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: 1136 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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Received December 18, 2017; Accepted January 08, 2018; Published January 11, 2018

Citation: Hartmann L, Schieweck O, Greie JC, Szabados F (2018) Human Campylobacter jejuni and Campylobacter coli Isolates: Demographic Pattern and Antimicrobial Susceptibility to Clinically Important Antimicrobials used in Livestock. J Med Microb Diagn 7: 269. doi:10.4172/2161-0703.1000269

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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/km². 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. lari 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 deoxycholate agar plates (mCCDA-Preston, OXOID®) 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 B-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® 42947 served as quality controls. Inhibition zone diameters of the four routinely used antimicrobials.

Statistical Analysis

Statistical analyses were performed using the χ²-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 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.

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; *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). Complete resistance rates 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].

| 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 (12.6) | <0.001 |
| C. jejuni | 4/1135 (0.4) |
| Tetracycline + Erythromycin + Ciprofloxacin | C. coli | 16/156 (10.3) | <0.001 |
| C. jejuni | 3/1135 (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.
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, β-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 Enterobacteriaceae 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 β-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 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 confinement 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.

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