ORIGINAL ARTICLE

Prevalence, Antimicrobial Resistance and Risk Factors for Thermophilic *Campylobacter* Infections in Symptomatic and Asymptomatic Humans in Tanzania

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Impacts

- In Tanzania, data on epidemiology of *Campylobacter* in humans are scant, whereas that on antimicrobial resistance of the organisms and risk factors for human infections are lacking.
- In this paper, we provide information on the epidemiology and antibiotic resistance profiles of *Campylobacter* infections in humans in the country.
- Presented data are useful in planning control measures for *Campylobacter* infections and guiding the antimicrobial therapy in *campylobacteriosis* cases requiring treatment.

Keywords:
Gastroenteritis; Cape Town protocol; phenotypic tests; polymerase chain reaction; disc diffusion; Eastern Tanzania

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Summary

The genus *Campylobacter* comprises members known to be a leading cause of foodborne gastrointestinal illness worldwide. A study was conducted to determine the epidemiology and antimicrobial resistance of *Campylobacter* in humans in Morogoro, Eastern Tanzania. Isolation of *Campylobacter* from stool specimens adopted the Cape Town protocol. *Campylobacter* isolates were preliminarily identified by conventional phenotypic tests and subsequently confirmed by matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry and polymerase chain reaction. Antimicrobial resistance testing employed the disc diffusion method. A small proportion of the test isolates was also subjected to agar dilution method. Risk factors for human illness were determined in an unmatched case–control study. Thermophilic *Campylobacter* were isolated from 11.4% of the screened individuals ($n = 1195$). The agreement between PCR and MALDI-TOF was perfect ($\kappa = 1.0$). Symptomatics and young individuals were infected with higher numbers than asymptomatic and adults, respectively. The majority (84.6%) of the isolates were *C. jejuni* and the remaining were *C. coli*. Isolates had highest resistance (95.6%) for colistin sulphate and lowest for ciprofloxacin (22.1%). The rates of resistance for other antibiotics (azithromycin, erythromycin, tetracycline, cephalothin, gentamycin, nalidixic acid, ampicillin, amoxycillin, norfloxacin, chloramphenicol) ranged from 44.1% to 89%. Comparison between disc diffusion and agar dilution methods indicated a good correlation, and the tests were in agreement to each other ($\kappa \geq 0.75$). Human illness was found to be associated with young age and consumption of chicken meat and pre-prepared salad. Our data indicate the presence of antibiotic-resistant thermophilic *Campylobacter* in humans in the study area. There is a need for routine investigation of the presence of the organisms in gastroenteritis aetiology, including determination of their antibiotic susceptibilities.

Introduction

Campylobacter- and Salmonella-mediated diarrhoeal diseases are leading bacterial causes of gastroenteritis worldwide (Rzewuska et al., 2010; Gripp et al., 2011; Newell et al., 2011; Haruna et al., 2013). Of the *Campylobacter* species, *C. jejuni* causes more than 83% of human symptomatic infections (Friedman et al., 2000; Snelling et al., 2005; Rajendran et al., 2012); majority of the remaining proportion being caused by *C. coli* (Rajendran et al., 2012). Occasional human campylobacteriosis is caused by *C. lari* (Rajendran et al., 2012). The organisms are transmitted to humans mainly by contaminated food of animal origin (Ogden et al., 2009), with raw/undercooked poultry meat being more incriminated (Lindmark et al., 2009). Contact with pets and farm animals has also been implicated as potential source of *Campylobacter* infection to humans. While different animal species are frequently colonized with *Campylobacter* asymptomatically, human infections result in acute and self-limiting intestinal infection (Takamiya et al., 2011). *C. jejuni* is also associated with post-infectious autoimmune neuromuscular disorder, Guillain-Barre′ syndrome and reactive arthritis (Blaser, 1997; Rajendran et al., 2012).

Campylobacter enteritis is in most cases self-limiting. Antimicrobial therapy is, however, required in severe and prolonged cases of enteritis, cases of bacteremia, septic arthritis and other extra intestinal infections in which erythromycin and ciprofloxacin are the drugs of choice (Guerrant et al., 2001; Gupta et al., 2004; McDermott et al., 2004). Apart from being a drug of choice for treatment of human campylobacteriosis, ciprofloxacin together with other fluoroquinolones has been used for symptomatic treatment of bacterial gastroenteritis (Allos, 2001). Increasing resistance of *Campylobacter* strains to macrolides (Gibreel and Taylor, 2006; Gallay et al., 2007), fluoroquinolones (Ghosh et al., 2013) and other antimicrobials suggests a need to monitor the *Campylobacter* resistance situation worldwide (Lehtopolku et al., 2012). The generated information will be useful in guiding antimicrobial treatment for severely ill patients with campylobacteriosis.

Case–control studies have frequently attributed sporadic human *Campylobacter* infections to different sources. Identified risk factors for the disease have included handling and/or consumption of poultry, consumption of raw and unpasteurized milk, drinking untreated water, drinking bottled water, eating salad vegetables, handling and cooking food, contact with animals, swimming in natural water bodies and travel abroad (Kapperud et al., 1992, 2003; Morgan et al., 1994; Fahey et al., 1995; Evans et al., 1996, 2003; Djuretic et al., 1997; Eberhart-Phillips et al., 1997; Kalman et al., 2000; Lehner et al., 2000; Studahl and Andersson, 2000; Rodrigues et al., 2001; Friedman et al., 2004; Carrique-Mas et al., 2005; Ethelberg et al., 2005; Schildt et al., 2006; Uyttendaele et al., 2006; Wingstrand et al., 2006; Stafford et al., 2007; Fajo′-Pascual et al., 2009). Some case–control studies have, however, reported contradictory results (Lindmark et al., 2009). While many studies demonstrate eating chicken at home as a risk factor, some found this behaviour to be protective (Adak et al., 1995; Eberhart-Phillips et al., 1997). A study in France found no association between consumption of chicken and human campylobacteriosis (Gallay et al., 2008). The authors identified consumption of undercooked beef as a risk factor. Identification of certain risk factors in some investigations but not in others might be an attribute of differences in sample size, study methodology or geographical and cultural differences (Rodrigues et al., 2001; Neimann et al., 2003; Wingstrand et al., 2006).

In Tanzania, as in many other African countries, there is dearth of information on bacteriological and epidemiological aspects of *Campylobacter*. The available information for both human and animal campylobacteriosis is sparse (Komba et al., 2013), thereby creating little awareness on the need to develop control strategies for this zoonosis. Consequently, the objective of this study was to determine the prevalence, antimicrobial susceptibility and risk factors for *Campylobacter* infections in humans.

Materials and Methods

Study area

This study was carried out in Morogoro Municipality, Morogoro region, Eastern part of Tanzania. The region occupies 7.99% of the total land area in the country which is 885 800 sq. km. As of the official 2012 population and housing census, the Municipality had a population of 315 866, 14.24% of the total population of Morogoro region (n = 2 218 492). Human stool specimens were obtained from Morogoro regional hospital, Sokoine University of Agriculture (SUA) dispensary and Upendo medical laboratory. The selected facilities represent 9.4% (3/32) of the total laboratories but serving more than 60% of patients in the municipality (Mwinuka, personal communication, 2011). Collected samples were analysed at microbiology laboratories of the Pest Management Centre of SUA.

Determination of prevalence of *Campylobacter* infections

Study design and sample size determination

A cross-sectional study design was adopted to establish the prevalence of *Campylobacter* in humans in the study area. The number of humans to be sampled was determined using the formula developed by Thrusfield (1995), that is

$n=Z^2p\ (1\text{-}p)/d^2$, where n is the sample size, Z is the multiplier from the normal distribution, p is the expected prevalence, and d is the desired absolute precision. The known prevalence of *Campylobacter* infections (p) in humans reported in a previous study in the country is 9.3% (Mdegela et al., 2006). With Z value of 1.96 at 95% confidence interval (CI) and desired precision (d) of 0.05, the calculated sample size (n) was 130.

Sample collection

Whole stool specimens were collected by medical personnel from individuals with enteric complaints (symptomatics) seeking medical attention in selected health facilities in the study area. Also samples were collected from individuals without enteric complaints (asymptomatics) in the study health facilities and other gatherings preferably academic institutions. During sampling, age, sex and place of residence for individuals' submitting samples were recorded. Study subjects below 15 years old were recorded as young and 15 years and above as adults (Mdegela et al., 2006). Following collection, samples were immediately placed in universal bottles containing Bolton broth (Oxoid Ltd., Basingstoke, Hampshire, England) and then stored at 4°C before being shipped to laboratories for analysis within 8 h post-sampling. In the laboratory, the stool samples were incubated at 37°C for 24 h after which they were subjected to the Cape Town protocol for primary isolation of thermophilic *Campylobacter*.

Isolation of Campylobacter

Following 24 h incubation of collected stool samples in Bolton broth, isolation of *Campylobacter* was carried out using Cape Town protocol developed by Le Roux and Lastovica (1998) with some modifications as indicated by Jacob et al. (2011). The technique involves culture of the organisms on an antibiotic-free blood agar after a filtration step. Briefly, a 0.45-μm-pore-size nitrocellulose filter (Sartorius Stedim Biotech GmbH 37070, Goettingen, Germany) was overlaid on the surface of blood agar (Oxoid LTD., Basingstoke, Hampshire, England) onto which 200 μl of enrichment broth containing the sample was dispensed and allowed to filter passively for 45 min at room temperature. The filter was then carefully removed with sterile forceps and discarded. Thereafter, the filtrate was spread evenly across the medium followed by incubation of the inoculated plates in microaerophilic atmosphere at 37°C for up to 72 h while checking for growth after every 24 h. Suspect *Campylobacter* colonies were subsequently subcultured on blood agar under similar conditions to obtain pure cultures which were then subjected to identification methods.

Identification of Campylobacter isolates

Preliminary identification: *Campylobacter* isolates were preliminarily identified based on phenotypic tests, namely growth atmospheric requirements, colonial characteristics, testing for Gram negativity using the KOH string test (3% potassium hydroxide on a glass slide), motility test and the sodium hippurate hydrolysis test for differentiation of *C. jejuni* from other thermophilic *Campylobacter*.

Confirmation of Campylobacter isolates: Following preliminary identification, *Campylobacter* isolates were further confirmed using a genome-based method, species-specific polymerase chain reaction (PCR), as well as a spectroscopic method, matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltronics with Bruker Daltonics MALDI biotyper software 2.0, Billerica, MA, USA). Genomic DNA to be used for PCR was extracted from bacterial suspensions by boiling at 100°C for 10 min. Primers F,5′CTATTTTATTTTTGAG TGCTTGTG3′ and R,5′GCTTTATTTGCCATTTGTTTTA TTA3′ (TAG COPENHAGEN A/S) were used to amplify the *mapA* gene (589 bp) of *C. jejuni*, whereas primers F,5′ATTTGAA AATTGCTCCAACTATG3′ and R,5′TGATTTTATTATTT GTAGCAGCG3′ (TAG COPENHAGEN A/S) were used to amplify the *ceuE* gene (462 bp) of *C. coli.* Each reaction was performed in a 50 μl total volume containing 10 μl primer mix (12 pmol of each primer), 25 μl Green master mix, 2 μl DNA template and 13 μl milli Q water. Amplification reactions were run in Biometra T3 thermocycler (Fisher Scientific, Loughborough, UK), with the following program: an initial denaturation at 95°C for 5 min followed by 34 cycles of denaturation at 94°C for 20 s, annealing at 50°C for 20 s and polymerization at 72°C for 1 min. A final extension was performed at 72°C for 5 min. Samples were then maintained at 4°C until processed. The amplification generated 589-bp and 462-bp DNA fragments corresponding to *C. jejuni* and *C. coli*, respectively. The PCR products were analysed on a 1.5% agarose gel (SeaKem, FMC BioProducts, Rockland, ME, USA) stained with 0.3 g/ml ethidium bromide and were visualized under UV light. A 100-bp ladder was used as a molecular size standard. All *Campylobacter* isolates were stored at −80°C in brain–heart infusion broth containing 15% v/v glycerol for future antimicrobial susceptibility tests and genetic typing.

Evaluation of antimicrobial resistance of *Campylobacter* isolates

In this study, antibiotic resistance testing of *Campylobacter* isolates was performed by disc diffusion method on

Muller-Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) as described by Luangtongkum et al. (2007), using *C. jejuni* NCTC 11168 for quality control purposes. The method is known to be reliable and easy tool for monitoring the prevalence of resistance in *Campylobacter* and a suitable alternative method to agar-based MIC methods (van Hees et al., 2007; Senok et al., 2007; Yang et al., 2008). Briefly, *Campylobacter* suspensions were prepared in sterile normal saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard. Sterile cotton-tipped swabs were inserted into the standardized inoculums, drained off and then used to transfer the inoculums onto well-dried Mueller-Hinton agar plates. Inoculated plates were dried in incubator for five minutes, and antibiotic discs were distributed over them using a BBL Sensi-disc dispenser. The plates were then incubated at 42°C for 48 h under microaerobic conditions. After 48 h of incubation, the diameters of inhibition zones were measured. Interpretation of results was guided by both standardized tables supplied by the National Committee on Clinical Laboratory Standards (currently known as Clinical and Laboratory Standards Institute) (NCCLS, 2002) and manufacturer's instructions. In this study, the most commonly used antimicrobial agents in livestock and humans in the study area and others that have been used and tested elsewhere (Pezzotti et al., 2003) were tested for resistance. They included twelve (12) different antimicrobials: nalidixic acid (NA, 30 μg), ciprofloxacin (CIP, 5 μg), gentamycin (CN, 10 μg), ampicillin (AMP, 10 μg), cephalothin (CL, 30 μg), amoxycillin (AML, 25 μg), norfloxacin (NOR, 10 μg), erythromycin (E, 15 μg), tetracycline (TE, 30 μg), colistin sulphate (CT, 10 μg), azithromycin (AZM, 15 μg) and chloramphenicol (C, 30 μg) (Oxoid, Hampshire, UK). A total of 136 human-derived isolates were tested for resistance to these different antimicrobials. An isolate that was resistant to two or more classes of antimicrobials was referred to as multi-drug resistant. A proportion (28/136) of the isolates tested for antimicrobial resistance by disc diffusion method was also subjected to agar dilution method as described earlier (Miflin et al., 2007). These were tested for resistance against four antimicrobial agents, that is ciprofloxacin, erythromycin, tetracycline and chloramphenicol (Oxoid).

Identification of risk factors associated with *Campylobacter* infections in humans

An unmatched case–control study was carried out to identify risk factors associated with *Campylobacter* infections in humans. Based on 95% confidence interval, power = 80.0%, case: control ratio of 1 : 5 and odds ratio of 2.46, it was estimated that a total number of 129 cases and 644 control individuals were to be involved in the study (Statcalc, Epi Info™ version 7, CDC, Atlanta, GA, USA).

Identification of 'cases' and 'controls'

For this particular study, a 'case' was defined as any individual (symptomatic or asymptomatic) confirmed in the laboratory to be positive for *Campylobacter* infection. On the other hand, a 'control' was defined as any individual (symptomatic or asymptomatic) confirmed in the laboratory to be negative for *Campylobacter* infection. Asymptomatics from the health facilities were cases with complaints other than those involving the gastrointestinal tract. Exclusion criteria for controls included (i) past history of campylobacteriosis, (ii) diarrhoea/abdominal pain in preceding month and (iii) travel history for the last two weeks.

Acquisition of the study subjects

In each participating health facility, individuals to be involved in the study were obtained as volunteers after explaining to them the purpose of the study. Any volunteer was requested to provide a written consent. In case of children, the consent forms were filled by either a parent or a guardian.

Interviews

All cases and controls were interviewed in person to collect information for determination of risk factors for *Campylobacter* infections in humans. The interviews were conducted by medical personnel involved in sample collection. A parent or guardian was interviewed if the individual was under 15 years of age. Each interview was designed to cover demographic and clinical information and specific exposures. The collected information included age, sex, area of residence, consumption of chicken and other meat, food-eating habits, kitchen hygiene and food-handling practices, type of water used for domestic purposes, hygienic practices, contact with animals, involvement in chicken farming, history of travelling and eating outside home, medications and history of chronic and enteric diseases.

Data analysis

Collected data in this study were analysed using EPI INFO statistical software version 7 (CDC, Atlanta, GA, USA). For prevalence and antimicrobial resistance data descriptive statistics (frequencies and cross-tabulations) were computed to determine proportions for different items. The chi-square test was used to determine the significance of

differences in proportions at $P \leq 0.05$. Agreement between different tests was determined using kappa statistic. The correlation between disc diffusion and agar dilution methods for antimicrobial resistance testing was determined by computing the correlation coefficient in MICROSOFT EXCEL® software (Redmond, WA, USA). Risk factor data were analysed in two steps: (i) univariate logistic regression was used to screen all potential risk factors for statistical significance at a *P*-value of ≤0.20 and (ii) statistically significant variables in univariate logistic regression were included in a multivariable logistic regression analysis based on a forward variable selection approach utilizing the likelihood ratio statistic and a *P*-value ≤0.05. The Mantel–Haenszel method was used to identify confounding factors. A factor was considered to have potential confounding effect if its magnitude of change was ≥25% in the coefficient estimates of other predictors. Goodness of fit of the final model was assessed using the Hosmer and Lemeshow test (Hosmer and Lemeshow, 2002). The final model was assessed for discrimination ability using receiver operating characteristic (ROC) curves, based on area under the curve (AUC) (Balk et al., 2006).

Ethical considerations

Ethical clearance (permit number: NIMR/HQ/R.8a/Vol. IX/1106) was sought from the Health Research Ethics Review Sub-Committee at the National Institute for Medical Research, Ministry of Health, Dar es Salaam. Qualified medical personnel were involved in collection of information and stool samples from study subjects after explaining the purpose of the study and obtaining written consent of the person to be screened or his/her parent/guardian. Confidentiality of the study participants was always adhered to, and after processing of samples, results were given to the medical personnel at respective health facility for further follow-up of the patient.

Results

Sample collection

Whole stool specimens were collected from 1195 human subjects: 898 symptomatic and 297 asymptomatic, respectively. The sex distribution was such that 586 individuals were males and 609 females. Of the sampled individuals, 251 (21%) were young (<15 years) and 944 adults (≥15 years). About fourteen per cent of the young individuals were under 5 years old.

Prevalence of *Campylobacter* organisms in study subjects

The overall prevalence of *Campylobacter* infection in humans was 11.4%. The prevalence levels by PCR and MALDI-TOF were identical ($P < 0.00$), and there was perfect agreement between the two methods ($\kappa = 1.0$). The prevalence was higher among symptomatic individuals (12.9%, $n = 898$) as opposed to asymptomatic individuals (6.7%, $n = 297$). There was no statistically significant difference in *Campylobacter* prevalence between males (10%, $n = 586$) and females (12%, $n = 609$). Young individuals had a significantly higher *Campylobacter* prevalence (16.7%) than adult individuals (10%). The majority (84.6%) of *Campylobacter* isolates obtained were *C. jejuni* and the remaining 15.6% *C. coli.*

Antimicrobial resistance profiles for *Campylobacter* isolates

A total of 136 human-derived *Campylobacter* isolates were tested for susceptibility to 12 different antimicrobials. A large proportion of the test isolates were resistant to colistin sulphate (CT), whereas a lower proportion of isolates were resistant to ciprofloxacin (CIP) as displayed in Fig. 1. The proportion of resistant isolates to the remaining 10 antimicrobials ranged from 44.10% to 89.0% (Fig. 1). All the test isolates displayed multi-drug resistance with 77.9% being resistant to more than half of the test antimicrobials and 19.9% showing resistance to all the test antimicrobials. Resistance to more than two classes of antimicrobials was in the following order: resistance to two classes (4.4%), resistance to three classes (11.0%), resistance to four classes (6.6%), resistance to five classes (18.4%), resistance to six classes (22.1%), resistance to seven classes (17.6%) and resistance to eight classes (19.9%). There were significantly higher proportions of resistant *C. coli* isolates than *C. jejuni* isolates for nalidixic acid and amoxycillin (Table 1). Comparison between disc diffusion and agar dilution methods showed good correlation. The correlation coefficients were 0.67 for ciprofloxacin, 0.58 for erythromycin, 0.86 for tetracycline and 0.53 for chloramphenicol, respectively. The two tests had excellent to perfect agreement on kappa statistics; the kappa values being 1.00 (0.96–2.96) for ciprofloxacin, 0.84 (1.06–2.74) for erythromycin, 1.00 (0.96–2.96) for tetracycline and 0.75 (1.08–2.58) for chloramphenicol.

Risk factors for *Campylobacter* infections in humans

Description of cases and controls

During December 2011 through April 2012, a total of 1195 individuals were available to participate in the risk analysis study, 898 being symptomatic and the remaining 297 asymptomatic. One hundred and thirty-six (136) individuals satisfied the criteria of a case, and 1059 individuals qualified as controls. The cases comprised of 12.9% of symptomatic and 6.7% of asymptomatic individuals, contributing 85.3% and 14.7%, respectively. The mean age

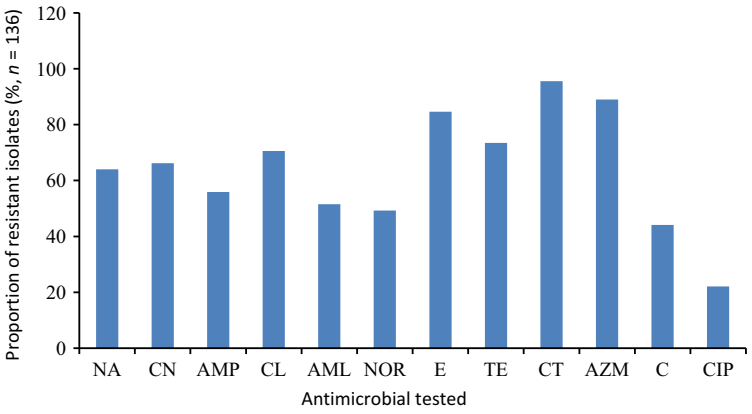

Fig. 1. Antimicrobial resistance patterns of human-derived thermophilic *Campylobacter* isolates (n = 136). Key: NA, nalidixic acid; CIP, ciprofloxacin; CN, gentamycin; AMP, ampicillin; CL, cephalothin; AML, amoxycillin; NOR, norfloxacin; E, erythromycin; TE, tetracycline; CT, colistin sulphate; AZM, azithromycin; C, chloramphenicol; n, number of isolates tested.

Table 1. Species comparison of antimicrobial resistance patterns of thermophilic *Campylobacter* isolates from humans

	Proportion of resistant isolates (%)		
Antimicrobial agent	*C. jejuni* (n = 115)	*C. coli* (n = 21)	*P*-value
NA	57.4	100	0.001
CN	62.6	85.7	0.10
AMP	53.0	71.4	0.12
CL	67.8	85.7	0.1
AML	47.8	71.4	0.05
NOR	47.8	57.1	0.43
E	84.3	85.7	0.87
TE	76.5	57.1	0.06
CT	94.8	100	0.28
AZM	89.6	85.7	0.60
C	41.7	57.1	0.20
CIP	20.9	28.6	0.43

NA, nalidixic acid; CIP, ciprofloxacin; CN, gentamycin; AMP, ampicillin; CL, cephalothin; AML, amoxycillin; NOR, norfloxacin; E, erythromycin; TE, tetracycline; CT, colistin sulphate; AZM, azithromycin; C, chloramphenicol; n, number of individuals in the group.

of the cases was 24.64 years (median, 22 years; range, 9 months to 62 years). Seventy-five (55.15%) cases were females and 61 (44.85%) were males. The age range for controls was between 10 months and 75 years (mean, 28.12 years; median, 25 years). Signs in symptomatic case individuals (n = 116) included abdominal cramps (11.20%), vomiting (9.5%), fever (16.38%), diarrhoea (22.4%), bloody diarrhoea (2.6%) and nausea (34. 48%). Signs in symptomatic control individuals (n = 782) included abdominal cramps (15.60%), vomiting (11.76%), fever (16.24%), diarrhoea (27.75%), bloody diarrhoea (5.5%) and nausea (34. 27%).

Univariate analysis of risk factors

Table 2 below displays results of the univariate analysis of risk factors for *Campylobacter* infections in humans. Presented factors in the table are only those which showed statistically significant association with increased risk for human infections during the analysis (specified at probability = 0.1). Briefly the risk for human campylobacteriosis increased with young age, consumption of poultry meat, eating prepared salad, drinking water from surface sources and working in a day care centre. Independent protective factors were using Western type toilet, contact with cattle and contact with sheep and goat. No statistically significant differences between cases and controls were detected when the following exposures were analysed: consumption of beef, pork, game meat, undercooked meat, fish, shellfish, sausages, prepared salads, unpeeled fruits, raw vegetables and milk or milk products including ice cream and yoghurt. Also cases and controls did not differ significantly with regard to kitchen hygiene parameters like cleaning of hands, chopping boards, knives or the countertop with soap and water during meat preparation and before use for other food items. Moreover, illness was not associated with the preparation of raw meat, working in a health facility, drinking untreated water, use of pit latrine or squat toilet, as well as contact with pigs, a cat, a dog, poultry, horse or wildlife. Cases and controls were also similar with respect to history of travel, recent use of antimicrobial agents, antacids and the use of regular medications.

Multivariate analysis of risk factors

Multivariate logistic regression analysis was performed to determine which variables were independently associated with *Campylobacter* positivity. The analysis involved all variables which were statistically significant in the univariate analysis. During multivariable logistic regression analysis, variables included in the final model as independent risk factors for human campylobacteriosis were age, consumption of poultry and eating pre-prepared salad (Table 3). Drinking water from a surface source, contact with a pig and working at a day care centre were associated

Table 2. Univariate analysis of risk factors for human campylobacteriosis

Risk factor	% positive in factor positive	% positive in factor negative	Odds ratio (95% CI)	*P*-value
Age category (Young/adult)	16.73 (*n* = 251)	10.0 (*n* = 944)	1.81 (1.22–2.69)	0.003
Eating poultry	19.64 (*n* = 550)	4.34 (*n* = 645)	5.40 (3.50–8.30)	0.00
Eating prepared salad	13.15 (*n* = 631)	9.40 (*n* = 564)	1.46 (1.01–2.10)	0.04
Drinking water from surface sources	14.29 (*n* = 894)	10.4 (*n* = 301)	0.70 (0.47–1.03)	0.07
Working at day care centre	26.67 (*n* = 15)	11.19 (1180)	2.70 (0.90–9.10)	0.07

%, percentage; *n*, number of individuals in the group; CI, confidence interval.

Table 3. Multivariate logistic regression analysis of risk factors for human campylobacteriosis

Risk factor	% positive in factor positive	% positive in factor negative	Odds ratio (95% CI)	*P*-value
Age category (Young/adult)	16.73 (*n* = 251)	10.0 (*n* = 944)	1.60 (1.04–2.44)	0.03
Eating poultry	19.64 (*n* = 550)	4.34 (*n* = 645)	5.32 (3.43–8.24)	0.00
Eating pre-prepared salad	13.15 (*n* = 631)	9.40 (*n* = 564)	1.53 (1.04–2.25)	0.03

%, percentage; *n*, number of individuals in the group; CI, confidence interval.

with human campylobacteriosis in univariate analysis, but did not remain significant in the multivariate analysis. The final model for assessment of risk factors associated with positivity to human campylobacteriosis passed the Hosmer–Lemeshow statistic test suggesting a good fit to the data. Similarly, the assessment of the predictive accuracy of the model based on the area under the curve (AUC) derived from the receiver operating characteristic curve analysis (AUC = 0.73) suggested that the model had good discriminating ability of predictors. Confounding factors were not detected during the model building process.

Discussion

The overall prevalence of *Campylobacter* infection among the sampled human subjects ($n = 1195$) in our study was 11.4%, with both symptomatic and asymptomatic individuals infected. The obtained proportion of *Campylobacter*-positive individuals is comparable to those reported in previous studies in Tanzania (Lindblom et al., 1995; Mdegela et al., 2006). Similarly, the observed high frequency of *C. jejuni* as compared to *C. coli* is in concordance with the results reported in many other studies in the country (Lindblom et al., 1995; Mdegela et al., 2006; Jacob et al., 2011) and elsewhere (Endtz et al., 1991; Gaudreau and Gilbert, 1997; Feizabadi et al., 2007; Gallay et al., 2007; Yang et al., 2008; Rajendran et al., 2012). A comparatively large proportion of infected individuals among symptomatics suggests that the organism is a significant cause of gastroenteritis.

A number of studies have reported antimicrobial resistance among *Campylobacter* isolates worldwide (Gupta et al., 2004; Jain et al., 2005; Gallay et al., 2007; van Hees et al., 2007; Nonga and Muhairwa, 2009; Tadesse et al., 2011; Ghosh et al., 2013). Results of the present study have also revealed different proportions of resistant isolates to a number of antimicrobials including macrolides and fluoroquinolones, the drugs of choice for antimicrobial treatment of human campylobacteriosis (Tadesse et al., 2011; Lehtopolku et al., 2012). Higher proportions of resistant isolates were observed for cephalothin, tetracycline, erythromycin, azithromycin and colistin sulphate, ranging from 70.6% to 95.6%. Relatively lower resistance rates were recorded for norfloxacin (49.3%), chloramphenicol (44.1%) and ciprofloxacin (the lowest, 22.1%). A more or less similar low frequency of resistance against ciprofloxacin was reported in France by Gallay et al. (2007). There are also studies that have found very low levels of resistance to ciprofloxacin among *Campylobacter* isolates (Deckert et al., 2013; Randrianirina et al., 2014). The low resistance to ciprofloxacin observed in our study emphasizes its continual usefulness for the treatment of *Campylobacter* gastroenteritis. Contrary to the finding in this study, studies elsewhere have reported moderate (Pigrau et al., 1997) and high (Li et al., 1998; Jain et al., 2005; Serichantalergs et al., 2007; Zhang et al., 2010; Ghosh et al., 2013; Di Giannatale et al., 2014) resistance rates of *Campylobacter* spp. to ciprofloxacin. These studies on the other hand observed low degrees (>25%) of resistance to erythromycin which is also contrary to the high degree of resistance (84.6%) recorded in this study. Gallay et al. (2007) pointed out that the resistance rate reported for erythromycin varies widely among studies. Zhang et al. (2010) reported 100% susceptibility of *Campylobacter* spp. to erythromycin. The observation in this study, which may partly be responsible for a higher prevalence of campylobacteriosis in children as erythromycin is a primary antimicrobial of choice for children, warrants reconsideration of use of macrolides as the drugs of choice in patients with severe gastroenteritis when *Campylobacter* is the presumed cause. Frequencies of resis-

tant *Campylobacter* isolates to other antimicrobials tested in this study have varied as previously reported in other studies (Gallay et al., 2007; de Jong et al., 2012; Ghosh et al., 2013). Differences in resistance seen in this study when compared to previous investigations could be due to changes over time but also could be an attribute of differences in exposure rates of the microbes to the different antimicrobials. Ghosh et al. (2013) points out that antimicrobial use for infections other than gastroenteritis and self-medication in the developing countries results into higher resistance levels as compared to levels in the developed countries.

It has been reported in previous studies that *C. coli* isolates are more resistant to antimicrobials than *C. jejuni* strains (Pezzotti et al., 2003; Taremi et al., 2006; Gallay et al., 2007; de Jong et al., 2012). In the present study, higher proportions of resistant *C. coli* isolates have been observed for nalidixic acid and amoxicillin. Our results, however, need caution during interpretation as the number of *C. coli* isolates was relatively low.

Our results point to a good correlation and agreement between agar dilution and disc diffusion methods for antimicrobial susceptibility testing of *Campylobacter*. The former method has been approved by the clinical and laboratory standards institute (CLSI) as a standard method for antimicrobial susceptibility testing of these organisms; however, it is time-consuming. The disc diffusion method, on the other hand, is relatively easy to perform and is less expensive. A good correlation between the two techniques, observed in this and previous works (Gaudreau and Gilbert, 1997; Miflin et al., 2007; Senok et al., 2007), forms the basis of considering the disc diffusion as an alternative susceptibility testing method for *Campylobacter*.

Associations of human campylobacteriosis with chicken consumption (Studahl and Andersson, 2000; Neimann et al., 2003; Friedman et al., 2004; Michaud et al., 2004; Wingstrand et al., 2006; Danis et al., 2009), young age (Lindblom et al., 1995; Mdegela et al., 2006) and commercial food items including vegetable salads (Michino and Otsuki, 2000; Evans et al., 2003), observed in this study, have been reported earlier. Some studies have reported a limited association to eating undercooked chicken (Ikram et al., 1994; Eberhart-Phillips et al., 1997; Stafford et al., 2007), while others with any type of chicken. Other studies have found increased risks with consumption of commercially prepared chicken (Eberhart-Phillips et al., 1997; Rodrigues et al., 2001; Evans et al., 2003; Friedman et al., 2004; Michaud et al., 2004; Unicomb et al., 2008; Tam et al., 2009). Factors that are likely to contribute to high prevalence in young individuals are poor hygiene and sanitation, contact with animals, malnutrition and low immunity because of first exposures (Mdegela et al., 2006). On the other hand, the association of human illness with eating foods prepared in commercial food establishments suggests the likelihood of cross-contamination (Dawkins et al., 1984) or undercooking in such settings. A finding that working in a day care was not associated with *Campylobacter* infections in humans could probably be a result of the low number of cases that worked in a day care.

Overall, we have reported the presence, risk factors and extent of thermophilic *Campylobacter* infections in humans, and resistance profiles of human-derived isolates to different antimicrobials in Eastern Tanzania. Prevalence results emphasize on routine microbiological isolation and identification of the organism in microbiology laboratories to investigate its role in gastroenteritis aetiology. These results show the need to plan and implement control procedures for *Campylobacter* organisms in both human and reservoir animal populations. The demonstration of high resistance to most of the antimicrobials necessitates ensuring control of indiscriminate use of antimicrobials both in livestock and in humans. We highly recommend determination of the antimicrobial resistance of the isolates on routine basis so as to help to guide the antimicrobial treatment approaches in cases of gastroenteritis.

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Conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

EVGK carried out the experiments, compiled the results and wrote the manuscript. LNN participated in carrying out the experiments. RHM, PLMM and HI participated in the design and helped to revise the manuscript. All authors approved the final version of the manuscript.

References

Adak, G. K., J. M. Cowden, S. Nicholas, and H. S. Evans, 1995: The public health laboratory service national case-control study of primary indigenous sporadic cases of *Campylobacter* infection. *Epidemiol. Infect.* 115, 15–22.

Allos, B. M., 2001: *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin. Infect. Dis.* 32, 1201–1206.

Balk, D., U. Deichmann, G. Yetman, F. Pozzi, S. I. Hay, and A. Nelson, 2006: Determining global population distribution: methods, applications and data. *Adv. Parasitol.* 62, 119–156.

Blaser, M. J., 1997: Epidemiologic and clinical features of *Campylobacter jejuni* infections. *J. Infect. Dis.* 176, 103–105.

Carrique-Mas, J., Y. Andersson, M. Hjertqvist, A. Svensson, A. Torner, and J. Giesecke, 2005: Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. *Scand. J. Infect. Dis.* 37, 101–110.

Danis, K., M. Di Renzi, W. O'Neill, B. Smyth, P. McKeown, B. Foley, V. Tohani, and M. Devine, 2009: Risk factors for sporadic *Campylobacter* infection: an all-Ireland case–control study. *Euro. Surveill.* 14, pii=19123.

Dawkins, H. C., F. J. Bolton, and D. N. Hutchinson, 1984: A study of the spread of *Campylobacter jejuni* in four large kitchens. *J. Hyg (Lond).* 92, 357–364.

Deckert, A. E., R. J. Reid-Smith, S. E. Tamblyn, L. Morrell, P. Seliske, F. B. Jamieson, R. Irwin, C. E. Dewey, P. Boerlin, and S. A. McEwen, 2013: Antimicrobial resistance and antimicrobial use associated with laboratory-confirmed cases of Campylobacter infection in two health units in Ontario. *Can. J. Infect. Dis. Med. Microbiol. Spring* 24, e16–e21.

Di Giannatale, E., G. Di Serafino, K. Zilli, A. Alessiani, L. Sacchini, G. Garofolo, G. Aprea, and F. Marotta, 2014: Characterization of antimicrobial resistance patterns and detection of virulence genes in *Campylobacter* isolates in Italy. *Sensors (Basel)* 14, 3308–3322.

Djuretic, T., P. G. Wall, and G. Nichols, 1997: General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. Communicable disease report. *CDR Review* 7, R41–R45.

Eberhart-Phillips, J., N. Walker, N. Garrett, D. Bell, D. Sinclair, W. Rainger, and M. Bates, 1997: Campylobacteriosis in New Zealand: results of a case-control study. *J. Epidemiol. Community Health* 51, 686–691.

Endtz, H. P., G. J. Ruijs, A. H. Zwinderman, T. Reijden, M. Biever, and R. P. Mouton, 1991: Comparison of six media, including a semisolid agar, for the isolation of various *Campylobacter* species from stool specimens. *J. Clin. Microbiol.* 29, 1007–1010.

Ethelberg, S., J. Simonsen, P. Gerner-Smidt, K. E. Olsen, and K. Mølbak, 2005: Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991–2001. *Am. J. Epidemiol.* 162, 1008–1015.

Evans, M. R., R. J. Roberts, C. D. Ribeiro, D. Gardner, and D. Kembrey, 1996: A milk-borne *Campylobacter* outbreak following an educational farm visit. *Epidemiol. Infect.* 117, 457–462.

Evans, M. R., C. D. Ribeiro, and R. L. Salmon, 2003: Hazards of healthy living: bottled water and salad vegetables as risk factors for *Campylobacter* infection. *Emerg. Infect. Dis.* 9, 1219–1225.

Fahey, T., D. Morgan, C. Gunneburg, G. K. Adak, F. Majid, and E. Kaczmarski, 1995: An outbreak of *Campylobacter jejuni* enteritis associated with failed milk pasteurisation. *J. Infect.* 31, 137–143.

Fajo′-Pascual, M., P. Godoy, M. Ferrero-Ca′ ncer, and K. Wymore, 2009: Case–control study of risk factors for sporadic *Campylobacter* infections in northeastern Spain. *Eur. J. Public Health* 20, 443–448.

Feizabadi, M. M., S. Dolatabadi, and M. R. Zali, 2007: Isolation and drug-resistant patterns of *Campylobacter* strains cultured from diarrheic children in Tehran. *Jpn. J. Infect. Dis.* 60, 217–219.

Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe, 2000: Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin, I., and M. J. Blaser (eds), Campylobacter, 2nd edn, pp. 121–135. ASM Press, Washington, DC.

Friedman, C. R., R. M. Hoekstra, M. Samuel, R. Marcus, J. Bender, B. Shiferaw, S. Reddy, S. D. Ahuja, D. L. Helfrick, F. Hardnett, M. Carter, B. Anderson, R. V. Tauxe, and Emerging Infections Program FoodNet Working Group, 2004: Risk factors for sporadic *Campylobacter* infection in the United States: a case-control study in FoodNet sites. *Clin. Infect. Dis.* 38 (Suppl. 3), 285–296.

Gallay, A., V. Prouzet-Mauleon, I. Kempf, P. Lehours, L. Labadi, C. Camou, M. Denis, H. de Valk, J. C. Desenclos, and F. Megraud, 2007: *Campylobacter* antimicrobial drug resistance among humans, broiler chickens, and pigs, France. *Emerg. Infect. Dis.* 13, 259–266.

Gallay, A., V. Bousquet, V. Siret, V. Prouzet-Mauleon, H. Valk, V. Vaillant, F. Simon, Y. Le Strat, F. Megraud, and J. C. Desenclos, 2008: Risk factors for acquiring sporadic *Campylobacter* infection in France: results from a national case-control study. *J. Infect. Dis.* 197, 1477–1484.

Gaudreau, C., and H. Gilbert, 1997: Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* 39, 707–712.

Ghosh, R., B. Uppal, P. Aggarwal, A. Chakravarti, and A. K. Jha, 2013: Increasing antimicrobial resistance of *Campylobacter jejuni* isolated from paediatric diarrhea cases in a tertiary care hospital of New Delhi, India. *J. Clin. Diagn. Res.* 7, 247–249.

Gibreel, A., and D. E. Taylor, 2006: Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* 58, 243–255.

Gripp, E., D. Hlahla, X. Didelot, F. Kops, S. Maurischat, K. Tedin, T. Alter, L. Ellerbroek, K. Schreiber, D. Schomburg, T. Janssen, P. Bartholomäus, D. Hofreuter, S. Woltemate, M. Uhr, B. Brenneke, P. Grüning, G. Gerlach, L. Wieler, S. Suerbaum, and C. Josenhans, 2011: Closely related *Campylobacter jejuni* strains from different sources reveal a generalist rather than a specialist lifestyle. *BMC Genom.* 12, 584.

Guerrant, R. L., T. Van Gilder, T. S. Steiner, N. M. Thielman, L. Slutsker, R. V. Tauxe, T. Hennessy, P. M. Griffin, H. DuPont, R. B. Sack, P. Tarr, M. Neill, I. Nachamkin, L. B. Reller, M. T. Osterholm, M. L. Bennish, and L. K. Pickering, 2001: Practice guidelines for the management of infectious diarrhea. *Clin. Infect. Dis.* 32, 331–351.

Gupta, A., J. M. Nelson, T. J. Barrett, R. V. Tauxe, S. P. Rossiter, C. R. Friedman, K. W. Joyce, K. E. Smith, T. F. Jones, M. A. Hawkins, B. Shiferaw, J. L. Beebe, D. J. Vugia, T. Rabatsky-Ehr, J. A. Benson, T. P. Root, and F. J. Angulo, 2004: Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerg. Infect. Dis.* 10, 1102–1109.

Haruna, M., Y. Sasaki, M. Murakami, T. Mori, T. Asai, K. Ito, and Y. Yamada, 2013: Prevalence and antimicrobial resistance of C*ampylobacter* isolates from beef cattle and pigs in Japan. *J. Vet. Med. Sci.* 75, 625–628.

van Hees, B. C., M. J. Veldman-Ariesen, B. M. de Jongh, M. Tersmette, and W. van Pelt, 2007: Regional and seasonal differences in incidence and antibiotic resistance of *Campylobacter* from a nationwide surveillance study in The Netherlands: an overview of 2000–2004. *Clin. Microbiol. Infect.* 13, 305–310.

Hosmer, D. W., and S. Lemeshow, 2002: Applied Logistic Regression, 2nd edn. John Wiley & Sons, New York.

Ikram, R., S. Chambers, P. Mitchell, M. A. Brieseman, and O. H. Ikam, 1994: A case–control study to determine risk factors for *Campylobacter* infection in Christchurch in the summer of 1992–3. *N. Z. Med. J.* 107, 430–432.

Jacob, P., R. H. Mdegela, and H. E. Nonga, 2011: Comparison of Cape Town and Skirrow's *Campylobacter* isolation protocols in humans and broilers in Morogoro, Tanzania. *Trop. Anim. Health Prod.* 43, 1007–1013.

Jain, D., S. Sinha, K. N. Prasad, and C. M. Pandey, 2005: *Campylobacter* species and drug resistance in a north Indian rural community. *Trans. R. Soc. Trop. Med. Hyg.* 99, 207–214.

de Jong, A., V. Thomas, S. Simjee, K. Godinho, B. Schiessl, U. Klein, P. Butty, M. Vallé, H. Marion, and T. R. Shryock, 2012: Pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food-producing animals. *J. Antimicrob. Chemother.* 67, 638–651.

Kalman, M., E. Szollosi, B. Czermann, M. Zimanyi, S. Szekeres, and M. Kalman, 2000: Milkborne *Campylobacter* infection in Hungary. *J. Food Prot.* 63, 1426–1429.

Kapperud, G., E. Skjerve, N. H. Bean, S. M. Ostroff, and J. Lassen, 1992: Risk factors for sporadic *Campylobacter* infections: results of a case-control study in southeastern Norway. *J. Clin. Microbiol.* 30, 3117–3121.

Kapperud, G., G. Espeland, E. Wahl, A. Walde, H. Herikstad, S. Gustavsen, I. Tveit, O. Natas, L. Bevanger, and A. Digranes, 2003: Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case–control study in Norway. *Am. J. Epidemiol.* 158, 234–242.

Komba, E. V. G., R. H. Mdegela, P. L. M Msoffe, and H. Ingmer, 2013: Human and animal *Campylobacteriosis* in Tanzania: a review. *Tanzan. J. Health Res.* 15, 1–13.

Le Roux, E., and A. J. Lastovica, 1998: The Cape Town protocol: how to isolate the most campylobacters for your dollars pounds franc yen etc. In: Lastovica, A. J., D. Newell, and E. E. Lastovica (eds), Proceedings of 9th International workshop on Campylobacter, Helicobacter and Related Organism, pp. 31–33. South Africa, Institute of Child health Cape Town.

Lehner, A., C. Schneck, G. Feierl, P. Pless, A. Deutz, E. Brandl, and M. Wagner, 2000: Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre. *Epidemiol. Infect.* 125, 13–16.

Lehtopolku, M., P. Kotilainen, P. Puukka, U. M. Nakari, A. Siitonen, E. Eerola, P. Huovinen, and A. J. Hakanen, 2012: Inaccuracy of the disk diffusion method compared with the agar dilution method for susceptibility testing of *Campylobacter* spp. *J. Clin. Microbiol.* 50, 52–56.

Li, C. C., C. H. Chiu, J. L. Wu, Y. C. Huang, and T. Y. Lin, 1998: Antimicrobial susceptibilities of *Campylobacter jejuni* and *coli* by using E-test in Taiwan. *Scand. J. Infect. Dis.* 30, 39–42.

Lindblom, G. B., C. Ahren, J. Changalucha, R. Gabone, B. Kaijser, L. A. Nilsson, E. Sjogren, A. M. Svennerholm, and M. Temu, 1995: *Campylobacter jejuni/coli* and enterotoxigenic *Escherichia coli* (ETEC) in faeces from children and adults in Tanzania. *Scand. J. Infect. Dis.* 27, 589–593.

Lindmark, H., S. Boqvist, M. Ljungström, P. Agren, B. Björkholm, and L. Engstrand, 2009: Risk factors for campylobacteriosis: an epidemiological surveillance study of patients and retail poultry. *J. Clin. Microbiol.* 47, 2616–2619.

Luangtongkum, T., T. Y. Morishita, A. B. El-Tayeb, A. J. Ison, and Q. Zhang, 2007: Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. *J. Clin. Microbiol.* 45, 590–594.

McDermott, P. F., S. M. Bodeis, F. M. Aarestrup, S. Brown, M. Traczewski, P. Fedorka-Cray, M. Wallace, I. A. Critchley, C. Thornsberry, S. Graff, R. Flamm, J. Beyer, D. Shortridge, L. J. Piddock, V. Ricci, M. M. Johnson, R. N. Jones, B. Reller, S. Mirrett, J. Aldrobi, R. Rennie, C. Brosnikoff, L. Turnbull, G. Stein, S. Schooley, R. A. Hanson, and R. D. Walker, 2004: Development of a standardized susceptibility test for *Campylobacter* with quality-control ranges for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem. *Microb. Drug Resist.* 10, 124–131.

Mdegela, R. H., H. E. Nonga, H. A. Ngowi, and R. R. Kazwala, 2006: Prevalence of thermophilic *Campylobacter* infections in humans, chickens and crows in Morogoro, Tanzania. *J. Vet. Med. B* 53, 116–121.

Michaud, S., S. Menard, and R. D. Arbeit, 2004: Campylobacteriosis, eastern townships, Quebec. *Emerg. Infect. Dis.* 10, 1844–1847.

Michino, H., and K. Otsuki, 2000: Risk factors in causing outbreaks of food-borne illness originating in schoollunch facilities in Japan. *J. Vet. Med. Sci.* 62, 557–560.

Miflin, J. K., J. M. Templeton, and P. J. Blackall, 2007: Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the South-East Queensland region. *J. Antimicrob. Chemother.* 59, 775–778.

Morgan, D., C. Gunneberg, D. Gunnell, T. D. Healing, S. Lamerton, N. Soltanpoor, D. A. Lewis, and D. G. White, 1994: An outbreak of *Campylobacter* infection associated with the

consumption of unpasteurised milk at a large festival in England. *Eur. J. Epidemiol.* 10, 581–585.

NCCLS, 2002: Perfomance standards for antimicrobial disk diffusion and dilution susceptibility tests for bacteria isolated from animals. National Committee for Clinical Laboratory Standards (NCCLS), Pennsylvania.

Neimann, J., J. Engberg, K. Molbak, and H. C. Wegener, 2003: A case–control study of risk factors for sporadic *Campylobacter* infections in Denmark. *Epidemiol. Infect.* 130, 353–366.

Newell, D. G., K. T. Elvers, D. Dopfer, I. Hansson, P. Jones, S. James, J. Gittins, N. J. Stern, R. Davies, I. Connerton, D. Pearson, G. Salvat, and V. M. Allen, 2011: Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. *Appl. Environ. Microbiol.* 77, 8605–8614.

Nonga, H. E., and A. P. Muhairwa, 2009: Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolates from free range domestic duck (*Cairina moschata*) in Morogoro municipality, Tanzania. *Trop. Anim. Health Prod.* 42, 165–172.

Ogden, I. D., J. F. Dallas, M. MacRae, O. Rotariu, K. W. Reay, M. Leitch, A. P. Thomson, S. K. Sheppard, M. Maiden, K. J. Forbes, and N. J. Strachan, 2009: *Campylobacter* excreted into the environment by animal sources: prevalence, concentration shed, and host association. *Foodborne Pathog. Dis.* 6, 1161–1170.

Pezzotti, G., A. Serafin, I. Luzzi, R. Mioni, and R. Perin, 2003: Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. *Int. J. Food Microbiol.* 82, 281–287.

Pigrau, C., R. Bartolome, B. Almirante, A. M. Planes, J. Gavalda, and A. Pahissa, 1997: Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. *Clin. Infect. Dis.* 25, 1414–1420.

Rajendran, P., S. Babji, A. T. George, D. P. Rajan, G. Kang, and S. S. Ajjampur, 2012: Detection and species identification of *Campylobacter* in stool samples of children and animals from Vellore, south India. *Indian J. Med. Microbiol.* 30, 85–88.

Randrianirina, F., E. H. Ratsima, L. Ramparany, R. Randremanana, H. C. Rakotonirina, T. Andriamanantena, F. Rakotomanana, S. Rajatonirina, V. Richard, and A. Talarmin, 2014: Antimicrobial resistance of bacterial enteropathogens isolated from stools in Madagascar. *BMC Infect. Dis.* 14, 104.

Rodrigues, L. C., J. M. Cowden, J. G. Wheeler, D. Sethi, P. G. Wall, P. Cumberland, D. S. Tompkins, M. J. Hudson, J. A. Roberts, and P. J. Roderick, 2001: The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. *Epidemiol. Infect.* 127, 185–193.

Rzewuska, K., D. Korsak, and E. Maćkiw, 2010: Antibiotic resistance of bacteria *Campylobacter* sp. *Przegl. Epidemiol.* 64, 63–68.

Schildt, M., S. Savolainen, and M. L. Hanninen, 2006: Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. *Epidemiol. Infect.* 134, 401–405.

Senok, A., A. Yousif, W. Mazi, E. Sharaf, K. Bindayna, A. Elnima el, and G. Botta, 2007: Pattern of antibiotic susceptibility in *Campylobacter jejuni* isolates of human and poultry origin. *Jpn. J. Infect. Dis.* 60, 1–4.

Serichantalergs, O., A. Dalsgaard, L. Bodhidatta, S. Krasaesub, C. Pitarangsi, A. Srijan, and C. J. Mason, 2007: Emerging fluoroquinolone and macrolide resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates and their serotypes in Thai children from 1991 to 2000. *Epidemiol. Infect.* 135, 1299–1306.

Snelling, W. J., M. Matsuda, J. E. Moore, and J. S. Dooley, 2005: *Campylobacter jejuni*. *Lett. Appl. Microbiol.* 41, 297–302.

Stafford, R. J., P. Schluter, M. Kirk, A. Wilson, L. Unicomb, R. Ashbolt, J. Gregory, and OzFoodNet Working Group, 2007: A multi-centre prospective case–control study of *Campylobacter* infection in persons aged 5 years and older in Australia. *Epidemiol. Infect.* 135, 978–988.

Studahl, A., and Y. Andersson, 2000: Risk factors for indigenous *Campylobacter* infection: a Swedish case-control study. *Epidemiol. Infect.* 125, 269–275.

Tadesse, D. A., P. B. Bahnson, J. A. Funk, S. Thakur, W. E. Morrow, T. Wittum, F. DeGraves, P. Rajala-Schultz, and W. A. Gebreyes, 2011: Prevalence and antimicrobial resistance profile of *Campylobacter* spp. isolated from conventional and antimicrobial-free swine production systems from different U.S. regions. *Foodborne Pathog. Dis.* 8, 367–374.

Takamiya, M., A. Ozen, M. Rasmussen, T. Alter, T. Gilbert, D. W. Ussery, and S. Knøchel, 2011: Genome Sequence of *Campylobacter jejuni* strain 327, a strain isolated from a turkey slaughterhouse. *Stand. Genomic Sci.* 4, 113–122.

Tam, C. C., C. D. Higgins, K. R. Neal, L. C. Rodrigues, S. E. Millership, S. J. O'Brien, and Campylobacter Case-Control Study Group, 2009: Chicken consumption and use of acid-suppressing medications as risk factors for *Campylobacter* enteritis, England. *Emerg. Infect. Dis.* 15, 1402–1408.

Taremi, M., S. D. M. Mehdi, L. Gachkar, S. MoezArdalan, K. Zolfaghari, and M. R. Zali, 2006: Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. *Int. J. Food Microbiol.* 108, 401–403.

Thrusfield, M., 1995: Veterinary Epidemiology, 2nd edn. Blackwell Science, Oxford, UK.

Unicomb, L. E., C. B. Dalton, G. L. Gilbert, N. G. Becker, and M. S. Patel, 2008: Age-specific risk factors for sporadic *Campylobacter* infection in regional Australia. *Foodborne Pathog. Dis.* 5, 79–85.

Uyttendaele, M., K. Baert, Y. Ghafir, G. Daube, L. De Zutter, L. Herman, K. Dierick, D. Pierard, J. J. Dubois, B. Horion, and J. Debevere, 2006: Quantitative risk assessment of *Campylobacter* spp. in poultry based meat preparations as one of the factors to support the development of risk-based microbiological criteria in Belgium. *Int. J. Food Microbiol.* 111, 149–163.

Wingstrand, A., J. Neimann, J. Engberg, E. M. Nielsen, P. Gerner-Smidt, H. C. Wegener, and K. Molbak, 2006: Fresh chicken

as main risk factor for campylobacteriosis, Denmark. *Emerg. Infect. Dis.* 12, 280–285.

Yang, J. R., H. S. Wu, C. S. Chiang, and J. J. Mu, 2008: Pediatric campylobacteriosis in northern Taiwan from 2003 to 2005. *BMC Infect. Dis.* 8, 151.

Zhang, M., Y. Gu, L. He, L. Ran, S. Xia, X. Han, H. Li, H. Zhou, Z. Cui, and J. Zhang, 2010: Molecular typing and antimicrobial susceptibility profiles of *Campylobacter jejuni* isolates from north China. *J. Med. Microbiol.* 59, 1171–1177.

