is no a general epidemiological cut-off value for colistin, the results are difficult to interpret. Using the former ECOFF of 2 mg/l (which is also the clinical breakpoint) resistance rates would have been highly influenced by differences in natural susceptibility (wildtype strains of *S. Enteritidis* and *S. dublin* are less susceptible for colistin). However, in Sweden 25% of *S. typhimurium* was colistin resistant with MIC above 4 mg/l. In Estonia, all *S. typhimurium* isolates were colistin susceptible.

Conclusion

In general, the antibiotic resistance of bacteria originated from animals and food depends on animal species, where higher resistance can be observed in swine and poultry compare to dairy cattle. The phenotypic antibiotic resistance of isolated *E. coli* is high in Estonia compared to Sweden and Denmark. The proportion of ESBL isolates were also high, especially in poultry meat. The proportion of *Campylobacter* resistant isolates originated from Estonian swine and poultry and food were similar to Denmark, Sweden and Netherlands. Higher resistance was observed among the isolates originated from Latvia and Lithuania. The resistance of *Salmonella* spp was higer compared to Sweden, Denmark and Netherland, but no resistance was developed to antibiotics used in human medicine. The annual antibiotic resistance monitoring program should be created following the example of Nordic countries and Netherland.

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