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QUO VADIS? – MONITORING *CAMPYLOBACTER* IN GERMANY

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Campylobacter is a poorly recognized foodborne pathogen, leading the statistics of bacterially caused human diarrhoea in Europe during the last years.

In this review, we present qualitative and quantitative German data obtained in the framework of specific monitoring programs and from routine surveillance. These also comprise recent data on antimicrobial resistances of food isolates. Due to the considerable reduction of *in vitro* growth capabilities of stressed bacteria, there is a clear discrepancy between the detection limit of *Campylobacter* by cultivation and its infection potential. Moreover, antimicrobial resistances of *Campylobacter* isolates established during fattening of livestock are alarming, since they constitute an additional threat to human health.

The European Food Safety Authority (EFSA) discusses the establishment of a quantitative limit for *Campylobacter* contamination of broiler carcasses in order to achieve an appropriate level of protection for consumers. Currently, a considerable amount of German broiler carcasses would not comply with this future criterion. We recommend *Campylobacter* reduction strategies to be focussed on the prevention of fecal contamination during slaughter. Decontamination is only a sparse option, since the reduction efficiency is low and its success depends on the initial contamination concentration.

Keywords: *Campylobacter* traceability, quantitative detection, prevalences in animal and food, antibiotic resistances, reduction strategies

Impact of *Campylobacter* as a food-associated pathogen

During the last few years, and hardly recognized by the public, *Campylobacter* is the most prevalent food-poisoning bacterium in Europe. The pathogen causes watery or bloody diarrhoea, which is frequently self-limiting after 4–7 days. Complications of the disease are reactive arthritis and peripheral neuropathies, e.g. the Guillain–Barré syndrome, which is estimated to occur in approximately 1 per 1000 cases (for a recent review on *Campylobacter* pathogenicity, see Ref. [1]). While a significant decrease in human infections caused by Salmonella could be observed, the frequency of human campylobacteriosis remained high during the last years, leading to more than 65,000 reported cases in 2010 in Germany (*Fig. 1*, [2]). The total number of reported human campylobacteriosis cases in 2011 was 70,560 in Germany ([2], last update 11.01.2012). This increase is most likely explained by the Shiga-toxin producing O104:H4 *E. coli* outbreak in Germany in 2011 and the elevated proportion of cases of diarrhoea investigated by medical practitioners. Hence, the true number of campylobacteriosis cases is probably significantly higher and estimated to reach more than four times the reported number [3]. In 2010, among cases with full typing details available, in particular *Campylobacter jejuni* were involved in human infections in Germany (90.8%), followed by *C. coli* (8.1%) and *C. lari* (1%). Only very few human cases were caused by *C. upsaliensis* (0.07%), *C. fetus* (0.02%), or others (0.01%).

Detection limitations of *Campylobacter* and the impact of viable but non-culturable (VBNC) forms

Campylobacter belongs to the ε-proteobacteria, is quite fastidious *in vitro*, and is limited to growth under microaerobic atmosphere and temperatures of 30–42 °C. The natural multiplication site of this bacterium is the intestine of endotherms, in particular poultry, but also mammals including humans [4]. Once outside the intestine, the bacterium is not capable of growth on food matrices. For several years, standard techniques are available to culture and detect *Campylobacter*. The ISO standard 10272:2006 details qualitative and quantitative procedures as well as a semiquantitative method for the detection of thermophilic *Campylobacter*. The high *in vitro* generation time of *Campylobacter* compared to competing intestinal flora constitutes the need for selection of *Campylobacter* by specific

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antibiotics, to which the bacterium exhibits intrinsic resistance. To improve the power of the detection strategy, two independent selective media are used in combination.

Outside the intestine, the bacterium is particularly confronted with oxidative and cold stress. However, stress situations impair the bacterium's capacity to subsequently multiply *in vitro,* thus hindering its proper detection. There are probably all kinds of intermediate states, which might also fail to grow *in vitro* while maintaining their infectious potential. But frequently observed and most vigorously discussed, *Campylobacter* transforms into a coccoid form, which definitely fails to grow *in vitro* [5]. However, such bacterial suspensions are capable of infection in various animal models, from which spiral and culturable *Campylobacter* were reisolated [6, 7]. Also, the invasion of human epithelial cells was shown using coccoids without capacity to grow on agar plates [8]. Hence, there is a clear discrepancy between the detection limit of *Campylobacter* by cultivation and its infectious potential, which constitutes a barrier for getting a realistic view about the transmission routes of this pathogen.

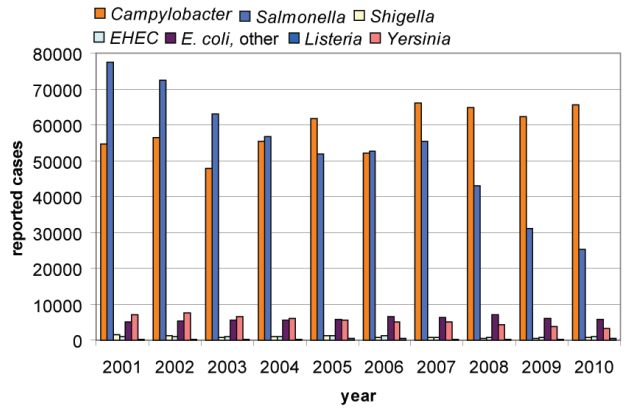

Fig. 1. Zoonotic infections in humans in Germany from 2001 until 2010 (data collected by the Robert-Koch Institute [2]). In 2010, the species causing campylobacteriosis were detected as *C. jejuni* (90.8%), *C. coli* (8.1%), *C. lari* (1%), *C. upsaliensis* (0.07%), *C. fetus* (0.02%), and others (0.01%).

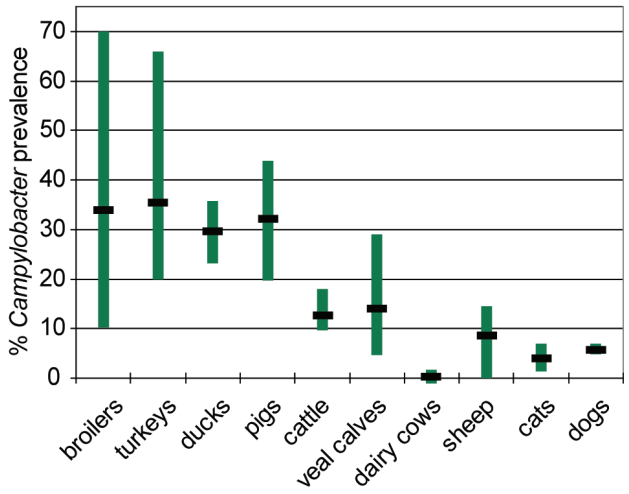

Fig. 2. Prevalence of *Campylobacter* ssp. in German livestock and pets (2004–2010). Vertical bar: distribution of prevalences detected; horizontal bar: mean. Data are from surveillance and zoonosis monitoring from 2 (ducks, dairy cows), 3 (sheep), 4 (turkeys), 5 (cats, dogs), 6 (cattle), or 7 (broilers, pigs) datasets (years) collected by the BfR.

Prevalence of *Campylobacter* in animals and food in Germany

In order to get insight into the distribution of infectious agents transmitted by animals and food, specific monitoring programs for *Campylobacter* were started in 2009 in Germany based on the European directive 2003/99/EC. By this directive, the European Member States are committed to gather, evaluate, and publish representative data on the prevalence and antimicrobial resistance of zoonotic agents in food, feed, and animals. The Federal Institute for Risk Assessment (BfR) coordinates this annual zoonosis monitoring program, in which the competent authorities of the Federal States take samples along the food chain according to a specific sampling plan involving primary production, slaughterhouses, and retail sampling. Hence, the National Reference Laboratory for *Campylobacter* routinely receives fresh *Campylobacter* isolates for further analysis in order to get a comprehensive picture about the national situation. Additional isolates come from routine sampling in the framework of surveillance of food producers and retail conducted by the federal authorities (according to regulation 2004/882/EC). The combined efforts intend to provide information about the source of *Campylobacter* contamination during food production. On this basis, suitable strategies to prevent dissemination of the infectious agents can be envisaged.

From data obtained in the last 7 years (2004 until 2010) in Germany, it is obvious that *Campylobacter* is most prevalent in poultry, such as broilers and turkeys, but also ducks, with mean values of around 30–40% and maxima of 36% (ducks) to 66–70% (turkeys and broilers) *(Fig. 2)*. The detection rates varied considerably from year to year, in particular for broilers. This is partly explainable by a variation in the quality of the samples, in terms of *Campylobacter* culturability (feces from boot socks, feces as fresh droppings, cecum samples, different transport conditions, and time). The lowest annual prevalence rate (10.2%) was found in 2009 using "boot socks", by which feces were taken from the floor of the chicken house, thereby bearing the risk of collecting *Campylobacter* after drying. Consistently, it was demonstrated that drying abrogates the culturability (and also viability?) of *Campylobacter* [9]. As expected from the combined results from poultry flocks, products from this origin are frequently contaminated with *Campylobacter*, ranging from around 20% positive fresh turkey meat samples, over 30–45% broiler fresh meat, to 30–65% duck meat during the years 2009 and 2010 *(Fig. 3)*. These data have to be considered as the lower limit of *Campylobacter* prevalence on these products (see also estimation below). In contrast, the mean prevalence of *Campylobacter* in pigs, cattle, and veal

calves ranged from 13% (cattle) and 14% (veal calves) to 32% (pigs) *(Fig. 2)*, whereas the respective meat *(Fig. 3)* was only rarely contaminated with *Campylobacter*. The different magnitudes of *Campylobacter* dissemination in meat is understandable with respect to different slaughtering techniques. The probability for fecal contamination is much higher during slaughter of chicken than of pigs [10, 11]. Since *Campylobacter* cells do not multiply on food, the initial bacterial contamination is most relevant for the magnitude of the risk of infection for the consumer.

Although poultry meat is considered to be the main cause for infection by *Campylobacter* (see below), direct contact with pets (dogs, cats, but also farm animals) has to be kept in mind as additional transmission route [12, 13]. The reported prevalence of *Campylobacter* in dogs and cats is quite stable and ranges at around 5% in Germany *(Fig. 2)*. The prevalence of *Campylobacter* in dairy cows (0.3%) and raw milk (0.9–1.9%) is reproducibly low, although *Campylobacter* infections after consumption of nonpasteurized milk have been reported repeatedly [14, 15].

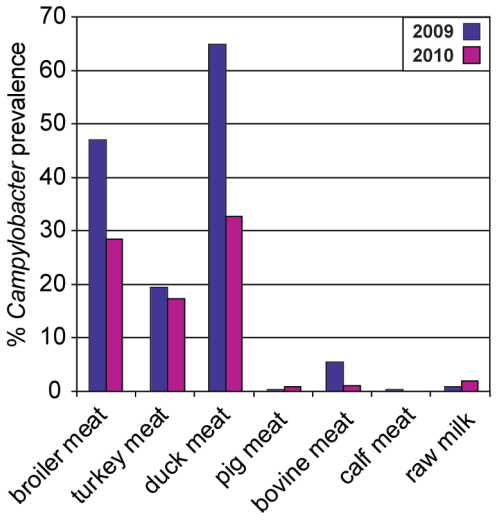

Fig. 3. Prevalence of *Campylobacter* ssp. in German food at retail (2009 and 2010). Data are from zoonosis monitoring, if available, otherwise from surveillance [43–45].

Source attribution of human *Campylobacter* infections

Campylobacter is considered to be a genetically highly variable organism, capable of horizontal gene transfer and frequent recombination. Furthermore, mixed populations of this organism (co-colonization of genetic variants of one species and/or multiple species) frequently colonize one host organism. The diversity of *Campylobacter* species and genotypes is certainly underestimated, since one or a few single colonies are routinely diagnosed. The existence of communities of high genetic flexibility probably enhances the bacterium's fitness for host switchover and adaptation to changing environments, for example, antimicrobial treatments during fattening. This feature of the bacterium aggravates direct genotypic matching of a single food isolate with the respective putative counterpart isolated from humans. As mentioned above, the capability of *in vitro* growth does not necessarily reflect the capability of host infection. By enrichment and isolation of the bacteria, the most dominant species capable of fastest *in vitro* growth in the respective selective medium is the one to be detected and identified. Using MLST (multilocus sequence typing), conserved "house-keeping" genes of *Campylobacter* isolates were sequenced and ordered in terms of similarity. On the basis of MLST data, the authors of a study from England estimated that 57% of all *C. jejuni* human infections were due to poultry products, 35% originated from cattle, 4% from sheep, less than 1.6% from wild birds, and less than 1% from pigs or from environmental water [16]. According to a study from Scotland, poultry accounted for 58–78% of all *C. jejuni* and for 40–56% of all *C. coli* human infections [17]. Turkeys contributed to only less than 1% to the *C. coli*-caused campylobacteriosis cases. *Campylobacter* from cattle was considered responsible for 10–12% of *C. jejuni* and 2–14% *C. coli* infections, while sheep was assigned for 8–26% *C. jejuni* and 40% *C. coli* infections. The contribution of *Campylobacter* from wild birds, pigs, and the environment as causative agent for human infection was also predicted to be low. In a Finnish study, *Campylobacter* from poultry were supposed to be equally responsible for human campylobacteriosis as *Campylobacter* from cattle [18]. The authors explained this phenomenon as due to the low prevalence of *Campylobacter* in Finnish poultry primary production, which is exceptional in Europe. For Germany, such a study is still missing. The public database for MLST (PubMLST, www.pubmlst.org) comprises currently less than 500 *C. jejuni* isolates from Germany, but probably (as for all other countries) only a part of the MLST results are submitted. Hence, the publication of a

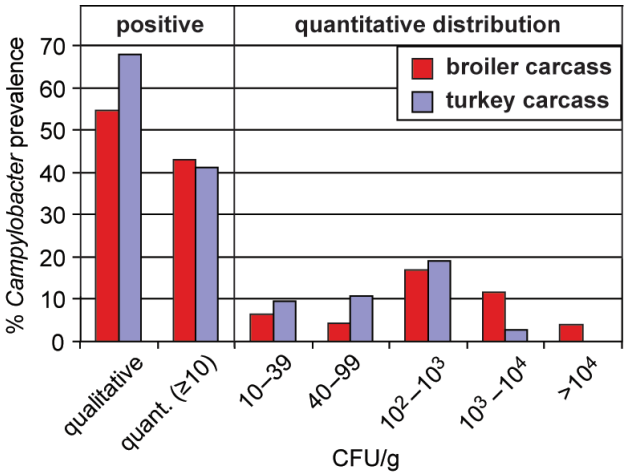

Fig. 4. *Campylobacter* detection on broiler and turkey carcasses in Germany according to ISO 10272–1 (qualitative) and ISO 10272–2 (quantitative). Broiler carcasses from the baseline study 2008 ($n_{qual.}$ = 432, $n_{quant.}$ = 432); turkey carcasses from the zoonosis monitoring 2010 ($n_{qual.}$ = 359, $n_{quant.}$ = 356) [22, 45].

similar analysis with specific source attribution of campylobacteriosis in Germany is expected. However, on the basis of the German prevalence data for *Campylobacter* in chicken and on carcasses, at least the contribution of poultry products to human *Campylobacter* infections is anticipated to be as high as estimated for England or Scotland. The EFSA has summarized the available MLST studies from different countries, estimating that 30–50% of the campylobacteriosis cases come from direct consumption and/or handling of chicken meat [3]. The major transmission route might be the consumption of undercooked meat and cross-contamination of meals during the preparation of fresh poultry meat [19]. Moreover, 50–80% of all human *Campylobacter* infections were attributed to the "chicken reservoir as a whole" [3]. The transmission route of *Campylobacter* for the latter phenomenon remains to be deciphered. In this context, also the transmission of *Campylobacter* via contaminated vegetables might play a role [3, 20].

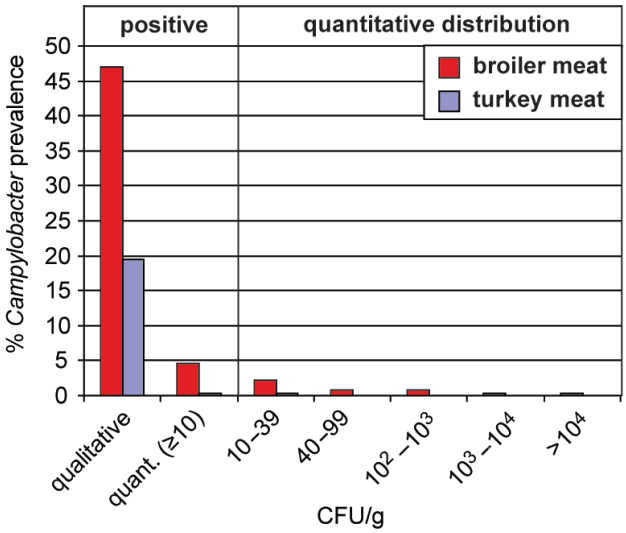

Fig. 5. *Campylobacter* detection on broiler and turkey meat in Germany according to ISO 10272–1 (qualitative) and ISO 10272–2 (quantitative). Broiler meat from the zoonosis monitoring 2009 ($n_{qual.}$=413, $n_{quant.}$=349); turkey meat from the zoonosis monitoring 2010 ($n_{qual.}$=399, $n_{quant.}$=564) [44, 45].

Qualitative versus quantitative detection of *Campylobacter*

The European baseline study conducted in 2008 was a systematic approach for the detection of *Campylobacter* on broiler carcasses by analyzing neck skin samples [21]. While using the qualitative method (ISO 10272–1) 55% of the broiler carcasses were *Campylobacter* positive, the enumeration method (ISO 10272–2) revealed *Campylobacter* on 43% of the carcasses. According to either method, 62% of the carcasses were *Campylobacter*-positive [22]. Assuming that this number is the "true number" of positives, 58% of these true positive carcasses (35.9% of all carcasses) were concordantly revealed by both methods. Further, 30% of the true positives (19% of all carcasses) were detected by the qualitative method and 12% (7.2% of all carcasses) were detected only using the quantitative method. It is expected that the qualitative method is superior to quantitative methods concerning sensitivity (the bacterium is first enriched before detection). In contrast, the quantitative method is advantageous in cases of inefficient suppression of competitive flora, outcompeting *Campylobacter* during enrichment. Direct dilution of the sample and plating on solid agar guarantee the immediate spatial separation of *Campylobacter* from competing cells.

Interestingly, the proportion of positive carcasses detected by only the quantitative method varied significantly between European Member States [23]. In Belgium, 68% of the total number of positive carcasses was detected only by the quantitative approach, followed by the Netherlands with 32% and Portugal with 16%. The presence of extended β-lactamase (ESBL) producing *E. coli* can hinder detection via the qualitative ISO method, since those cefoperazone-resistant bacteria grow in Bolton broth used as pre-enrichment medium. Therefore, the data might implicate a different dissemination of ESBL-producing *E. coli* and/or other resistant background flora in broilers from different European countries. Currently, the use of Preston broth (additionally containing polymyxin B, e. g. for inhibition of ESBL *E. coli*) for samples with expected high background flora or, alternatively, direct plating of, for example, cecal samples is debated. In the latter samples, *Campylobacter* is supposed to be present as extremely vital cells in high numbers, justifying the omission of an enrichment step. Still, Bolton broth is accepted to be the most sensitive medium for enrichment of stressed *Campylobacter.*

How can the data from broiler and turkey carcasses be interpreted compared to those from meat? Does the reduction of *Campylobacter* counts detected on meat products indicate safety? From the data obtained from poultry carcasses, it is obvious that broiler carcasses manifest higher *Campylobacter* loads than turkey carcasses. While 15.5% of broiler carcasses had *Campylobacter* concentrations higher than 1000 CFU/g, this accounted for only 2.8% of the turkey carcasses (*Fig. 4*). However, also turkey carcasses were frequently contaminated with *Campylobacter*, as documented with 68% positively tested samples via qualitative detection. Only one sample was positive according to the quantitative method but failed to be positive after enrichment (0.7% in contrast to 12% of positive broiler carcasses). This might suggest that, in contrast to broilers, background flora did not pose a problem for the detection of *Campylobacter* in turkeys. It also implicates that the prevalence on broiler carcasses is underestimated by using only the qualitative method.

Comparing the *Campylobacter* contamination of broiler carcasses with fresh meat at retail, the qualitative data do not suggest a reduction of *Campylobacter* prevalence on broiler meat *(Fig. 5)*. However, while interpreting the quantitative data, a significant reduction of the amount of culturable *Campylobacter* on meat was observed. First,

Campylobacter is predominantly transferred to the meat product via fecal contamination on the surface during slaughter. Part of the meat products were devoid of skin (e.g. breast filet), thereby contributing to a real decrease in *Campylobacter* concentration. Second, part of the products might have been frozen (the term "fresh meat" also includes frozen meat). Freezing is considered to be a physical decontamination process, leading to a 2 log reduction of *Campylobacter* concentration after 3 weeks of freezing [3]. Third, stressed and nonculturable cells of *Campylobacter* do not grow *in vitro* on selective medium and are not accessible for common detection methods [24]. Under meat storage conditions (4 °C), the number of culturable *Campylobacter* on chicken skin was reduced by 2 log within the first 2–5 days depending on the strain tested [25]. The quantitative distribution of the *Campylobacter* concentrations on carcasses peaked at 100–1000 CFU/g *(Fig. 4)*. The results hint at a 3 log *Campylobacter* reduction when fresh carcasses are compared with fresh meat. We hypothesize that one of the major contributors for reduction of *Campylobacter* on meat versus carcasses might be an "apparent" reduction due to loss of culturable bacteria, as also observed by Chaisowwong et al. [8]. Further analysis is needed to estimate the "true" reduction caused by death and/or removal of *Campylobacter* cells. For this purpose, it is necessary to develop appropriate methods for the detection of stressed and viable but nonculturable *Campylobacter* that do not grow on selective media. In any case, with respect to constant high chicken-derived *Campylobacter* infection rates, the residual culturable *Campylobacter* found on poultry meat together with those that are nonculturable and/or dead must be considered a sufficient threat for human infections. As a conclusion, when setting a quantitative value (microbiological criterion) as an efficient strategy for the reduction of *Campylobacter*, this value is most appropriately monitored at the slaughterhouse. On meat products, the *Campylobacter* counts do not sufficiently reflect the infection risk for consumers, at least not on the quantitative level.

Antimicrobial resistance in *Campylobacter*

In the framework of the zoonosis monitoring, we characterized the antimicrobial resistance profiles of the isolates from food matrices and animal origin in 2009 and 2010 (*Figs 6* and *7*). Over 1000 German *Campylobacter* isolates were subjected to antimicrobial resistance profiling. In order to cover all relevant food chains with a representative number of samples, maintaining practicability for the Federal States, the monitoring program focuses every year on a subset of pathogen–matrix combinations. In 2009, *Campylobacter* isolates were analyzed from chicken (feces from laying hens and broilers) and broiler meat as well as from veal calves (colon) and from raw milk at farm. In 2010, the same was done for isolates from turkeys at slaughter (cecum, carcass) and turkey meat at retail, and again from raw milk. Seven antimicrobials representing five different classes were tested using the microdilution method and a European-wide standardized microplate format (EUCAMP). The results were interpreted using epidemiological cut-off values according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, *Table 1*).

Our data show that *Campylobacter* isolated from food and animals exhibit high resistance, in particular to fluoroquinolones and tetracycline (*Figs 6* and *7*). The proportion of resistant isolates depended on the origin and *Campylobacter* species. In general and previously observed by others [26], *C. coli* were significantly more resistant than *C. jejuni*. Resistance to ciprofloxacin was frequently observed in around 40% of the *C. jejuni* isolates from veal calves and broiler meat as well as to over 90% for *C. coli* isolates from

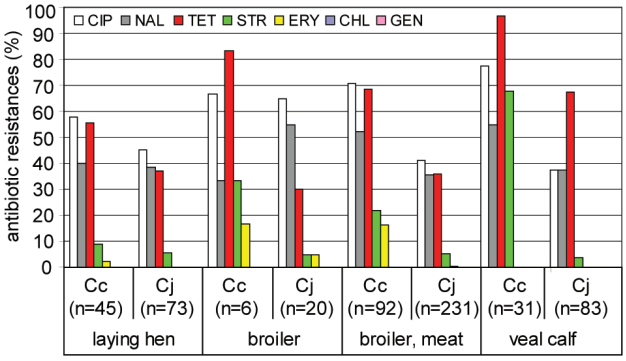

Fig. 6. Antimicrobial resistances of *Campylobacter* isolates from laying hen, broiler, broiler meat, and veal calf. CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; STR, streptomycin; ERY, erythromycin; CHL, chloramphenicol; GEN, gentamicin; Cj, *C. jejuni*; Cc, *C. coli*; *n*, number of tested isolates. Isolates stem from the zoonosis monitoring program 2009; isolates from broiler meat stem from both monitoring and surveillance.

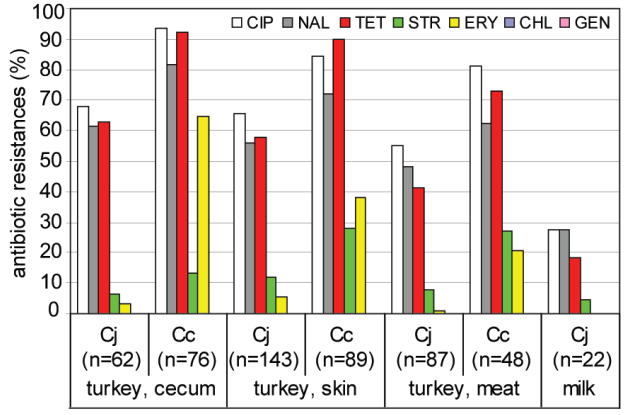

Fig. 7. Antimicrobial resistances of *Campylobacter* isolates from turkey cecum, skin, and meat, and from milk. CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; STR, streptomycin; ERY, erythromycin; CHL, chloramphenicol; GEN, gentamicin; Cj, *C. jejuni*; Cc, *C. coli*. Isolates stem from the zoonosis monitoring program 2010. Isolates from turkey meat and raw milk originate from both monitoring and surveillance. Due to the low number isolates from raw milk, isolates obtained in 2009 and 2010 were pooled.

Table 1. Test range of antibiotic concentrations and interpretation criteria for *C. jejuni* and *C. coli*

Class	Antimicrobial	Cut-off #(μg ml^{-1})	Range of test concentrations		Reference
			Minimum (μg ml^{-1})	Maximum (μg ml^{-1})	
Aminoglycoside	GEN	1*/2**	0.125	16	2007/516/EG
	STR	2*/4**	1	16	2007/516/EG
(Fluoro-)quinolone	NAL	16*/32**	2	64	EUCAST
	CIP	1	0.06	4	2007/516/EG
Tetracycline	TET	2	0.25	16	2007/516/EG
Macrolide	ERY	4*/16**	0.5	32	2007/516/EG
Phenicol	CHL	16	2	32	EUCAST

**C. jejuni*, ***C. coli* cut-off values were defined according to the European decision 2007/516/EG or according to EUCAST (www.eucast.org). CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; STR, streptomycin; ERY, erythromycin; CHL, chloramphenicol; GEN, gentamicin; #, values higher than the cut-off value indicate resistance.

turkey cecum samples. In veal calves, nearly all *C. coli* (97%) and 68% of the *C. jejuni* isolates were tetracycline-resistant. In isolates from poultry origin, tetracycline resistance of *C. coli* ranged between 56% (laying hens) and over 90% (turkey, cecum and veal calves) and that of *C. jejuni* between 30% (broiler) and 68% (veal calves).

But also resistances to streptomycin and erythromycin were considerable (*Figs 6* and *7*). The species-specific degree of resistance was especially obvious for the prevalence of resistance against erythromycin and streptomycin. While 65% *C. coli* isolates were erythromycin-resistant when isolated from turkey cecum, this accounted for 40% *C. coli* from turkey skin and around 20% from turkey meat. In contrast, the proportion of erythromycin-resistant *C. jejuni* from the same origins ranged between 1.1% and 5.6%. An analogous situation was revealed for streptomycin resistance, for which species differences were most pronounced in isolates from veal calves. Here, 68% of the *C. coli* exhibited streptomycin resistance, while this was the case for only 4% of the respective *C. jejuni* isolates. The reason for this phenomenon remains to be elucidated.

The fact that isolates from milk had rather low overall resistance rates suggested that antibiotic administration to dairy cows might have been lower than to the other tested farm animals. However, due to the low prevalence of *Campylobacter* in raw milk, only few isolates were tested ($n = 6$ in 2009, $n = 16$ in 2010).

Also, the number of *Campylobacter* isolates for antimicrobial resistance analysis from broiler flocks was rather low ($n = 26$), since boot socks appear to be an inadequate collection device for culturable *Campylobacter* from feces (see above). Further representative data on German broilers will be available soon from the monitoring conducted in 2011 on the basis of cecal samples. But nevertheless, the statistics of antimicrobial resistance on *Campylobacter* from broiler meat collected in 2009 was deduced from a sufficiently large number of isolates ($n = 323$). Since broiler meat is supposed to constitute the major source of infection for human campylobacteriosis, *Campylobacter* isolates from this origin should principally match the prevalence of antimicrobial resistances of human isolates. When comparing data from human isolates collected by the Robert-Koch institute (data from 2005–2007, [27]), a low amount of both *C. jejuni* and *C. coli* human isolates (5–8%) showed resistance to chloramphenicol and gentamicin, which was not the case in isolates from food and animals. However, the overall antimicrobial resistances of *C. jejuni* isolates from broiler correlated well with those from isolates of human origin ($R^2 = 0.82$), while those of *C. coli* did not ($R^2 = 0.47$). This may suggest that a considerable proportion of *C. coli* originated from a different source than broiler. Alternatively, or in addition, it might be indicative of a higher adaptive potential of *C. coli*, more rapidly losing and/or gaining new antimicrobial resistances when facing changing environments (human host).

In conclusion, the prevalence of antimicrobial resistances in *Campylobacter* from food and food-producing animals is alarming, because resistant bacteria can frequently be transmitted to the human host. Although most of the *Campylobacter* infections do not require antimicrobial treatment, severe cases, especially in immunocompromised patients, demand effective antibiotics for treatment of campylobacteriosis. Thus, the high antimicrobial resistance rates found in *Campylobacter* constitute an additional risk, to which the consumer is exposed to upon transmission of this pathogen via food. These data again demonstrate that there is an urgent need to minimize antimicrobial treatment in primary production.

Reduction strategies

Since broiler chickens constitute the main source for *Campylobacter* infections, reduction strategies focus on production of broiler meat. According to a mathematical model, the EFSA estimates that a reduction of human

campylobacteriosis by 50% or 90% can be achieved if a microbiological criterion of 1000 or 500 CFU/g carcass skin, respectively, is established [3]. Currently, 15.5% of the broiler carcasses and 2.8% of the turkey carcasses in Germany would not comply with the upper limit of 1000 CFU/g (*Fig. 4*). Hence, a quantitative reduction of *Campylobacter* on chicken carcasses is crucial. In principle, the prevalence of foodborne zoonotic pathogens can be reduced at different levels of the food chain, such as primary production (prevention of pathogen entry into the food processing chain), slaughtering process (prevention of fecal contamination), and post slaughtering (decontamination).

Unlike Salmonella, *Campylobacter* is not vertically transmitted from breeder flock to progeny but its dissemination is merely horizontal [28]. Therefore, hygienic measures in primary production are essential to avoid spread of the bacterium and are considered to be key strategies for reduction of *Campylobacter* loads on food [3]. The probability of *Campylobacter* colonization increases with the age of the chicken [3]. Recent evidence was provided that the latter can be explained by the combined effect of colonization resistance of young chickens mediated by maternal antibodies and the probability of *Campylobacter* exposure [29]. Intriguingly and currently inexplicable, newly hatched chicken were highly susceptible towards *Campylobacter* colonization, although the level of maternal antibodies was the highest. However, resistance was established within 3 days and lasted for over 3 weeks. More work is needed to understand the interplay between *Campylobacter*, the host immune system, and microbiota, which are key players in defining the colonization capacity of the bacterium [30].

Campylobacter-specific bacteriophages could potentially be exploited to decrease bacterial concentration in poultry prior to slaughter. Using different bacteriophages, a transient 1.5–5 log reduction in cecal *Campylobacter* concentrations, peaking approximately 2 days post administration, was observed in chickens (reviewed in Ref. [31]). Hence, current research aims to understand the molecular and phenotypic variety of different types of natural bacteriophages [32–35] in order to rationally design an appropriate cocktail for efficient reduction of *Campylobacter* in practice.

Campylobacter is accepted to be primarily a superficial contamination, which occurs during slaughtering [36]. The bacterium was occasionally also found inside the muscle of poultry meat collected at retail, however, quantitatively in very low numbers [37], and it is yet unclear if these *Campylobacter* recently originated from skin (via lesions) or had systemically been transmitted via blood. Freezing for 3 weeks is considered to be a physical decontamination strategy, which results in a decrease of *Campylobacter* counts by 2 logs. In contrast, there is an increasing demand for fresh, nonfrozen meat on the market. It is established that the bacterium tightly adheres to skin and meat surfaces. Since *Campylobacter* was found in deep crypts of the chicken skin [38], it is expected that, once spread over the surface of the chicken, its removal is rather complicated. Indeed, chemical decontamination resulted only in a reduction of *Campylobacter* concentration by around 1 log (on average) depending on the chemical and concentration used [3, 39].

Hence, the prevention of fecal contamination during slaughter appears to be the most efficient strategy for limiting the spread of the pathogen to food. Short-term feed withdrawal before slaughter for reduction of the amount of intestinal content is one of the means already implemented in practice [40]. When does fecal contamination take place most predominantly? Contamination of skin and feathers during transport due to leakage of feces is to be considered. However, quantitatively it is not comparable with the amount of feces distributed during slaughter. A study clearly showed the effect of contamination by fecal exit during defeathering. The cloacae of one group of chicken were closed by plugging and suturation after electrocution and scalding [41]. Post defeathering, *Campylobacter* counts on the carcass were determined quantitatively. While the control group was 100% *Campylobacter*-positive with an average of 4.5 log *Campylobacter* per carcass, 89% of the cloacae-sewed chicken were negative, with a residual 11% positive carcasses bearing 2.5 log *Campylobacter* on average per carcass. These results demonstrate that the main contamination stems from feces escape from the respective chicken during slaughter. Recently, variations in the slaughter process were tested for efficiency of reduction in fecal contamination during picking. The effect of hanging broiler carcasses with the vent down to allow escaped feces to fall on the ground rather than disseminate across the carcass was characterized by using the same standard shackle line [42]. With this approach, the plucking fingers probably exerted vigorous movement of the broiler carcass in all spatial dimensions during defeathering, which is ideal in the propagation of escaped feces across the carcass surface instead of ensuring its loss by gravity. Consistently, this method was shown to be ineffective in prevention of *Campylobacter* spread [42]. In future trials, it remains to be shown whether the (in principle) promising upside-down hang can be combined with a cloacal plugging device inserted from below the shackle line or even with concomitant evisceration from below in order to efficiently prevent fecal spread. Moreover, alternative spray-/splash-scalding processes have to be developed, lacking a common scalding tank for all carcasses, which leads to cross-contamination. In general, *Campylobacter* spread from feces to the carcass has to be prevented and should be in the focus of reduction strategies. This would not only lead to reduction of *Campylobacter* but also of any other potentially harmful intestinal microbes.

Conclusions

There is a clear gap in *Campylobacter* traceability and knowledge on its potential to colonize various hosts. More

research is needed to understand its success as a foodborne pathogen, which might be related to its enormous capability to develop genetic variants. Furthermore, the frequent antimicrobial resistance established during fattening has to be considered a significant threat to human health. The most realistic prevalence data on *Campylobacter* are obtained from fresh samples (e.g. at the slaughterhouse), where detection by cultivation methods is appropriate. We recommend reduction strategies to be focused on the prevention of fecal contamination during slaughter. Decontamination presents only a limited option, since the reduction efficiency is low and its success depends on the initial contamination concentration.

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