Campylobacter spp. as emerging food-borne pathogen - incidence, detection and resistance

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ABSTRACT

Campylobacter jejuni and Campylobacter. coli are the leading cause of bacterial food-borne enteric infection with still increasing incidence in the most developed countries. Consuming and/or handling poultry meat is the most consistent risk factor, linked to the high prevalence of campylobacters in retail poultry meat. Recent data about the incidence of human campylobacteriosis and prevalence of C.jejuni and C. coli in poultry meat are presented. Important aspects of Campylobacter transmission along the food chain are discussed – physiological specificities possibly enabling adaptation and survival in the food production environment as well as the emerging resistance to antimicrobial agents used in veterinary and human medicine. Recent advances in detection and identification methods of Campylobacter spp. are mentioned as a basis for preventive strategies to bring these food-borne pathogens under control. Recent risk assessments show that mitigation strategies could be applied at different points from food-animals production to the final consumption of foods. Educating the consumers is important, since critical control point remains the hygiene in the final food preparation.

Keywords: Campylobacter jejuni, Campylobacter coli, food-borne pathogens, incidence, detection, resistance, poultry meat;

INTRODUCTION

Emerging and re-emerging food-borne pathogens

Pasteur’s discoveries experimentally confirmed for the first time the involvement of microorganisms in food spoilage and specific human diseases. From that time a broad spectrum of pathogenic microorganisms in food and water supplies have been discovered (1). In fact, the spectrum of microbial food-borne pathogens has been changing over time. Well-established pathogens with known transmission routes have been controlled or even eliminated and new ones have emerged (2). True emergence, however, is rare. More common is re-emergence, where a known microorganism causes new types of infections, is associated with new vehicles, or appears in new geographic locations (3). Some food-borne pathogens are considered emerging because the role of food in their transmission has been recognized only recently or become more common (4).

Some food-borne pathogens developed new virulent strains by transfer of mobile virulence factors, the other one developed the ability of adaptation and better survival in adverse environmental conditions during food production, processing and preservation. New pathogens became emerge because of changing ecology and changing technology that connects a potential pathogen with the food chain (2).

Important reasons for emerging microbial food-borne pathogens and diseases are changes in modern lifestyle and human demographics affecting nutritious behaviour of population. Eating outside home in-advance-prepared meals in restaurants, canteens or even street vendors, but also home-prepared foods is combined by not always sufficient food safety education of food suppliers and consumers (5, 6, 7). On the other side, larger migrations of people, animals, foodstuffs and other commodities in globalized form of food production and distribution introduce the pathogens into new geographic areas (8, 9, 10). Nevertheless, emergence of new food-borne pathogens is a function of changes in host susceptibility (11). The population of highly susceptible persons is
expanding worldwide because of ageing, malnutrition, increasing number of immuno-compromised patients and other underlying medical conditions (4). Besides the changing spectrum of microbial food-borne pathogens and illnesses they cause, other characteristics can be recognized, such as global dissemination of some food-borne pathogens in pandemic form, their increasing antimicrobial resistance, identification of opportunistic pathogens and a new type of large and highly dispersed food-borne outbreaks, which may only be detected when microbial strains are collected, subtyped and compared in well-developed infectious disease surveillance systems (2, 9, 12).

Campylobacter spp. are zoonotic bacteria with the natural habitat in gastrointestinal tract of warm-blooded mammals and birds. They become the leading cause of food-borne infection in many countries (13). Human campylobacteriosis shares many of the general characteristics of food-borne infections, mentioned above. However, in some aspects campylobacters are specific. The objective of this paper is a brief review of current data about the increasing incidence of human Campylobacter infections with connection to the high prevalence of thermophilic campylobacters in food products, especially poultry meat. Important facts of Campylobacter spp. transmission along the food chain are discussed, such as their resistance to ecological factors influencing the survival in the food environment as well as their increasing resistance to antimicrobial agents used in veterinary and human medicine. A development of detection and identification procedures for thermophilic Campylobacter spp. in/from food is briefly reviewed.

### EPIDEMIOLOGY

#### Incidence of Campylobacter infections

The first observation of Campylobacter-like microorganisms as agents of disease dates more than a century ago. In 1886, Escherich reported the uncultivable Campylobacter-like forms in stool samples of children with diarrhea (14). Later campylobacters (called Vibrio until the reclassification of V. fetus subsp. jejuni to C. jejuni in 1963) were many times identified in pathological animal tissues and reported in the veterinary literature, but it was not until the 1970’s that certain campylobacters were confirmed as causes of illness in humans. In 1977, C. jejuni was confirmed as a cause of food-borne gastrointestinal disease (15). So campylobacters are unique among enteropathogenic bacteria as a century elapsed between their first observations and routine isolation from different environments. Following a development of selective media and isolation procedures as well as the introduction of enhanced monitoring in developed countries the incidence of campylobacteriosis, particularly due to C. jejuni and C. coli, has been steadily increasing in the last 10 years (14).

Besides C. jejuni and C. coli, another two thermophilic species, C. lari and C. upsaliensis, and also C. hyointestinalis have been identified as agents of human gastrointestinal disease (16). The members of closely related Arcobacter (mainly A. butzleri and A. cryoaerophilus) and Helicobacter (H. pullorum) of the family Campylobacteriaceae could also be associated with human gastrointestinal disorders (16) and food transmission (17, 18).

Currently, the incidence of reported Campylobacter infections in northern European countries has been 60-90 cases per 100,000 inhabitants. Since these diagnosed and reported infections represent only a fraction of the total number of the actual infections, the true incidence could be 10-100 fold higher (19). In twelve member states of European Union, in overall, there has been an increase by 33% of the reported cases of human campylobacteriosis during the years 1996 and 2000 (20). C. jejuni is responsible for more than 90% of the cases. However, in some south European countries, Croatia and Bosnia and Herzegovina, the clinical implication of closely related thermophilic C. coli could be much higher, 30-40% (6, 10, 21-22). It was recently reported also from England and Wales sentinel surveillance scheme that risk factors for infection by C. coli are different from those for infection by C. jejuni (23) and C. coli health burden is probably greater than previously thought (24).

In The United States and in a number of developed European countries (the whole Scandinavia, the Netherlands, Ireland, United Kingdom etc.) the reported incidence rates for campylobacters surpassed those of salmonellae from patients suffering gastroenteritis already some years ago (20, 25, 26). This is significant, since routine screening for Campylobacter is still rare due to the difficulties associated with the isolation and culture of C. jejuni, most common human pathogen. Until very recently there have been no national reference centres for Campylobacter even in developed countries analogous to that which have been established for Salmonella much earlier (27). In addition, the nature of the disease is often that of self-limiting diarrhoea, which, in normal healthy adults would not warrant a trip to a physician or hospital, and therefore remains unreported. It has been estimated that, for every case
of *Campylobacter* infection reported to national surveillance in England, there are a further seven in the community (28). It is the nature of the disease, the fastidiousness of the organism, and the lack of uniform testing that provide difficulty in identifying the precise scope of the *Campylobacter* infection problem (29).

The exact reasons for the increasing incidence of human campylobacteriosis in developed countries are also not known. It is true that better surveillance systems and diagnostics could have an influence but they are not the main reason (26). The others could be traced to the changes in consumption of some foods, particularly fresh poultry meat. It is considered to be an important source of *Campylobacter* and related organisms (30).

**Poultry meat as a vehicle of human Campylobacter infections**

*Campylobacters* are widely distributed and occur in most warm-blooded domestic, production and wild animals. Their natural habitat is also the intestinal tract of food animals - especially poultry, cattle, pigs and sheep. Different vehicles for transmission of human *Campylobacter* infections have been identified, but it is estimated that around 80% of them are transmitted by food (25, 31). Undercooked or cross-contaminated poultry meat, untreated drinking water, and raw milk have been sources of *Campylobacter* outbreaks (30, 32).

However, the vast majority of *Campylobacter* infections are reported as sporadic individual infections with no easily discernable pattern. The most consistent risk factor in a number of case-controls studies worldwide has been consumption of, cross-contamination from or contact with raw or undercooked poultry, accounting for a range of approximately 10% to 50% of cases. The risky poultry was specifically that eaten outside of the home, chicken livers and other organ meats and consumption of barbecued meat (33-35).

It was reported recently from several European countries, that the prevalence of *Campylobacter* spp. on raw chicken meat from retail sail could vary in relation to the geographic location, season and method of isolation, etc., but was frequently as high as 80% (30, 36-40).

There were no official data available, on the extent of poultry meat contamination with *Campylobacter* spp. in Slovenia and Bosnia and Herzegovina. However, the results of one of our study indicated high extent of retail poultry meat contamination in both regions (41, 42, 43).

The sources of meat contamination are mainly intestines of birds, which are easily colonized with *Campylobacter* spp., mainly thermophilic *C. jejuni* and *C. coli*. Reservoirs of horizontal transmission in commercial flocks include infected animals via faecal droppings, unchlorinated drinking water, rodents, beetles, wild birds, farm-workers etc. Dry poultry feeds are not considered as a source of campylobacters, since their survival in dry conditions outside the intestines is very poor. Literature review of the importance of vertical transmission of campylobacters in poultry is not consistent yet (44-55). Biosecurity measures such as improved hygiene barriers, pest and rodent control, staff education, chlorinated water or even more specialized procedures as the treatment of chicks with commensal bacteria or immunization of birds reduce

*Campylobacter* spp. colonization in poultry flocks.

Slaughter and particularly further processing provide the opportunity for reducing *Campylobacter* on poultry carcasses. However, in crucial steps, such as the evisceration, bacterial counts increase due to faecal recontamination, but decline again during subsequent chilling (13, 56, 57). It is evident from different studies that microbial survival on poultry carcasses during processing is mainly a function of scalding temperature, chlorine level in chilling water and age of scalding and chilling water (58, 59). An additional phenomenon should be mentioned. Chickens from *Campylobacter* - free flocks are frequently contaminated with *Campylobacter* spp. during transport, slaughtering and/or processing (36, 60-62). This is important, since there is no processing step (except unacceptable thermal treatment or irradiation), which could eliminate *Campylobacter* from chicken carcasses. It is true, that these bacteria cannot multiply on the meat surface, but they could be very infectious, so minimal contamination could result in disease. It was shown in a volunteer study, that the infectious dose could be as low as 800 cells (63). It is obvious, that only a general strategy including attempts “from farm to table” can ensure safety of food products.

Two important aspects of this are briefly reviewed in the next two paragraphs - first, our knowledge of the ecology and resistance mechanisms of campylobacters against environmental stresses during food production and processing and secondly, a development of rapid, sensitive and reliable detection/identification methods of pathogenic campylobacters in/from different matrices.

**Ecology and resistance of Campylobacter spp. to environmental stresses**

*Campylobacters* are physiologically fragile organisms. When observed under a microscope they appear as small, slender, spirally-S-curved or coccoid-shaped thin, non-sporogenic Gram-negative rods. They have
characteristic corkscrew-like motility due to uni- or bipolary attached flagella (64-65). Only recently, after genetic and biochemical evidence (66, 67) also a microscopic demonstration of polysaccharide capsule surrounding the surface of C. jejuni cells was reported (68, 69).

The oxidative metabolism of Campylobacter spp. is microaerophilic with the best growth in a low oxygen environment, such as 5% O2, 10% CO2 and 85% N2. Their survival outside the gut, at the atmospheric concentration of oxygen, is poor. The optimum growth temperature of C. jejuni is 42 °C, the approximate body temperature of the chicken. It cannot grow under 30 °C, although physiological activity can be retained far below the minimal growth temperature (70). However, it usually grows slowly even under optimal growth conditions and is recognized as a poor growth competitor (71).

From many model experiments the organism is known to be sensitive to different environmental conditions, particularly drying, freezing, salting, osmotic pressure, oxygen concentrations above 5% and also to a wide variety of chemical sanitizers and dezinfecants (72). In this context, Campylobacter spp. appear to be unlikely food-borne pathogens, since most of these are relatively robust organisms, as a consequence of the necessity to survive the inimical conditions imposed by food processing and food preservation practices. Campylobacters seem to lack many of the adaptive responses to environmental stresses known to enable survival of other food-borne pathogens such as the global stationary phase stress response factor RpoS and the oxidative stress response factor SoxRS (73, 74). A high degree of hyper variable sequences has been revealed by complete sequencing of the C. jejuni small genome (67). The high genetic heterogeneity is also believed to be involved in the survival strategy (75). Recently, an evidence of inducible adaptive tolerance response (ATR) as well as the existence of protein extracellular signaling molecules in Campylobacter stress response has been published (76, 77). Probably these mechanisms contribute to the survival of campylobacters along the food producing chain and hide at least one part of the answer of the so-called “Campylobacter paradox” (29): How can an organism of such limited hardiness and growth capabilities be responsible for an ever-increasing level of human food-borne disease?

There are also specific physiological forms, which could improve the survival of microbial cells in the food processing environment. The first could be biofilms, which can protect constituent cells, including those of human pathogens, from the environmental stresses and antimicrobial agents (78-80). Little is known about pathogenic C. jejuni behaviour as a constituent microorganism in biofilms, but recent report (81) indicated that biofilm cells of C. jejuni are less resistant to stress that their planktonic counterparts and confirmed the suggestion of Park (74) that campylobacters lack many of the adaptive stress-resistance responses common in other bacteria.

Another physiological phenomenon which could enhance survival in unfavoured conditions is a transition of spiral-shaped Campylobacter to the more resistant coccoid form, usually accompanied by a transformation into so called viable but non-culturable state (VBNC) (82). This has been described in many non-differentiating, mainly Gram-negative bacteria. It is a resting or dormant stage, induced by environmental stresses including changes and limitations in nutrient availability, temperature, oxygen saturation, osmotic pressure etc. It is not clear until now, if VBNC response could be analogously compared to the stress responses of the differentiating bacteria (e.g. spore formation), as a genetically programmed physiological response of some bacteria which enhances survival during environmental stress. To confirm this hypothesis, the VBNC cells should be transformed from the dormant survival state into actively metabolising cells. While many reports confirm a transformation into VBNC cells, reports of both in vivo and in vitro resuscitation are much more sceptic (83). The retention of infectivity of VBNC forms also remains a topic, which needs further research because current data in the literature are still controversial. VBNC C. jejuni was proven to cause an infection after a passage through an animal host (84, 85), but others reported, that the role of VBNC in campylobacter epidemiology may be negligible (86-89). At least it is clear, that the transformation of spiral cells into coccoid and the subsequent response, i.e. changes in culturability and infectivity of cells could be strain and stress specific and effected by environmental conditions, such as temperature, pH, osmotic stress, the availability of nutrients etc. (74, 90-92).

The effect of usual stress situations in food processing, heat shock (55 oC, 3 min) and oxidative stress (3 mM H2O2 for 10 min or prolonged incubation at atmosphere oxygen concentration) on non-starved and starved cells of Campylobacter jejuni from different growth phases, were tested. Viability as assessed with the Bacterial Viability Kit LIVE/DEADâ BacLightTM dying before fluorescent microscopy and culturability of the cells (CFU ml-1) from both growth phases showed that starvation increased heat but not oxidative resistance. High temperature and oxidative stress invoked quick
transformation from culturable spiral shaped to nonculturable spiral and cocccoid cells (94). However, the formation of cocccoid cells has not improved the survival of the cells during oxidative stress (91). Much more research is needed to elucidate how the unusual physiological features and stress-response of campylobacters influence their role as food-borne pathogens.

Even not concerning infectivity and more theoretical debates of the role of VBNC forms in bacterial life cycles, these forms could have important practical consequences on reliability of Campylobacter detection in foods. Since classical microbiological techniques relying on cultivation of microorganisms still present a basis for most food microbiology procedures in routine laboratories, these non-culturabe forms remain undetected, but PCR-based methods amplify their DNA. Some important events in a development of detection and identification procedures for Campylobacter spp. in foods are mentioned below.

**DETECTION AND IDENTIFICATION OF CAMPYLOBACTER FROM FOODS**

Campylobacters are zoonotic bacteria mainly transmitted by food of animal origin. Methods have therefore been developed for their isolation from animal, food and clinical specimens. I will focus here on the procedures used in food examination.

For the isolation of campylobacters from any source, the crucial step was a development of selective culture media, which started in the 1970’s and still continues nowadays. Basal nutrient components are supplemented by two groups of supplements. The first are complex substrates such as blood, charcoal, ferrous sulphate, sodium metabisulphite, sodium pyruvate etc., mainly added to protect campylobacters from the oxidative stress during cultivation. The second group of supplements are antimicrobial agents for suppression of accompanying microflora of Gram-negative organisms, such as trimethoprim, polymyxin and novobiocin, and of Gram-positive organisms, such as vancomycin, teicoplanin, bacitracin, rifampicin etc. (95). In blood free isolation media, the usual component is charcoal ceferozarzone deoxycholate, which could be used with different formulations of antimicrobial agents to get the most selective conditions for isolation of different Campylobacter and other Campylobacter-like organisms, particularly Arcobacter spp. and Helicobacter pullorum, such as mCCDA, CAT agar etc. (30, 71, 96, 97).

Many Campylobacter enrichment broths and enrichment protocols have been developed, but only a few have been adopted as standard methods. A horizontal method for detection of thermotolerant Campylobacter was standardised (98) and revised later. It is split into two parts, detection and enumeration method. However, even the revision could not overcome some basic problems of phenotypic detection/identification of campylobacters. It takes 5-7 days and is often problematic because of fastidious growth requirements and few distinguishing biochemical characteristics of campylobacters. Identification is hindered by subjective interpretation of biochemical tests and atypical phenotypes of some strains. For example, the differentiation between C. jejuni and C. coli mainly relies on the ability of C. jejuni to hydrolyse the hippurate, but certain C. jejuni strains fail to do so (99-102).

These limitations might be overcome by more specific methods, including immunological and nucleic acid-based detection methods. PCR technology, which seems most promising, is an extensively used genetic approach for rapid and sensitive detecting of a number of food-borne pathogens (103-106). For detection of Campylobacter spp. in food products it was introduced already twelve years ago (107). Theoretically, PCR should be able to detect a single copy of a target gene, but in practice, many substances react as DNA polymerase inhibitors, thereby lowering PCR detection capacity. The pre-enrichment step lengthens the time of analysis, but dilutes PCR inhibitors from food and increases the number of target viable organisms. Another solution for detecting only viable cells is reverse transcriptase PCR (RT-PCR) targeting mRNA (108), but it requires skilled operator. In fact, the appropriate sample preparation is still crucial for all amplifications. Several procedures to remove PCR inhibitors and/or concentrate bacteria and/or release target DNA have been applied, like filtering, washing, and termal or enzyme lysing (109-111), bouyant density centrifugation (1112) and immunomagnetic separation (113-116), combined with different detection techniques (117-120). The wide range of sample preparation methods cited in the literature reflects that a method, appropriate for one type of sample or procedure may not be adaptable to the others. We also studied different protocols for sample and/or DNA preparation to increase sensitivity of PCR detection of Campylobacter in Preston enrichment broth. Buoyant density centrifugation (BDC) with Percoll prior to simple heat lysis of cells and flaA amplification improved PCR detection of Campylobacter for 100-1000-fold comparing to procedures without BDC (121).
Similarly, a number of PCR protocols targeting different genes within Campylobacter or specifically within C. jejuni and C. coli, have been applied for detecting and identifying these organisms. Linton et al. developed a PCR assay specific for the genus Campylobacter (122) as well as PCR assays specific for C. jejuni and C. coli (123). Several flaA typing procedures have been developed with considerable variations in DNA preparation technique, primer design and annealing temperatures. To allow a direct comparison of results obtained in different laboratories the consensus pair of primers has been developed for identification and typing of C. jejuni and C. coli strains (124).

Although many PCR detection and identification protocols have been already developed for microbiological examination of foods, including Campylobacter detection and identification, PCR analyses are still not widely adopted. There are many reasons for this (125). Among others, more comparative evaluations and validations of alternative methods and standardised PCR protocols in food microbiology are strongly needed (126-128). Since one of the limitations of practical use of PCR procedures in routine microbiological laboratories is also the susceptibility of PCR to DNA contamination and consequently, the need of skilled operators and proper infrastructure of the laboratory, commercially prepared reagents would be appreciated. First commercial diagnostic PCRs for the identification of C. jejuni and C. coli are already available as well as the results about their selectivity and utility (129). However, much more development on different fields of work (education, cost reduction, formal acceptance through national and international regulations etc.) is needed to make these assays more widely accessible.

**ANTIBIOTIC RESISTANCE OF CAMPYLOBACTER – RELATIONSHIP AMONG ANIMAL, FOOD AND HUMAN ISOLATES**

Besides the increasing prevalence of bacterial food-borne pathogens including Campylobacter, there is another growing concern – their resistance to antibiotics used in veterinary and human medicine. It is now generally accepted that the main risk factor for accumulation of resistant bacteria is an extensive use of antibiotics, not only for therapy and prevention of infections in humans and animals, but also in sub-therapeutic doses in animal feed to promote growth, increase feed efficiency and decrease waste production (130-140). Despite legislation targeted at controlling the use of antimicrobials in food-producing animals in recent years there has been significant increase in developed countries in the occurrence of resistance in different food-borne bacteria including Campylobacter spp. (141). Zoonotic bacteria can carry antibiotic resistance genes from the food-producing environment via food directly to the consumer (142).

A case study for this could be emerging resistance to fluoroquinolones of Campylobacter from food animals, foods of animal origin and human isolates in different countries of the world (37, 138-140, 143-150). It can be assumed from many studies, that an evident increase in bacterial resistance followed the introduction of fluoroquinolones in veterinary medicine in the 1990s. The prevalence of resistant isolates is usually higher from animals and foods comparing to human isolates as well as in C. coli comparing to C. jejuni. Geographically, the resistance to fluoroquinolones remain at relatively lower rates in northern European countries comparing to the southern European countries. High antimicrobial resistance of C. coli from food animals–producing environment could possibly be related to the large prevalence of this species not only in pigs but also in poultry and chicken meat. This is reported from some countries in the southern part of Europe (40, 41, 43). The resistance might have determined a selective effect on one population compared with the other as a consequence of routine antimicrobial treatments administered to the animals at the farms. It is also from genetic studies, that the acquisition of resistance to fluoroquinolone antimicrobials in Campylobacter, unlike in other Gram-negative bacteria, does not require stepwise accumulation of gyrA mutations and overexpression of efflux pumps, but is mainly mediated by single-step point mutations in quinolone resistance-determining (QRDR) of gyrA in the presence of a constitutively expressed multidrug efflux pump, CmeABC. Resistant mutants are competitive in the colonization of their hosts and may persist even in the absence of antimicrobial selection pressure. This highlights the need for extra effort to prevent the occurrence and spread of fluoroquinolone -resistant campylobacters (151).

A useful tool to confirming the link among emerging fluoroquinolone resistance of Campylobacter from food animals, foods of animal origin and human isolates are microbial typing methods, such as automated ribotyping (RiboPrinting), pulsed-field gel electrophoresis (PFGE) using different restriction enzymes and some PCR-based typing methods (152). They give stable profiles of isolates from different sources over time (153). Some
authors confirmed identical or very similar fingerprints of resistant campylobacters from animal and human sources (146, 154, 155), but more research is needed to elucidate as well the importance of other reservoirs of resistant strains, possibly common to food animals and humans, like wild animals, pets, waters etc. (156-159). Macrolide resistance of Campylobacter spp. has already been studied in many countries but limited or no increase was documented for human, mainly C. jejuni isolates (160, 161). Much higher prevalence has been found for C. coli, particularly for C. coli isolated from pigs (162). But, in Bosnia and Herzegovina, extremely high erythromycin resistance rates in clinical isolates (more than 30%) persisted since 1998, despite the fact, that source of C. coli probably other than pigs (139, 140).

The resistance to other antimicrobial agents including tetracycline, aminoglycoside and chloramphenicol is generally low. However, resistance is high to beta-lactam antimicrobials and sulphonamides (163-165). The increasing resistance to fluoroquinolones was reported also for clinical isolates of Campylobacter spp. in Slovenia and Bosnia and Herzegovina (138-140). However, the prevalence and antibiotic resistance of campylobacters from food are still not monitored routinely, but during the four-year research project we introduced standardised and molecular methods for detection, identification, typing and antibiotic resistance testing of thermostolerant Campylobacter spp. from poultry meat samples. We compared the results about antimicrobial susceptibility testing of campylobacters from retail poultry meat and human clinical isolates collected in the same time frame (2001-2003) . Atimicrobial susceptibility testing against eight different antibiotics used in veterinary and/or human medicine was included in study. The portion of ciprofloxacin resistant human clinical isolates has increased in Slovenia as well as in Bosnia and Herzegovina in the last five years from 10.5% and 15% in the year 1998, to nearly 50% and 32% in 2002, respectively. But, while ciprofloxacin resistance of clinical isolates in Slovenia still lower than in poultry meat isolates (58.2% of resistant strains among isolates from years 2001-2003), in Bosnia and Herzegovina situation was different: Campylobacter isolates from poultry meat and farm animals had lower extent of ciprofloxacin resistance(27% and 31%, respectively) (140, 166). In the case of ciprofloxacin and erythromycin resistance, MICs were determined by E-test. The resistance to ciprofloxacin was confirmed also by MAMA-PCR detecting mutations in quinolone resistance determining region (QRDR) of the gyrA gene of C. jejuni and C. coli (167). These results indicate that poultry meat is indeed an important source of antibiotic resistance in thermostolerant campylobacters. Moreover, multiple antibiotic resistance was found as a critical point of tested food isolates (168). Our results show that we need a monitoring system of the prevalence and antibiotic resistance of zoonotic bacteria from human, animal and food samples on a national or regional level to better understand the epidemiology of animal, food and human strains and to assure safety of our food products.

RISK ASSESSMENTS FOCUSING CAMPYLOBACTER SPP. IN FOOD PRODUCTS

The increasing incidence of human campylobacteriosis and high prevalence of pathogenic Campylobacter spp. in food, mainly chicken meat have induced targeted interventions to bring the infections under control (169). According to the principles of Codex Alimentarius Commission (170) a quantitative risk assessment has been prepared to assess the effect of different mitigation strategies on the number of human campylobacteriosis in Denmark associated with thermophilic Campylobacter species in chickens. Mathematical simulations showed that the incidence of campylobacteriosis associated with consumption of chicken meals could be reduced 30 times by introducing a 2 log reduction of the number of Campylobacter on the chicken carcasses (for example by freezing). Similar reduction can be achieved by reducing Campylobacter flock prevalence (with strict hygienic barriers or biosecurity zones) or by improving kitchen hygiene level approximately 30 times, for example with preventing cross-contamination by washing the cutting boards (171). Besides the increasing incidence of human campylobacteriosis and prevalence of Campylobacter spp. in poultry slaughter houses and retail stores, the emerging fluoroquinolone resistance of Campylobacter spp. induced more risk assessments of the impact of antibiotic resistantpathogensonhumanhealth(172, 173). Food and Drug Administration’s Center for Veterinary Medicine (CVM) developed a risk assessment model, which has quantitatively demonstrated that resistance development in bacteria from food-producing animals presents a risk to human health. However, the model quantified the level of risk due to consumption of chicken, contaminated with fluoroquinolone resistant campylobacters, but has not quantified the impact of the spread of these pathogens from chicken to other foods or other environmental reservoirs of human infection, due to lack of data.
In conclusion, changes in food production, preservation, marketing and consumption, a new knowledge about the microorganisms and a development of efficient techniques for their detection and differentiation from already known pathogenic species, have enabled the identification of some new emerging food-borne pathogens. Thermophilic campylobacters, mainly *C. jejuni* and *C. coli*, are the leading cause of zoonotic enteric human infection in developed countries. There is an evident steady increase in the number of reported human campylobacteriosis in developed European countries. Many case-control studies indicated that consuming or handling poultry meat is the most consistent risk factor, related to the high prevalence of *Campylobacter* in retail poultry meat.

Strict hygienic and biosecurity measures in poultry rearing, transporting of animals, slaughtering and further processing of poultry carcasses can decrease the number but not completely eliminate pathogenic *Campylobacter* on retail poultry meat. Educating the general consumers should be an important action in attempts to reduce *Campylobacter* infections, since the last critical control point for assuring safety remains the hygiene of the final food preparation.

More scientific knowledge about “*Campylobacter paradox*”, i.e. its physiological specificities enabling adaptation and survival in different ecological niches, is needed. Interdisciplinary and international programmes should focus on monitoring and controlling the use of antimicrobial agents in food-animals production as well as physico-chemical procedures in food processing and subsequent microbial resistance development.

Finally, further epidemiological and microbiological studies combining novel molecular approaches are needed to define more precisely the natural sources and transmission routes of campylobacters to humans. This will be a basis of more efficient mitigation strategies to decrease the number of human Campylobacter infections.

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