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Human *Campylobacter jejuni* and *Campylobacter coli* Isolates: Demographic Pattern and Antimicrobial Susceptibility to Clinically Important Antimicrobials used in Livestock

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Abstract

Background: Campylobacteriosis is the leading zoonotic disease in developed countries with *C. jejuni* and *C. coli* being the two predominant causative pathogens. It has been shown that quinolone consumption in livestock is associated with increased quinolone resistance of *Campylobacter* isolates from food producing animals and infected patients. However, susceptibility testing of clinical isolates is not commonly performed and, consequently, resistance rates of human *C. jejuni* and *C. coli* isolates in areas of high consumption of antimicrobials in livestock may be undervalued. A strong association between *C. jejuni* infections and patients` age and gender has been highlighted by several authors. However, there is still little information on the demographic pattern in *C. coli* infections.

Methods: 1135 *C. jejuni* and 156 *C. coli* human isolates were obtained within a rural region of Germany. The study area was characterised by intensive swine and poultry farming involving high consumption of clinically important antimicrobials. Isolates were analysed for susceptibility to amoxicillin, ciprofloxacin, tetracycline and erythromycin using the EUCAST disc diffusion method. Furthermore, data were stratified with respect to patients age and gender.

Results: Contrary to male-biased distribution in *C. jejuni* isolates, *C. coli* was the predominant species in female patients with a maximum female surplus in young children and middle-aged adults. Resistance rates of *C. coli* vs. *C. jejuni* to amoxicillin, ciprofloxacin, tetracycline and erythromycin were 46.2% vs. 48.3%, 62.8% vs. 64.5%, 68.6% vs. 35.2% and 14.7 vs. 0.6%, respectively. Resistance rates were found to correlate with usage of these antimicrobials in livestock.

Conclusion: The high prevalence of *C. coli* in female patients may point to sex-specific behavioural or physiological aspects. The observed high to moderate resistance rates of *Campylobacter* isolates warrant prudent use of antimicrobials in livestock as well as routine susceptibility testing of human isolates to ensure efficacy of antimicrobial therapy.

Keywords: *C. jejuni*; *C. coli*; Livestock; Antimicrobial consumption; Antimicrobial resistance

Introduction

C. jejuni and *C. coli* are recognized as major bacterial pathogens of human gastroenteritis [1]. Consumption of contaminated food such as poultry or pork is regarded the main source of infection, followed by drinking of untreated water or unpasteurized milk and contact with farm animals [2]. While infection can occur at any age, higher incidence rates of campylobacteriosis have been observed in male patients, especially in infants and young adults [3]. However, most studies do not discriminate between *C. jejuni* and *C. coli*, which could mask species-specific differences due to the lower frequency of less common *C. coli* infections.

As campylobacteriosis is mainly a mild and self-limiting disease, antimicrobial therapy is usually not recommended except in elderly and immunocompromised patients [4]. Macrolides and quinolones are regarded as the most active agents for treatment of campylobacteriosis followed by tetracyclines or amoxicillin [5]. However, the emergence of drug-resistant *Campylobacter spp.* has decreased efficacy of antimicrobial therapy and poses a worrisome public health concern regarding the potential transmission of resistance genes [6]. In addition, resistance to quinolones or macrolides also seems to be associated with an adverse course of campylobacteriosis [7]. Surveillance data indicate that indiscriminate usage of antimicrobials in livestock could be the major cause for the dissemination of resistant *Campylobacter* strains mainly by selecting for quinolone-, tetracycline- and erythromycin-resistant strains or by induction of intrinsic ß-lactamases [8]. In order to compare antimicrobial consumption between countries with different sizes of livestock populations, national sales data of active agents (mg) are normalised by the estimated weight of livestock at treatment (PCU). According to surveillance data from the European Medicines Agency (EMA) total consumption in German livestock ranked third among EU member states in 2011 with 211 mg/PCU. Although consumption decreased significantly to only 98 mg/PCU until 2015, which was mainly related to lower use of penicillin and tetracyclines, it remained the fifth highest in the EU [9]. Moreover, consumption of critically important quinolones even increased by 13% from 2011 until to date [10]. However, data elucidating the impact of high consumption of antimicrobials in livestock on local resistance rates of *C. jejuni* and *C. coli* are scarce.

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Methods

Strain collection

47.985 faecal samples were collected between beginning of September 2014 and end of June 2016 from approximately 150-200 medical practices, outpatient clinics and hospitals in the North-West region of Lower-Saxony (Weser-Ems), Germany. The study population included approximately two million inhabitants mostly living in rural areas with low population densities of less than 165 inhabitants/km^2. Approximately 50% of German poultry and swine livestock populations are farmed in the study area, which accordingly involves high antimicrobial consumption and makes the region the leading consumer of veterinary antimicrobials in Germany. Among the isolates obtained, 1755 *Campylobacter spp.* were identified corresponding to 1135 *C. jejuni* (87.9%), 156 *C. coli* (12.1%), two *C. laridis* and one *C. upsaliensis* after correction for duplicates. The latter two species were excluded from further analysis due to their low frequency. The origin of isolates from the study region was verified by use of the patients' postal code on the doctor`s referral.

Isolation and identification of *Campylobacter spp*

Suspensions of fresh faeces were streaked onto modified charcoal cefoperazone desoxycholate agar plates (mCCDA-Preston, OXOID™, Thermo Scientific, Wesel, Germany). Inoculated plates were incubated together with a control strain (*C. jejuni* ATCC™ 33560) for 48 h at 42°C in a microaerobic environment using a commercially airtight container and atmosphere generator (GENbox™ and GENbag™ microaer, bioMérieux, Nürtingen, Germany). Suspect growth was confirmed by a positive cytochrome oxidase reaction (Bactident™, Merck, Darmstadt Germany). Isolated colonies were identified to the species level by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using the VITEK™ MS database (bioMérieux, Nürtingen, Germany) as described previously [11,12].

Susceptibility testing

Antimicrobial susceptibility testing (AST) was performed according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST). In brief, the inoculum was adjusted to 0.5 McFarland standard using a calibrated photometric device (DensiCHECK®, bioMérieux, Nürtingen, Germany) and streaked onto Mueller Hinton agar plates supplemented with 5% (w/v) defibrinated horse blood and 20 mg/l ß-NAD (MH-F, OXOID, Wesel, Germany) followed by placement of discs supplemented with either ampicillin 10 µg, ciprofloxacin 5 µg, erythromycin 15 µg or tetracycline 30 µg (BD Sensi-Discs™, Becton Dickinson, Sparks, USA). *C. jejuni* ATCC™ 33560, *Escherichia coli* ATCC™ 25922 and *Haemophilus influenzae* ATCC™ 42947 served as quality controls. Inhibition zone diameters for ciprofloxacin, erythromycin and tetracycline were measured with a ruler and classified as sensitive or resistant according to the latest EUCAST breakpoint tables for *C. jejuni* and *C. coli*. Classification of zone diameters for ampicillin and imipenem was performed according to the EUCAST breakpoints for Enterobacteriacae. Susceptibility to amoxicillin was inferred from ampicillin. For the purpose of this study, a completely resistant or completely susceptible isolate was defined as being resistant or susceptible to all four antimicrobials. Co-resistant isolates were defined as resistant to at least two of the four antimicrobials. Completely resistant isolates involved supplemental testing with amoxicillin clavulanate 20/10 µg and imipenem 10 µg from the same manufacturer as mentioned above. Isolates with insufficient growth were excluded from further analysis. A separate collection of 47 *C. coli* and 328 *C. jejuni* isolates collected between July 2016 and December 2016 was used to demonstrate the distribution of zone diameters of the four routinely used antimicrobials.

Statistical Analysis

Statistical analyses were performed using the χ^2-test, Fisher`s exact test and t-test where appropriate (Prism version 7.0 GraphPad software, San Diego California, USA). A P value<0.05 was considered as statistically significant.

Results

Demographic data

Approximately 95% of isolates originated from the study area as judged by the patients` postal codes. Approximately 75% and 25% of isolates were obtained from outpatients and hospitalised patients, respectively, with no significant difference between *C. jejuni* and *C. coli* isolates (p=0.8). There was also no significant association between hospitalisation of patients and observed antimicrobial resistance patterns of clinical isolates except for higher amoxicillin resistance of *C. coli* isolates from inpatients (Table 1). A median age of 37 years was found in patients with *C. jejuni* and *C. coli* infections. The mean age of patients did also not differ significantly with 39 years and three months in *C. jejuni* infections versus 40 years and two months in *C. coli* infections (p=0.69). More than 50% of C. *jejuni* and C. *coli* isolates were found in patients younger than 39 years featuring a maximum in young adults between 20-29 years (Figures 1 and 2). 630 males (55.5%; n=1135) and 505 female (44.5%) patients were infected with *C. jejuni* while 81 female (51.9%; n=156) and 75 male (48.1%) patients were infected with *C. coli* resulting in a male-to-female (M-F) ratio of 1.2 and 0.9, respectively. Species-specific differences in gender distribution were statistically not quite significant (p=0.08). Age groups comprising patients with *C. jejuni* infection were almost all male-dominated featuring a slight male surplus in young children and distinct surplus in middle-aged adults (Figure 1). In contrast, a female surplus was observed in most age groups with *C. coli* infections including a maximum female excess rate in children between 5-9 years and middle-aged adults between 40-49 years (Figure 2).

Antimicrobial susceptibility testing results

Ciprofloxacin performed poorly in *C. jejuni* and *C. coli* isolates with resistance rates of more than 60%, respectively (Table 2). *In vitro* activity

Antimicrobial resistance	Origin of isolates	No. of *C. jejuni* (%)	No. of *C. coli* (%)
Erythromycin-resistant	Inpatient	1/7 (14.3)	5/23 (21.7)
	Outpatient	322/1128 (28.5)	37/133 (27.8)
Ciprofloxacin-resistant	Inpatient	197/732 (26.9)	28/98 (28.6)
	Outpatient	126/403 (31.2)	14/58 (24.1)
Tetracycline-resistant	Inpatient	107/399 (26.8)	25/107 (23.3)
	Outpatient	216/736 (29.3)	17/49 (34.7)
Ampicillin- resistant	Inpatient	154/548 (28.1)	26/72 (36.1)[a]
	Outpatient	169/587 (28.8)	16/84 (19.0)

Table 1: Antimicrobial resistance rates of *C. jejuni* and *C. coli* isolates from hospitalised patients and outpatients showing no significant species-specific differences except for higher amoxicillin-resistance of *C. coli* isolates from inpatients; [a] p=0.019.

of tetracycline was just as low in *C. coli* while it was still active in about 70% of the *C. jejuni* isolates. Amoxicillin showed *in vitro* activities of only 50% in both species. Erythromycin was found to be the most active agent in both species with a minimal resistance rate less than 1% in *C. jejuni* and a moderate resistance rate of 15% in *C. coli* (p<0.001). Only 10% of *C. coli* isolates were found to be completely susceptible as compared to 25% of *C. jejuni* isolates (p<0.001). Complete resistance was almost exclusively found in *C. coli* isolates (p<0.001). *C. coli* isolates were also significantly more resistant to tetracycline and erythromycin as compared to *C. jejuni* (p<0.001). Only 0.4% of *C. jejuni* isolates were found to be co-resistant to ciprofloxacin and erythromycin, whereas a co-resistance rate of 10.9% was found in *C. coli* isolates (p<0.001).

Erythromycin resistance in *C. coli* was significantly associated with co-resistance to tetracycline as compared to erythromycin-susceptible isolates (p=0.039). One completely resistant *C. jejuni* isolate and seven out of 12 completely resistant *C. coli* isolates (58%) were still susceptible to amoxicillin-clavulanate. All of the completely resistant *Campylobacter* isolates were susceptible to imipenem.

Average inhibition zone diameters (mm) including a 95% confidence interval of 328 *C. jejuni* and 47 *C. coli* isolates were 15.4 (14.4-16.4) vs. 14.5 (12.2-16.7) for amoxicillin, 16.0 (14.6-17.4) vs. 13.7 (10.1-17.3) for ciprofloxacin, 24.0 (22.4-25.6) vs. 15.7 (11.9-19.6) for tetracycline and 30.3 (29.9-30.7) vs. 27.9 (26.3-29.5) for erythromycin, respectively (Figures 3 and 4).

Discussion

To date, only few studies have investigated antimicrobial susceptibility of human *C. jejuni* and *C. coli* isolates using the recently standardized EUCAST disc diffusion method. A comparable French study on a nationwide collection of 1997 *C. jejuni* and 419 *C. coli* isolates reported a similarly low erythromycin resistance rate of 0.45% in *C. jejuni* while erythromycin resistance rate in *C. coli* isolates was found to be only 9.3% [13]. Resistance to erythromycin in *Campylobacter spp.* is conferred by stepwise mutations in the 23S rRNA after prolonged exposure to macrolides or by activation of the CmeABC efflux pump [14]. The higher prevalence of erythromycin-resistant *C. coli* isolates in our collection may be related to common usage of macrolides in German swine livestock, the main reservoir of *C. coli*. In fact, macrolides are the second most commonly administered antimicrobial in fattening pigs in Germany [15], which may effectively select for erythromycin-resistant *C. coli* isolates, if given repeatedly during the fattening period. Furthermore, it has been shown that administration of macrolides in pigs led to a marked increase of erythromycin-resistant *Campylobacter* isolates even after only four days of treatment [16]. A high prevalence of erythromycin-resistant human isolates of *C. coli* (38%) has also been reported from Spain, the second largest pig producing country in Europe, which seems to align with exorbitant macrolide consumption (24 mg/PCU) [17]. In contrast, almost no erythromycin-resistant *C. coli* isolates have been reported from Sweden and Norway where pig production and use of macrolides in livestock is minimal (0.4 mg/PCU and <0.01 mg/PCU, respectively) [18].

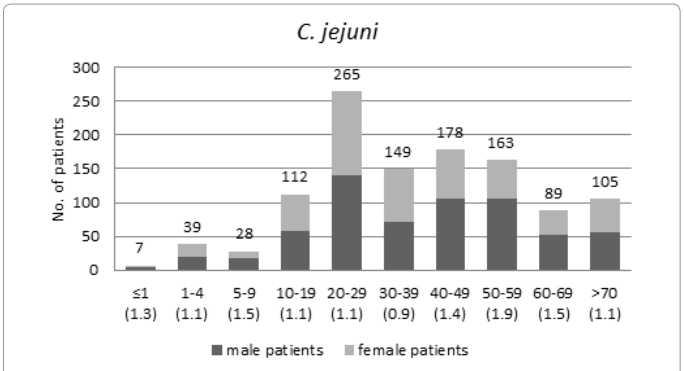

Figure 1: Gender distribution of *C. jejuni* isolates in different age group showing a slight male surplus in children and distinct male surplus in adults above 40 years of age while sex ratio in adolescents and young adults is almost even. Male-female ratio per age group is set in parenthesis.

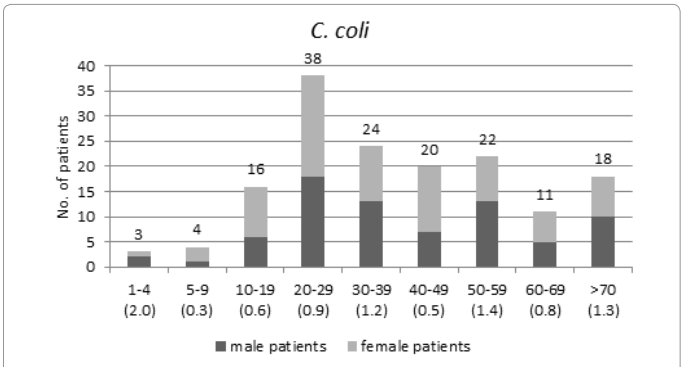

Figure 2: Gender distribution of *C. coli* isolates in different age groups showing a marked female surplus rate in children between 5-9 years and adults between 40-49 years. Male-female ratio per age group is set in parenthesis.

Antimicrobial agent	*Campylobacter spp.*	Rate of resistant isolates (%)	P value
Completely susceptible	*C. coli*	16/156 (10.3)	<0.001
	C. jejuni	280/1135 (24.7)	
Ampicillin 10 µg	*C. coli*	72/156 (46.2)	0.618
	C. jejuni	548/1135 (48.3)	
Ciprofloxacin 5 µg	*C. coli*	98/156 (62.8)	0.699
	C. jejuni	732/1135 (64.5)	
Tetracycline 30 µg	*C. coli*	107/156 (68.6)	<0.001
	C. jejuni	399/1135 (35.2)	
Erythromycin 10 µg	*C. coli*	23/156 (14.7)	<0.001
	C. jejuni	7/1135 (0.6)	
Ampicillin + Ciprofloxacin	*C. coli*	54/156 (34.6)	0.293
	C. jejuni	447/1135 (39.4)	
Ampicillin + Tetracycline	*C. coli*	53/156 (34.0)	0.003
	C. jejuni	254/1135 (22.4)	
Ampicillin + Erythromycin	*C. coli*	15/156 (9.6)	<0.001
	C. jejuni	3/1135 (0.3)	
Ciprofloxacin + Tetracycline	*C. coli*	75/156 (48.1)	<0.001
	C. jejuni	365/1135 (32.2)	
Ciprofloxacin + Erythromycin	*C. coli*	17/156 (10.9)	<0.001
	C. jejuni	5/1135 (0.4)	
Tetracycline + Erythromycin	*C. coli*	20/156 (12.8)	<0.001
	C. jejuni	4/1135 (0.4)	
Tetracycline + Erythromycin + Ciprofloxacin	*C. coli*	16/156 (10.3)	<0.001
	C. jejuni	3 (0.3)	
Ampicillin + Erythromycin + Ciprofloxacin	*C. coli*	14 (8.9)	<0.001
	C. jejuni	3 (0.3)	
Ampicillin + Erythromycin + Tetracycline	*C. coli*	14 (8.9)	<0.001
	C. jejuni	2 (0.2)	
Ampicillin + Tetracycline + Ciprofloxacin	*C. coli*	44/156 (28.2)	0.049
	C. jejuni	241/1135 (21.2)	
Completely resistant	*C. coli*	14/156 (8.9)	<0.001
	C. jejuni	2/1135 (0.2)	

Table 2: Antimicrobial susceptibility of *C. jejuni* and *C. Coli* isolates showing high resistance rates of *C. jejuni* and *C. coli* isolates to amoxicillin and ciprofloxacin as well as significantly increased resistance rates of *C. coli* isolates to tetracycline and erythromycin.

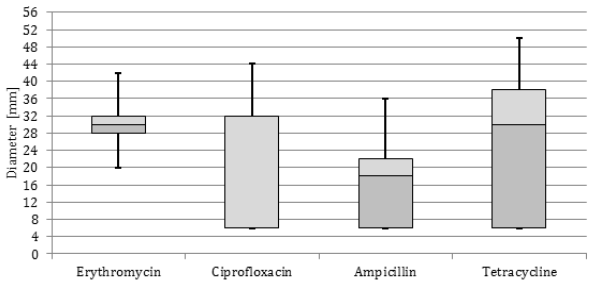

Figure 3: Box plots displaying distribution of inhibition zone diameters of four antimicrobials in 328 *C. jejuni* isolates.

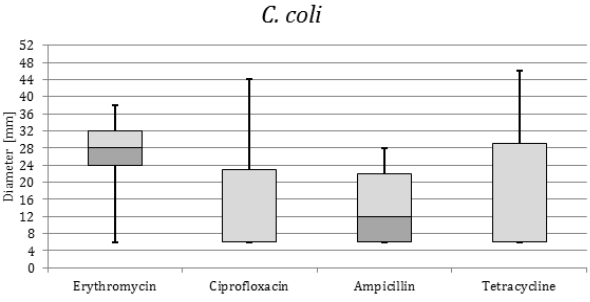

Figure 4: Box plots displaying distribution of inhibition zone diameters of four antimicrobials in 47 *C. coli* isolates.

Resistance of *C. coli* isolates to ciprofloxacin in our region was as high as compared to the French study while the quinolone resistance rate in our *C. jejuni* isolates was even 9% higher [13]. Quinolones are frequently administered in German poultry, the main reservoir of *C. jejuni* [19]. Increased quinolone resistance rate in *C. jejuni* isolates seems to be in accord with higher fluoroquinolone consumption in German livestock, which corresponded to 1.1 mg/PCU in 2015, thereby being three times as high as in France [9]. Significantly lower resistance rates of only 38% and 20% have been reported from Sweden and Norway, where consumption of quinolones in 2015 was below 0.02 mg/PCU. High prevalence rates of quinolone-resistant *Campylobacter spp*. of more than 90% have been reported from Spain and Portugal, thereby coinciding with high quinolone consumption of 8 and 9 mg/PCU in 2015, respectively [20].

The tetracycline resistance rate in French *C. coli* isolates was 70.2%, which was quite similar to our results, while resistance rate in French *C. jejuni* isolates was approximately 12% higher. This seems puzzling as tetracycline consumption does not differ significantly between Germany and France [9]. However, application data indicate that tetracyclines are more frequently administered in French cattle and poultry livestock [21], which represent important reservoirs for *C. jejuni*. Very high tetracycline resistance rates of up to 59% and 79% for *C. jejuni* and up to 79% and 93% for *C. coli* and have been reported from Italy and Spain, respectively, which seem to coincide with high consumption of tetracyclines (93.0 and 134.9 mg/PCU, respectively) [9]. Tetracycline resistance in *Campylobacter spp*. is mainly conferred by the plasmid-encoded *tet(O)* gene and active efflux by the CmeABC pump [5]. *C. coli* isolates in our study showed a significantly higher tetracycline resistance rate as compared to *C. jejuni*, which is consistent with EU surveillance data [17]. As active efflux and *tet(O)* gene transfer are not species-specific, the excess of tetracycline resistance in *C. coli* may rather be related to synergistic activity of different resistance mechanisms. Tetracycline resistance in our *C. coli* isolates was significantly more often associated with erythromycin resistance as compared to erythromycin-susceptible isolates. Thus, tetracycline resistance in *C. coli* may be enhanced by co-resistance to erythromycin. This hypothesis is also corroborated by a study on *C. coli* isolates from Norwegian pig farms showing only minimal resistance rates to tetracycline and erythromycin, which is in line with very low veterinary usage of both agents in Norway [18].

A worrisome 10.9% of our *C. coli* isolates were found to be co-resistant to ciprofloxacin and erythromycin. This is an alarming finding regarding the potential transmission of resistance genes as both agents are listed among the most critically antimicrobials for human medicine [6]. Furthermore, co-resistance to ciprofloxacin and erythromycin significantly limits oral therapy in patients with campylobacteriosis as amoxicillin-clavulanate was also only effective in 58% of completely resistant *C. coli* isolates. As a result, carbapenems remain the last line of defence against completely-resistant isolates.

Resistance rates of French *C. coli and C. jejuni* isolates to amoxicillin were only 34.8% and 9.6%, respectively. The lower prevalence of amoxicillin-resistant isolates in the French study may be related to lower penicillin consumption in France livestock, which corresponded to only 8.3 mg/PCU in 2015 compared to 38 mg/PCU in Germany. Low amoxicillin resistance rates have also been observed in Norway and Finland, which is in line with lower penicillin consumption of only 1.6 and 9.6 mg/PCU in 2015, respectively [9]. In contrast, high resistance rates to amoxicillin were observed in Italy where consumption of penicillin is more than twice as high as in Germany [22]. However, mechanisms of penicillin resistance in *Campylobacter spp.* are not yet fully understood as resistance rates of strains are highly variable [5]. Nevertheless, ß-lactamase induction may be the most likely explanation for higher amoxicillin resistance rates in our collection. It is noteworthy that all completely resistant isolates were still susceptible to imipenem as the first carbapenem-resistant Enterobacteriacae have been recently found in several swine and poultry farms in Germany [23]. Although carbapenems are not licensed for treatment in German livestock, high consumption of ß-lactams may select for carbapenem-resistant strains in the animal reservoir.

The fraction of *C. coli* in our collection was markedly higher as compared to recent surveillance data from the European Union (EU) member states and Germany, reporting a relative proportion of only 8% for *C. coli* on average [3,17]. However, only 53% and 67% of notified cases of campylobacteriosis in the EU member states and Germany were differentiated to the species level, respectively, which may result in undervaluing of *C. coli* infections. Higher consumption of pork or turkey, which are frequently contaminated with *C. coli* may have also contributed to high prevalence of *C. coli* in our collection [24]. Furthermore, the majority of our study population lived in rural areas, which has been identified as a potential risk factor for *C. coli* infections due to exposure to species-specific reservoirs such as standing water. Large-scale swine farming in our study region may have also contributed to the high prevalence of *C. coli* isolates via run-off of water or higher exposure of patients while visiting or living on a farm [25].

It has been shown that patients with *C. coli* infections were on average seven years older than patients with *C. jejuni* infections [26]. Nevertheless, the reason for this age difference yet remain unclear. Furthermore, the reported species-specific age difference was not observed in our study, so that it may have rather been influenced by behavioural aspects than biological age.

Prevalence of *C. jejuni* and *C. coli* in our study reached a maximum in young adults between 20-29 years. This finding is in accord with several epidemiological studies reporting highest incidence rates of campylobacteriosis in young adults [3,27]. This finding has been explained with a change in eating habits in this period of life such as consuming more chicken and eating out more frequently after leaving home [28,29]. In addition, the overall male bias of campylobacteriosis has been related to male-specific ineptness in kitchen and poultry-handling but also immunological deficiencies making male patients more prone to *Campylobacter* infections than female patients [30]. However, if stratified by age and patient`s gender, an almost even M-F ratio was observed for *C. jejuni* in patients aged between 10 years and 40 years, including even a slight female surplus in adults aged between 30-39 years. This finding has been explained by rising incidence rates of campylobacteriosis in female patients at this age due to either higher exposure to *Campylobacter spp.* from young children under their care but also to sex hormones [27]. In fact, incidence rates of female campylobacteriosis have been shown to rise and fall noticeably with the onset of puberty and the end of childbearing age and there is also scientific evidence that female sex hormones promote growth of *Campylobacter spp.* [31].

Contrary to gender distribution in *C. jejuni*, more female than male patients were found to be infected *with C. coli* including a marked female surplus among young children between 5-9 years although based on low case numbers and adults aged 40-49 years. The observed female excess among young children with *C. coli* infections is contrary to the observed male-biased sex ratio in *C. jejuni* infections at this age. As gender differences regarding nutrition or sex hormones at this age seem less likely, the underlying cause for this species-specific difference in young children remains unclear. It may be associated with immunological differences between female and male children at this age. The observed higher prevalence of *C. coli* infections in adult female patients has also been observed in an epidemiological study from Luxembourg and may rather reflect sex specific behavioural or nutritional habits [29].

According to our data, approximately one third of patients with campylobacteriosis were hospitalized, which contrasts with German surveillance data and a case-control study in a comparable rural region of Germany, which report lower hospitalisation rates of 18% and 24%, respectively [28]. The higher hospitalisation rate in our study may be explained with higher morbidity of campylobacteriosis due to antimicrobially resistant isolates [7]. However, our results show that hospitalisation rates were not associated with increased resistance patterns of *Campylobacter* isolates. To our mind, the higher hospitalisation rate in our study may rather be related to enhanced hospitals confinements of patients with unclear gastroenteritis as well as higher frequency of stool examinations in hospitalised patients.

Authors' Disclosures of Potential Conflicts of Interest

The authors declare no conflict of interest.

References

1. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM (2015) Global Epidemiology of Campylobacter Infection. Clin Microbiol Rev 28: 687-720.
2. Silva J, Leite D, Fernandes M, Mena C, Gibbs PA, et al. (2011) *Campylobacter* spp. as a foodborne pathogen: A review. Front Microbiol 2: 12.
3. Schielke A, Rosner BM, Stark K (2014) Epidemiology of campylobacteriosis in Germany - insights from 10 years of surveillance. BMC infectious diseases 14: 30.
4. Ternhag A, Asikainen T, Giesecke J, Ekdahl K (2007) A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with Campylobacter species. Clin Infect Dis 44: 696-700.
5. Wieczorek K, Osek J (2013) Antimicrobial resistance mechanisms among Campylobacter. Biomed Res Int 2013: 12.
6. Aarestrup FM (2015) The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. Philos Trans R Soc Lond B Biol Sci 370: 12.
7. Helms M, Simonsen J, Olsen KE, Molbak K (2005) Adverse health events associated with antimicrobial drug resistance in Campylobacter species: a registry-based cohort study. J Infect Dis 191: 1050-1055.
8. Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, et al. (2009) Antibiotic resistance in Campylobacter: emergence, transmission and persistence. Future Microbiol 4: 189-200.
9. Anonymous European Medicines Agency (2017) European surveillance of veterinary antimicrobial consumption. Sales of veterinary antimicrobial agents in 30 European countries in 2015: 177.
10. Wallmann JBA, Bode C, Köper LM, Heberer T (2017) Abgabemengenerfassung antimikrobiell wirksamer Stoffe in Deutschland 2016. Deutsches Tierärzteblatt 12: 1650-1659.
11. Szabados F (2012) Whole-cell matrix-assisted laser desorption ionization time-of-flight mass spectrometry applications in medical microbiology. J Med Microb Diagn 02: 2.
12. Szabados F, Tix H, Anders A, Kaase M, Gatermann SG, et al. (2012) Evaluation of species-specific score cutoff values of routinely isolated clinically relevant bacteria using a direct smear preparation for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based bacterial identification. Eur J Clin Microbiol Infect Dis 31: 1109-1119.
13. Sifre E, Salha BA, Ducournau A, Floch P, Chardon H, 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.
14. Gibreel A, Taylor DE. (2006) Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. J Antimicrob Chemother 58: 243-255.
15. van Rennings L, von Munchhausen C, Ottilie H, Hartmann M, Merle R, et al. (2015) Cross-sectional study on antibiotic usage in pigs in Germany. PLoS One 10: 28.
16. Juntunen P, Heiska H, Olkkola S, Myllyniemi AL, Hanninen ML (2010) Antimicrobial resistance in *Campylobacter coli* selected by tylosin treatment at a pig farm. Vet Microbiol 146: 90-97.
17. Anonymous EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control (2017) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. 15: 100-120.
18. Kempf I, Kerouanton A, Bougeard S, Nagard B, Rose V, et al. (2017) Campylobacter coli in organic and conventional pig production in France and Sweden: Prevalence and antimicrobial resistance. Front Microbiol 8: 9.
19. Humphrey TJ, Jorgensen F, Frost JA, Wadda H, Domingue G, et al. (2005) Prevalence and subtypes of ciprofloxacin-resistant Campylobacter spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. Antimicrob Agents Chemother 49: 690-698.
20. Anonymous (2017) NORM/NORM-VET 2016. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. 86-88.
21. De Briyne N, Atkinson J, Pokludova L, Borriello SP (2014) Antibiotics used most commonly to treat animals in Europe. Vet Rec 175: 8.
22. Giacomelli M, Salata C, Martini M, Montesissa C, Piccirillo A (2014) Antimicrobial resistance of Campylobacter jejuni and Campylobacter coli from poultry in Italy. Microb Drug Resist 20: 181-188.
23. Fischer J, Rodriguez I, Schmoger S, Friese A, Roesler U, et al. (2013) Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. J Antimicrob Chemother 68: 478-480.
24. El-Adawy H, Ahmed MF, Hotzel H, Tomaso H, Tenhagen BA, et al. (2015) Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* recovered from organic turkey farms in Germany. Poult Sci 94: 2831-2837.
25. Fitzenberger J, Uphoff H, Gawrich S, Hauri AM (2010) Urban-rural differences

of age- and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005-June 2006. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European Communicable Disease Bulletin 15: 7.

26. Bessede E, Lehours P, Labadi L, Bakiri S, Megraud F (2014) Comparison of characteristics of patients infected by Campylobacter jejuni, Campylobacter coli, and Campylobacter fetus. J Clin Microbiol 52: 328-330.

27. Gillespie IA, O'Brien SJ, Penman C, Tompkins D, Cowden J, et al. (2008) Demographic determinants for Campylobacter infection in England and Wales: Implications for future epidemiological studies. Epidemiology and Infection 136: 1717-1725.

28. Rosner BM, Schielke A, Didelot X, Kops F, Breidenbach J, et al. (2017) A combined case-control and molecular source attribution study of human Campylobacter infections in Germany, 2011-2014. Scientific reports 7: 12.

29. Mossong J, Mughini-Gras L, Penny C, Devaux A, Olinger C, et al. (2016) Human campylobacteriosis in Luxembourg, 2010–2013: A case-control study combined with multilocus sequence typing for source attribution and risk factor analysis. Scientific reports 6: 20939-20951.

30. Strachan NJ, Watson RO, Novik V, Hofreuter D, Ogden ID, et al. (2008) Sexual dimorphism in campylobacteriosis. Epidemiology and Infection 136: 1492-1495.

31. Yokoyama M, Hinode D, Masuda K, Yoshioka M, Grenier D (2005) Effect of female sex hormones on Campylobacter rectus and human gingival fibroblasts. Oral Microbiol Immunol 20: 239-243.