



## American Journal of Microbiology and Immunology (ISSN: 2474-2910)



# Prevalence and antibiotic resistance of *Campylobacter coli* and *Campylobacter jejuni* in Greek swine farms

Dimitrios Papadopoulos<sup>1</sup>, Evanthia Petridou<sup>1</sup>, <sup>(†)</sup> Georgios Filioussis<sup>1</sup>, Theofilos Papadopoulos<sup>1</sup>, Konstantinos Papageorgiou<sup>1</sup>, Maria Chatzistilianou<sup>2</sup>, Spyridon K. Kritas<sup>1</sup>

<sup>1</sup>Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece; <sup>2</sup>Clinic of Pediatrics-Immunology and Infectious Disease, Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

### ABSTRACT

*Campylobacter species* are one of four key global causes of human diarrheal diseases, according to W.H.O. It is considered to be the most common bacterial cause of human gastroenteritis in the world. The objective of this study was to estimate the prevalence of *Campylobacter coli* (*C. coli*) and *Campylobacter jejuni* (*C. jejuni*) in Greek commercial swine farms, and describe the antimicrobial resistance of the isolated strains. A total of 1,000 rectal swabs (50 per farm) were collected from twenty swine farms in Greece. Ten rectal samples had been randomly collected from each of five age-groups (suckling piglets, nursery pigs, grower pigs, finisher pigs, sows). Isolation of *Campylobacter* spp. was performed using the ISO 10272-1:2017. A PCR method, based on the amplification of *mapA*<sub>*C.jejuni*</sub> and *ceuE*<sub>*C.coli*</sub> specific genes, was used for identification of the isolated strains. All isolates were tested for their susceptibility against gentamycin, erythromycin, ciprofloxacin, tetracycline and meropenem; EUCAST guidelines were used for the interpretation. The results showed that 16 out of the 20 farms (80%) and 491 (49%) of the samples were positive for *Campylobacter* spp. Prevalence of *C.coli* was 38% (95% CI 35.1-41.1) and of *C.jejuni* 10.9% (95% CI 9.1-13.0). Sows were 1.4 times more likely to be colonized by *Campylobacter* spp than sucking piglets ( $p<0.05$ ) while nursery and grower pigs were 2.14 and 2 times more likely to be colonized than sows ( $p<0.001$ ). However, colonization was not associated with farm size. High rates of resistance were recorded for tetracycline (67,3%), while 18,1%, 7,3% and 3.9% of the isolates were resistant in ciprofloxacin, erythromycin and gentamycin respectively. Thirty-two of the isolates (6,52%) were classified as multidrug resistant; resistance to meropenem was not found. Our findings indicate high prevalence of *C.coli* and *C.jejuni* in Greek pig farms with high resistant rates to tetracycline and ciprofloxacin; this constitutes a potential reservoir for resistance genes spread to the community.

**Keywords:** *Campylobacter*, Antimicrobial Resistance, Minimum Inhibitory Concentration (MIC), Antimicrobial Susceptibility Testing (AST), pigs, zoonosis

### \*Correspondence to Author:

Dimitrios Papadopoulos  
Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

### How to cite this article:

Dimitrios Papadopoulos, Evanthia Petridou, Georgios Filioussis, Theofilos Papadopoulos, Konstantinos Papageorgiou, Maria Chatzistilianou, Spyridon K. Kritas. Prevalence and antibiotic resistance of *Campylobacter coli* and *Campylobacter jejuni* in Greek swine farms. American Journal of Microbiology and Immunology, 2020 5:6

 **eSciPub**  
eSciPub LLC, Houston, TX USA.  
Website: <http://escipub.com/>

## Introduction

*Campylobacter* spp. infections constitute a zoonosis of worldwide distribution, with serious repercussions for public health and with significant socio-economic impact <sup>1</sup>. *C. jejuni* and *C. coli* are the most commonly isolated species and recognized as the most important and with greater pathogenic potential from the viewpoint of food safety. In 2017, 246,158 confirmed cases of campylobacteriosis in humans were reported in the EU, which accounted to an average notification rate of 64.8 per 100,000 population <sup>2</sup>.

Although human campylobacteriosis is usually mild and self-limiting, an acute infection can have serious long-term consequences, including the peripheral neuropathies, Guillain–Barré syndrome <sup>3</sup>, Miller Fisher syndrome <sup>4</sup>, and functional bowel diseases, such as irritable bowel syndrome <sup>5</sup>. Campylobacteriosis in pigs is associated with neonatal and suckling piglet diarrhea, while in older animals clinical signs are rarely present.

Farm animals are the major reservoir of *Campylobacter* species. Poultry and pigs are recognized as the most important reservoirs of *Campylobacter* <sup>6</sup> and *C. coli* is the predominant species in pigs <sup>7</sup>. When treatment of human campylobacteriosis is needed, quinolones, macrolides and tetracyclines are the drugs of choice <sup>8</sup>.

Moreover the World Health Organization (WHO) identified *Campylobacter* as one of the high priority antimicrobial resistant pathogens, for its resistance in fluoroquinolones <sup>9</sup>. The resistance to both macrolides and fluoroquinolones is of public health concern as there are currently limited options in the choice of treatment of *Campylobacter* infections. In the EU, surveillance and monitoring of *Campylobacter* in humans, animals, and food is one of the public health priorities. However there is a relatively low number of studies concerning the prevalence and antimicrobial resistance of *Campylobacter* in pigs, comparing to poultry.

## Materials and Methods

### Sample collection

Twenty (coded A - T) commercial, farrow to finish, pig farms from the continental country, that were registered in the National Record of pig farmers association were randomly selected for sampling. The median size of the farm was 550 breeding sows (230-1600 breeding sows). Farms were categorized in two groups depending on their breeding sows capital, small farms with 400 sows or less and big farms with 401 sows and more. Sample collection lasted from April 2017 until February 2018.

From each farm a total of 50 samples were collected, 10 rectal swabs from pigs each of the following five age groups and living in different pens : group 1-suckling pigs (age 0-28 days), group 2-nursery pigs (age 29-70 days), group 3-grower pigs (age 71-105 days), group 4-finisher pigs (age 106 days to slaughter), group 5-sows (over 9 months of age). All animals sampled did not show any clinical sign of diarrhea.

In total 1,000 samples were collected, 50 samples per farm and 200 samples per age group.

All rectal swabs were placed in Modified Cary Blair (MCB) medium <sup>10</sup> and transferred under 4-6°C, in less than 24h to the laboratory.

### *Campylobacter* isolation and identification

The Detection Procedure B from the ISO 10272-1:2017 <sup>11</sup> method, was used for the detection and isolation of *Campylobacter* spp. from pig feces. Samples were added to the liquid enrichment medium (Preston broth, OXOID) and incubated in a microaerobic atmosphere at 41,5 °C for 24 h. From the enrichment culture obtained a loop of 10µL, and inoculated in the selective mCCD agar (Oxoid) and incubated at 41,5 °C in a microaerobic atmosphere and examined after 44 h to detect the presence of suspect *Campylobacter* colonies. One suspected *Campylobacter* colony was examined from each mCCD agar plate, for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed

by detection of oxidase activity and aerobic growth test at 25 °C.

Speciation of *Campylobacter* strains was made according to the 2nd version of the protocol for PCR amplification of *C.jejuni* and *C.coli*<sup>12,13</sup>. A multiplex PCR protocol targeting the identification of *Campylobacter jejuni* and *Campylobacter coli* based on the amplification of the two genes, *mapA<sub>C.jejuni</sub>* and *ceuE<sub>C.coli</sub>*, was performed. In addition, a 16S primer set has been included as quality assurance of the DNA-preparation and analysis (internal control). The *C.jejuni* ATCC 33560 and *C.coli* ATCC 33559 were used for Quality Control.

### Antimicrobial Susceptibility Testing (AST)

Agar dilution method was used for the antimicrobial susceptibility testing. All *Campylobacter* isolates were tested for their susceptibility to 5 antimicrobials from 5 different antimicrobial classes. Antimicrobials tested included gentamycin, erythromycin, ciprofloxacin, tetracycline and meropenem (Sigma Aldrich).

Muller Hinton agar supplemented with 5% mechanically defibrinated horse blood and 20mg/L β-NAD was used for *Campylobacter* isolates susceptibility testing.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables version 8.1 for *Campylobacter jejuni* and *Campylobacter coli* were used for the interpretation of the results<sup>14</sup>. As regards gentamicin and meropenem, tables of the same version for *Enterobacteriaceae* were used.

Antibiotics exhibiting phenotypic resistance to more than three antibiotics from different classes were regarded as Multidrug Resistant<sup>15</sup>.

The *C.jejuni* ATCC 33560 and *C.coli* ATCC 33559 were used for the Quality Control, during AST.

### Statistical Analysis

A prospective study was conducted to estimate the prevalence and the antimicrobial resistance profile of *Campylobacter* species. Prevalence was calculated as a fraction of samples with

positive result in microbiological test. Prevalence ratios (PR), 95% confidence intervals (CI), and P values were calculated. The outcome variables were *Campylobacter* spp, *C. coli* and *C. jejuni* positive samples and the explanatory variables were age group and farm size (small or big farm). A P value of ≤0.05 was considered statistically significant. STATA 13 (STATA CORP LP, College Station, Texas, USA) was used for statistical analysis.

## Results and Discussion

### Prevalence of *Campylobacter*

*Campylobacter* was isolated from 16 of the 20 sampled farms (80%). From the 1,000 rectal samples collected, 491 samples were positive for *Campylobacter*. In total, 49,1 % (95% CI, 36,7-60,6) of the samples were positive for *Campylobacter* species (380 *C.coli*, 109 *C.jejuni*, 2 later sequenced as *C. lari*). Prevalence of *C.coli* was 38% (95% CI 35,1-41,1) and of *C.jejuni* 10,9% (95% CI 9,1-13,0). Prevalence of *Campylobacter* spp among each of the 20 farms ranked from 0 to 78%, while prevalence of *C. coli* ranked from 0 to 68% and of *C. jejuni* from 0 to 28%. We found significant differences in prevalence of *Campylobacter* spp, *C. coli* and *C. jejuni* among the 20 studied farms ( $p < 0.05$ ). (Table 1)

Table 2 presents the prevalence of *Campylobacter* spp in among different age groups. Bivariate analysis showed that suckling piglets were 0.7 times less likely to be colonized by *Campylobacter* spp, than sows, while nursery and grower pigs were 2.14 and 2.01 times respectively more likely to be colonized comparing to sows ( $p < 0.05$ ). Interestingly, finisher pigs were 1.34 times more likely to be colonized by *Campylobacter* spp comparing to sows ( $p < 0.05$ ). Regarding *C. coli* nursery and grower pigs were 2.91 and 2.6 times, respectively, more likely, more likely to be colonized comparing to sows ( $p < 0.05$ ). However finishing pigs were 1,51 times more likely to be colonised comparing to sows. Regarding *C. jejuni* we did not identify statistical difference in colonization among the different age groups.

Moreover farm size seems not to be a risk factor for colonization *Campylobacter* spp.

### Antimicrobial susceptibility

130 isolates (26.48%) were susceptible to all 5 antibiotics tested (table 2). Moreover 62,5% of group 1 isolates were susceptible to all 5 antibiotics, while only 16.44% and 19.57% of group 2 and group 3 isolates were regarded as susceptible to all 5 antibiotics (table 3). 280 isolates (57.03%) and 49 isolates (9.98%) were resistant to 1 and 2 classes of antibiotics. 32 isolates (6.52%) were regarded as Multidrug Resistant by showing resistance in three different classes of antibiotics. Most MDR isolates were found in age group 3 and 4 (10.87% and 7.69%, respectively for each age group).

All clinical isolates (n=491), tested were susceptible to meropenem (table 4). Very high rates of resistance were recorded for

tetracycline. For *C.jejuni* isolates (n=109) 57.8% (n=63), were resistant to tetracycline while for *C.coli* isolates (n=380) resistance to tetracycline was recorded in 70,0% (n=266). In total 330 of the *Campylobacter* spp isolates (n=491) were resistant in tetracycline (62,71%). Medium rates of resistance were recorded for ciprofloxacin. For *Campylobacter* spp 18.13% of the isolates were resistant in ciprofloxacin. Resistance rates were higher for *C.jejuni* comparing to *C.coli*, with 22.94% and 16.84% respectively. Low and very low resistance rates were recorded for erythromycin and gentamycin. For erythromycin 7.33% (n=36) of all *Campylobacter* isolates were resistant. 9.17% (n=10) of *C.jejuni* and 6.84% (n=26) of *C.coli* isolates were resistant in macrolides. As regards gentamicin 3.87% (n=19) of *Campylobacter* spp isolates were resistant. The rate of gentamicin resistance for *C.jejuni* was 3.67%, (n=4) and for *C.coli* 3.95% (n=15).

**Table 1: Prevalence of *Campylobacter* spp, *Campylobacter coli* and *Campylobacter jejuni* in Greek pig farms**

Farm	Pigs tested (N)	<i>Campylobacter</i> spp			<i>Campylobacter coli</i>			<i>Campylobacter jejuni</i>		
		Positive samples (N)	PR (%)	95% CI	Positive samples (N)	PR (%)	95% CI	Positive samples (N)	PR (%)	95% CI
A	50	32	64	49.8-76.1	28	56	42-69.1	4	8	3-19.6
B	50	29	58	43.9-70.9	20	40	27.3-54.2	9	18	9.6-31.3
C	50	31	62	47.8-74.4	25	50	36.3-63.7	6	12	5.4-24.4
D	50	29	58	43.9-70.9	24	48	34.5-61.8	4	8	3-19.6
E	50	29	58	43.9-70.10	24	48	34.5-61.8	5	10	4.2-22
F	50	36	72	57.9-82.8	29	58	43.9-70.9	7	14	6.8-26.7
G	50	25	50	36.3-63.7	22	44	30.9-58	3	6	1.9-17.2
H	50	28	56	42-69.1	24	48	34.5-61.8	4	8	3-19.6
I	50	28	56	42-69.1	26	52	38.2-65.5	2	4	1-14.8
J	50	26	52	38.2-65.5	19	38	25.6-52.2	7	14	6.8-26.7
K	50	28	56	42-69.1	22	44	30.9-58	6	12	5.4-24.4
L	50	0	0	NA	0	0	NA	0	0	NA
M	50	39	78	64.3-87.5	34	68	53.8-78.5	5	10	4.2-22
N	50	36	72	57.9-82.8	27	54	40.1-67.3	9	18	9.6-31.3
O	50	34	68	53.8-79.5	18	36	23.9-50.2	15	30	18.9-44.1
P	50	0	0	NA	0	0	NA	0	0	NA
Q	50	38	76	62.2-85.9	29	58	43.9-70.9	9	18	9.6-31.3
R	50	0	0	NA	0	0	NA	0	0	NA
S	50	23	46	32.7-59.9	9	18	9.6-31.3	14	28	17.2-42.1
T	50	0	0	NA	0	0	NA	0	0	NA
Total	1000	491	49	46-52	380	38	35-41.1	109	10.9	9.1-13

**Table 2: Prevalence of *Campylobacter* spp, *Campylobacter coli* and *Campylobacter jejuni* per age group in Greek pig farms**

Age group	Pigs tested (N)	<i>Campylobacter</i> spp			<i>Campylobacter coli</i>			<i>Campylobacter jejuni</i>		
		Positive samples (N)	PR (%)	95% CI	Positive samples (N)	PR (%)	95% CI	Positive samples (N)	PR (%)	95% CI
Suckling piglets	200	48	24	18.6-30.4	35	17.5	12.8-23.4	13	6.5	3.8-10.9
Nursery pigs	200	146	73	66.4-78.7	125	62.5	55.6-69	21	10.5	6.9-15.6
Grower pigs	200	138	69	62.2-75	112	56	49-62.7	26	13	9-18.4
Finisher pigs	200	91	45	38.7-52.5	65	32.5	26.3-39.3	26	13	9-18.4
Sows	200	68	34	27.7-40.9	43	21.5	16.3-27.8	23	11.5	7.8-16.7
<b>Total</b>	<b>1000</b>	<b>491</b>	<b>49</b>	<b>46-52</b>	<b>380</b>	<b>38</b>	<b>35-41.1</b>	<b>109</b>	<b>10.9</b>	<b>9.1-13</b>

**Table 3 Number and proportion of isolates to different classes of antibiotics**

Resistance to antibiotic classes	N	%
0	130	26.48
1	280	57.02
2	49	9.98
3	32	6.52
<i>Total</i>	491	100

**Table 4 Number and proportion of isolates to different classes of antibiotics per age group**

Age group	Resistance to different classes of antibiotics					Total
		0	1	2	3	
1	N	30	18	0.00	0.00	48
	%	62.50	37.50	0.00	0.00	100.00
2	N	24	95	21	6	146
	%	16.44	65.07	14.38	4.11	100.00
3	N	27	80	16	15	138
	%	19.57	57.97	11.59	10.87	100.00
4	N	24	51	9	7	91
	%	26.37	56.04	9.89	7.69	100.00
5	N	25	36	3	4	68
	%	36.76	52.94	4.41	5.88	100.00
Total	N	130	280	49	32	491
	%	26.48	57.02	9.98	6.52	100.00

**Table 5 Antimicrobial resistance of *Campylobacter coli*, *Campylobacter jejuni* and *Campy* spp per age group**

Age Groups	Campylobacter coli isolates N=380						Campylobacter jejuni isolates N=109					Campylobacter spp isolates N=491				
		Cip	Ery	Tet	Mer	Gm	Cip	Ery	Tet	Mer	Gm	Cip	Ery	Tet	Mer	Gm
1	N	0	0	12	0	0	0	1	5	0	0	0	1	17	0	0
	%	0	0	34.29	0	0	0	7.69	38.46	0	0	0	2.08	35.42	0	0
2	N	26	6	89	0	5	9	5	15	0	0	38	11	104	0	5
	%	20.8	4.8	71.2	0	4	42.86	23.81	71.43	0	0	23.97	7.53	71.43	0	3.42
3	N	20	10	90	0	7	9	1	18	0	2	29	11	108	0	9
	%	17.86	8.93	80.36	0	6.25	34.62	3.85	69.23	0	7.69	21.01	7.97	78.26	0	6.52
4	N	13	8	47	0	1	2	2	16	0	1	15	10	63	0	2
	%	20	12.31	72.31	0	1.54	7.69	7.69	61.54	0	3.85	16.48	10.99	69.23	0	2.2
5	N	5	2	28	0	2	5	1	9	0	1	10	3	38	0	3
	%	11.63	4.65	65.12	0	4.65	21.74	4.35	39.13	0	4.35	14.71	4.41	55.88	0	4.41
Total	N	64	26	266	0	15	25	10	63	0	4	89	36	330	0	19
	%	16.84	6.84	70	0	3.95	22.94	9.17	57.8	0	3.67	18.13	7.33	67.21	0	3.87

**Table 6 Antimicrobial Resistance phenotypes**

Resistance phenotypes	N	%
Cip	20	5.54
Tet	255	70.64
Ery	6	1.66
CipEry	3	0.83
CipGm	2	0.55
CipTet	32	8.86
EryTet	8	2.22
GmTet	3	0.83
CipEryTet	18	4.99
CipGmTet	13	3.60
EryGmTe	1	0.28
Total	361	100

In our study three MDR phenotypes were identified (table 5). The resistance CipEryTet phenotype was the most common as it was found in 18 *Campylobacter* spp isolates (4,97%). The resistance phenotype CipGmTet followed, as it was identified in 13 isolates (n=3.59%). In one isolate (0,28%) we identified resistance in erythromycin, gentamicin and tetracycline.

21 *Campylobacter* spp isolates (5,75%), were resistant to both erythromycin and ciprofloxacin, drugs of choice for treatment of invasive human campylobacteriosis (table5).

Moreover statistical analysis showed that *C.jejuni* isolates from big farms, over 401 sows were 1.74 times (1.14-2.67), more likely to be resistant in ciprofloxacin, comparing to isolates from smaller farms (table 6). Regarding *C.coli* isolates from small farm were 5.9 times (1.3-26.5) more likely to be resistant in erythromycin. We did not identify connection between MDR isolates with certain age groups or with the farm size.

## Discussion

This study was designed to assess the prevalence and the patterns of antimicrobial resistance of *Campylobacter* spp isolates from farrow-finisher pig farms in Greece, and among the different age groups that represent the different stages of pig production in Greece. Fluoroquinolones like ciprofloxacin and macrolides like erythromycin, classified as category I and category II antimicrobials, respectively, are of particular importance since patients with campylobacteriosis are generally treated with these agents.

The results of this study emphasize on the significant prevalence of *Campylobacter* spp in Greek commercial farms. In total, 49,1% of the samples were positive for *Campylobacter* species (380 *C.coli*, 109 *C.jejuni*, 2 later sequenced as *C. lari*) The predominant species was *C.coli* with 77.4% of the isolates followed by *C.jejuni* with 22.2%.

Similar significant prevalence rates of *Campylobacter* in pigs have been identified in

many studies that have been performed in countries like France <sup>7</sup>, United Kingdom <sup>16</sup>, the United States <sup>17</sup> at slaughterhouse level and Japan <sup>18</sup> at farm level. The 2018 EFSA-ECDC report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks for the year 2017 (<sup>19</sup>), reported that only 2% of the pigs samples tested (n=2,481), were positive for *Campylobacter* spp, at slaughterhouse level. The same report for 2016 <sup>20</sup>, reported 17,6% of positive for *Campylobacter* spp pigs samples (n=3,817).

The AMR rates found in our study and more specifically the high rates of tetracycline resistance and the low and very low rates of erythromycin and gentamicin resistance, match with the results on the EFSA-ECDC summary report on antimicrobial resistance for 2017 <sup>21</sup>. The median EU resistance rates for tetracycline was 51.5%, and for erythromycin and gentamicin 15.6 % and 7.7% in 979 *C.coli* isolates from fattening pigs. As regards fluoroquinolones like ciprofloxacin we have recorded a significant lower rate of resistance comparing to 52.3% of the EFSA report.

The EU median for MDR *Campylobacter coli* according to EFSA report was 21.2%. In our study the proportion of MDR *Campylobacter* spp isolates was relatively low (6.52%). The most common MDR phenotypes that were identified in our study (CipEryTet and CipGmTet) match with the recognized MDR phenotypes of the EFSA report.

This is the first study in Greece, that provides data on *Campylobacter* prevalence in pig farms and on their antimicrobial resistance. Moreover it provides useful data for the microorganism prevalence and resistance in the different age groups that reflect the productive stages of pig farming in farrow to finish farms.

Based on the findings in this study, we conclude that pigs are an important reservoir for *Campylobacter* species and especially for *C.coli*, and can play important role in the spread of the pathogen. The role of pigs in human *Campylobacter* infections has not been

investigated in detail in Greece. More studies on *Campylobacter* epidemiology in pig farms and pork products is needed.

Furthermore, the high rates of resistance to antimicrobials like tetracycline, and the medium rates of resistance to fluoroquinolones, highlights the need for further and systematic monitoring of *Campylobacter* isolates of pig origin.

**Acknowledgement:** Research was funded by the Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece.

We declare that we have no conflicts of interest

## References:

1. Havelaar, A.H., Nauta, M.J., Manges, M.J.J., de Koeijer, A.G., Bogaardt, M.J., Evers, E.G., Jacobs-Reitsma, W.F., Pelt, W., van Wagenaar, J.A., de Wit, G.A., van der Zee, H., 2005. Costs and benefits of controlling campylobacter in The Netherlands — integrating risk analysis, epidemiology and economics. RIVM Report 250911009/2005.
2. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017
3. Jackson BR, Zegar JA, Lopez-Gatell H, Sejvar J, Arzate F, Waterman S, Nunez AS, Lopez B, Weiss J, Cruz RQ, Murrieta DY, Luna-Gierke R, Heiman K, Vieira AR, Fitzgerald C, Kwan P, Zarate-Bermudez M, Talkington D, Hill VR, Mahon B. 2014. Binational outbreak of Guillain-Barre syndrome associated with *Campylobacter jejuni* infection, Mexico and USA, 2011. *Epidemiol Infect* 142:1089–1099. doi:10.1017/S0950268813001908
4. Koga M, Gilbert M, Li J, Koike S, Takahashi M, Furukawa K, Hirata K, Yuki N. (2005). Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry. *Neurology* 64:1605–1611. doi:10.1212/01.WNL.0000160399.08456.7C
5. Gradel KO, Nielsen HL, Schonheyder HC, Ejlsertsen T, Kristensen B, Nielsen H. (2009). Increased short-and long-term risk of inflammatory bowel disease after *Salmonella* or *Campylobacter* gastroenteritis. *Gastroenterology* 137:495501. doi:10.1053/j.gastro.2009.04.001
6. Quintana-Hayashi, M. P., and Thakur, S. (2012). Longitudinal study of the persistence of antimicrobial-resistant *Campylobacter* strains in distinct swine production systems on farms, at slaughter, and in the environment. *Appl. Environ. Microbiol.* 78, 2698–2705. doi: 10.1128/AEM.07723-11
7. Avrain, L., Humbert, F., Sanders, P., Vernozzy-Rozand, C., and Kempf, I. (2004). Antimicrobial resistance in *Campylobacter* from pigs in French slaughterhouses. *Rev. Med. Vet.* 155, 156–158
8. Sifre, E., Salha, B. A., Ducournau, A., Floch, P., Chardon, H., Megraud, F., et al. (2015). EUCAST recommendations for antimicrobial susceptibility testing applied to the three main *Campylobacter* species isolated in humans. *J. Microbiol. Methods* 119, 206–213. doi: 10.1016/j.mimet.2015.10.018
9. WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015, WHO, Geneva (2018)
10. Massipab C., Guet-Revilletab H., Grareab M., Sommetcd A., Duboisab D. (2018). Enhanced culture recovery of *Campylobacter* with modified Cary-Blair medium: A practical field experience. *Journal of Microbiological Methods* 149: 53-54. doi:10.1016/j.mimet.2018.05.001
11. ISO 10272-1:2017, Microbiology of the food chain- Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method. Geneva : ISO.
12. Protocol for PCR amplification of *C.jejuni* and *C.coli*, recommended by the EURL-AR, 2<sup>nd</sup> version, 2013.
13. M. Denis, C. Soumet, K. Rivoal, G. Ermel, D. Blivet, G. Salvat and P. Colin (1999). Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Letters in Applied Microbiology*, 29, 406–410
14. European Committee on Antimicrobial Susceptibility Testing . Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1, 2018.
15. Magiorakos A.-P., Srinivasan A., Carey R. B., et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection.* 2012;18(3):268–281. doi: 10.1111/j.1469-0691.2011.03570.x.
16. Milnes, A. S., Stewart, I., Clifton-Hadley, F. A., Davies, R. H., Newell, D. G., Sayers, A. R., et al. (2008). Intestinal carriage of



- verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiol. Infect.* 136, 739–751. doi: 10.1017/S0950268807009223
17. Tadesse, D. A., Bahnson, P. B., Funk, J. A., Thakur, S., Morrow, W. E., Wittum, T., et al. (2011). Prevalence and antimicrobial resistance profile of *Campylobacter* spp. isolated from conventional and antimicrobial-free swine production systems from different U.S. regions. *Foodborne Pathog. Dis.* 8, 367–374. doi:10.1089/fpd.2010.0665
  18. Haruna, M., Sasaki, Y., Murakami, M., Mori, T., Asai, T., Ito, K., et al. (2013). Prevalence and antimicrobial resistance of *Campylobacter* isolates from beef cattle and pigs in Japan. *J. Vet. Med. Sci.* 75, 625–628. doi: 10.1292/jvms.12-0432
  19. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal* 2017;15(12):5077. doi: 10.2903/j.efsa.2017.5077
  20. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal* 2018;16(12):5500. doi: 10.2903/j.efsa.2018.5500
  21. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). 2019. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. *EFSA Journal* 2019;17(2):5598. doi: 10.2903/j.efsa.2019.5598.

