

Human and animal *Campylobacteriosis* in Tanzania: A review

ERICK V.G. KOMBA^{1*}, ROBINSON H. MDEGELA¹, PETER L.M. MSOFFE¹ and HANNE INGMER²

¹Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania

²Department of Veterinary Disease Biology, Faculty of Life sciences, University of Copenhagen, Frederiksberg C, DK-1870, Denmark

Abstract: The thermotolerant species of *Campylobacter* have become very important in public health, particularly as agents of infectious diarrhoea in human beings. Though the mechanism by which they cause disease is yet to be fully explained, they have been recognized as the leading cause of bacterial enteritis in both developed and developing countries. The organisms colonize different animal species without causing any symptoms of disease; and humans acquire infections through contact with or consumption of contaminated meat especially raw/undercooked poultry meat. The growing trend of antibiotic resistant *Campylobacter* isolates continues to pose significant public health challenges. In this review we present the available information generated in Tanzania about *Campylobacter* infections in humans and animals. We conducted a structured literature search of PUBMED and ScienceDirect electronic databases and identified 15 articles. Studies on humans reported *Campylobacter* infections in both symptomatic and asymptomatic subjects; with higher prevalence in children under the age of five years. Studies on animals found colonization of both domestic and wild species. Among isolates, some demonstrated antimicrobial resistance. The available information for both human and animal *Campylobacteriosis* in the country is sparse. It however provides an insight of the bacteriological and epidemiological aspects of *Campylobacter* infections in the country and eventually creates more awareness on the need to develop control strategies. Since the organism is zoonotic its control strategies should adopt the “One Health” approach involving collaborative efforts from veterinary and human medicine.

Keywords: *Campylobacter*, infections, diarrhoea, epidemiology, humans, animals, Tanzania

Introduction

Increasing meat consumption around the world goes together with increased concerns and challenges to meat hygiene and safety (Sofos & Geornaras, 2010). One of the major safety concerns is contamination by *Campylobacter*, a leading bacterial cause of diarrhoea in humans worldwide, of meat products particularly poultry. *Campylobacter* species reside in the gut of domesticated warm-blooded animals and birds as part of the intestinal microbiota (Ketley, 1997). Of particular importance to humans is their colonization of animals used in food production, including poultry, cattle, sheep and swine (Blaser, 1997; Moore *et al.*, 2005). The most frequently isolated species, *Campylobacter jejuni*, is now recognized as the leading bacterial cause of food-borne disease in both developed and developing countries (Guerrant *et al.*, 1990; Skirrow, 1990; Butzler, 2004). In addition, infection with *C. jejuni* is the most frequent cause of a form of neuromuscular paralysis known as Guillain-Barré syndrome (Parkhill *et al.*, 2000).

The development of health programmes aimed at controlling diseases, including campylobacteriosis, requires adequate information on the disease including its epidemiology. The absence of national surveillance programmes for *Campylobacter* infections, particularly in developing countries, makes it difficult to give an accurate picture of the true incidence for some populations (Senok & Botta, 2009). Thus, resulting into substantial gaps in knowledge about the epidemiology of campylobacteriosis in developing countries. Here we summarize available studies on epidemiological and bacteriological aspects of campylobacteriosis in humans and animals in Tanzania. We further

* Correspondence: Erick V.G. Komba; E-mail: ekomba@suanet.ac.tz

identify some challenges that need to be addressed by researchers in the country as far as studies on *Campylobacter* organisms are concerned.

Methods

We developed a search algorithm to obtain papers which described *Campylobacter* infections in Tanzania. More specifically the used algorithm to search the literature had the following key words: “*Campylobacter*” and “Tanzania” and (humans or children or diarrhoea or animals or goats or cattle or chickens or poultry or pigs or meat or wild animals or environment). The database PubMed provided by the United States National Library of Medicine and ScienceDirect provided by Elsevier, were used. A search through reference lists of initially obtained articles provided some additional research documents on *Campylobacter* not listed in the PubMed and ScienceDirect databases. A total of 15 papers, published between 1993 to the time of the searches (July, 2011), were obtained and summarized in the present review. During the review of published literature, the data extracted included types and sources of samples, isolation and identification methods, isolation rates and antimicrobial susceptibility profiles (one paper).

Types and sources of samples

Faecal samples have been identified as the specimens of choice for the isolation of *Campylobacter* species in patients presenting with gastrointestinal symptoms (Senok & Botta, 2009). In reported studies on *Campylobacter* in humans, these samples were collected from patients complaining of enteric problems (symptomatic) seeking for medical services in health facilities within the study areas. Studies on *Campylobacter* infections in children (Lindblom *et al.*, 1995; Kingamkono *et al.*, 1999) also involved collection of faecal samples from asymptomatic human subjects. Studies on detection of *Campylobacter* in animals involved collection of faecal samples from both domestic (goats, cattle, ducks, chickens and pigs) and wild (crows, mice and chimpanzees) species. From avians the samples were obtained either through cloacal swabs (live birds), collection of droppings in poultry houses or obtaining caecal contents from intestines of killed birds. Investigations on contamination levels of meat products (Nonga *et al.*, 2009, 2011) involved collection of swabs. Sample collection in all the studies considered the fastidious nature of the microorganism, and so ensured favourable transport and storage conditions including use of transport and enrichment media in the pre-analytical phase.

Isolation/detection of *Campylobacter*

For detecting *Campylobacter* species from different samples, several official protocols are available. However, most medical microbiology laboratories use conventional diagnostic procedures, such as culture and microscopy, for routine detection of enteric pathogens. These procedures include enrichment steps, use of selective culture media, biochemical identification, serotyping, and resistance profiling (Corry *et al.*, 2003; de Boer *et al.*, 2010). Such analyses have been the gold standard for many bacterial pathogens as they have allowed characterization at species and subspecies levels (Persson *et al.*, 2011). Conventional diagnostic methods for *Campylobacter* require that suspected stool specimens are cultured on selective agar at 42°C under microaerophilic conditions for up to 72 hours before a negative report is issued.

Molecular methods provide a means for sensitive and rapid detection of enteric pathogens. However, their broad applications remain limited due to their assumed high costs, inhibition caused by faecal constituents (Monteiro *et al.*, 1997), and the need for specialized laboratories. The

investigations reported by the publications summarized in this review employed mainly culture methods, particularly the qualitative (enrichment) method and direct plating method. A single study (Kaur *et al.*, 2011) employed molecular biological method in combination with enrichment culture. The enrichment media employed in most of the studies were mainly Bolton broth, *Campylobacter* enrichment broth (CEB) and Preston broth. The isolation medium employed in most of the studies was mainly charcoal cefaperazone deoxycholate agar (CCDA) plates. Antibiotic free blood agar (in combination with filtration) was also used in some investigations (Kaur *et al.*, 2011; Jacob *et al.*, 2011). A study by Jacob *et al.* (2011) revealed that the Cape Town protocol (combining filtration and culture on antibiotic-free blood agar) resulted into significantly higher prevalence than the Skirrow's protocol (culture on antibiotic containing agar). The superiority of the Cape Town protocol over the Skirrow's protocol has been documented previously in South Africa (Lastovica *et al.*, 2002). The authors pointed out that the protocol increases isolation of both the number of strains and the number of *Campylobacter* spp. and species of the related genera *Arcobacter* and *Helicobacter* from stool samples.

Identification and typing of *Campylobacter*

Phenotypic methods were adopted in identifying the *Campylobacter* isolates in the reviewed studies. *Campylobacter* species were mainly identified based on growth temperature preferences, growth in microaerophilic environment, colonial morphology, Gram staining and biochemical characterization of urease, catalase, and oxidase production, as well as sensitivity to nalidixic acid and cephalothin. The studies adopted hippurate hydrolysis test to distinguish between *C. jejuni* and other species. According to some researchers, however, proper phenotypic identification of *Campylobacter* isolates, especially differentiation between *C. jejuni* and *C. coli* based on the hippurate test, might be difficult and could result in false isolate identification (Rönner & Lindmark, 2007; Nakari *et al.*, 2008). One of the cited studies in this review found that only 74.1% of 243 isolates identified by phenotypic tests to be *C. jejuni* were confirmed by Polymerase Chain Reaction (PCR) (Mdegela *et al.*, 2006).

The typing of *Campylobacter* isolates adopted serotyping method (Lindblom *et al.*, 1995) and DNA based molecular techniques (Mdegela *et al.*, 2006; Chuma, 2008; Kaur *et al.*, 2011).

Antimicrobial resistance testing

Of the reviewed articles only one study on *Campylobacter* colonization in ducks by Nonga & Muhairwa (2009) addressed the issue of antimicrobial susceptibility of the *Campylobacter* isolates. Resistance profiles of the isolates to different antimicrobials were determined by the disk diffusion method (Bauer & others 1966) on Mueller-Hinton agar. The authors tested the isolates against the following antimicrobials: streptomycin (10µg), amoxicillin (10µg), ampicillin (10µg), ciprofloxacin (5µg), cefuroxime sodium (30µg), gentamicin (10µg), cloxacillin (5µg), tetracycline (30µg), nitrofurantoin (30µg), amikacin (30µg), erythromycin (15µg) and norfloxacin (10µg) (Span Diagnostic, Surat India). The bacterial growth inhibition zone was measured to assess resistance of the isolates using guidelines stipulated by the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

***Campylobacter* infections in humans**

Campylobacter species are carried in the intestinal tracts of mammals and birds. Sources of human infections include raw milk, contaminated water, direct contact with pets, and foods, particularly poultry (Pepe *et al.*, 2009). *Campylobacter jejuni* and *C. coli* are the species that account for the

majority of human infections. To date a total of eight studies have been conducted to investigate on *Campylobacter* infections in humans in Tanzania. Prevalence ranging between 0 (Gasco'n et al., 2000; Vargas et al., 2004) and 21.6% (Jacob et al., 2011) have been reported (Table 1).

Table 1: Overall and species specific prevalence of *Campylobacter* in different studies on humans in Tanzania

Age group	Disease status (sample size)	Prev (%)	Species prevalence (%)			References
			<i>C. jejuni</i>	<i>C. coli</i>	others	
U-5	Symptomatic (394)	18	80	20		Lindblom et al., 1995
	Asymptomatic (278)	12	94	6	0	
Adults	Symptomatic (82)	1	-	-	-	Kingamkono et al., 1999
	Asymptomatic (247)	1	-	-	-	
U-5	Asymptomatic (445)	9.4	-	-	-	Gasco'n et al., 2000
U-5	Symptomatic (103)	0	-	-	-	Vargas et al., 2004
	Asymptomatic (206)	0	-	-	-	
U-5	Symptomatic (348)	2.56	-	-	-	Kusiluka et al., 2005
	Symptomatic (103)	0	-	-	-	
All	Symptomatic (484)	1.9	100	0	0	Mdegela et al., 2006
All	Symptomatic (632)	9.3	96.6	3.4	0	Chuma, 2009
U-5	Symptomatic (268)	19	78.4	19.6	2	Jacob et al. 2011
All	Symptomatic (176)	21.6	92.1	7.9	0	

Key: U-5=under five years; Prev = Prevalence

Consistently studies have reported higher prevalence in young individuals as compared to adults (Lindblom et al., 1995; Mdegela et al., 2006). It has been mentioned previously that in developing countries campylobacteriosis is a disease of young children (Khalil et al., 1993; Molbak & Hojlyng, 1988), while in industrialized countries it is a disease affecting mainly adults (Kaijser, 1988). A study involving children under the age of 5 years showed that males had higher prevalence as compared to females (Chuma, 2009). Studies involving symptomatic and asymptomatic human subjects found no significant differences in infection with *Campylobacter* (Lindblom et al., 1995; Gasco'n et al., 2000). Lindblom et al. (1995) however found a high prevalence of *Campylobacter* infection in symptomatic children under the age of 18 months as opposed to asymptomatic counterparts. Similarly investigators elsewhere in other developing countries noticed that *Campylobacter* is as common in faeces from symptomatic children as from asymptomatic ones but they found a difference among those under the age of 18 months (Steele et al., 1988, Ringertz et al., 1980, Haq & Rahman 1991). The reason for this has been claimed to be that in developing countries repeated infections in young children induce immunity (Blaser et al., 1985).

In most of the studies carried out in the country, *C. jejuni* was the dominant species isolated and *C. coli* was less frequently isolated, although the ratio of *C. coli* to *C. jejuni* varied considerably among studies. It has also been reported that in industrialized countries infections caused by *C. jejuni* account for a large proportion of cases of human bacterial gastroenteritis (Friedman et al., 2000).

Campylobacter colonization in poultry

Today, thermophilic *Campylobacter* remain the most common cause of acute bacterial enteritis in the world (Moore et al., 2005). Ingestion of contaminated chicken or poor food handling practices associated with raw chicken represents the primary route of transmission to humans. Despite all the

acquired knowledge on *Campylobacter* organisms, including the publication of the complete genome sequence for *C. jejuni* (Parkhill *et al.*, 2000), the prevalence of human infections remains high and we still have major problems in producing poultry that are free of *Campylobacter*. The ability of these bacteria to grow at 42°C perhaps reflects their adaptation to the gut of some types of birds (Crushell *et al.*, 2004). Broiler chicks become colonized at a very early stage in their lives and prevalence of colonization among poultry flocks can reach up to 100% in some areas.

From 1993 to 2011, five different studies were conducted to assess the extent of colonization of chickens (Kazwala *et al.*, 1993; Mdegela *et al.*, 2006; Chuma, 2009; Jacob *et al.*, 2011) and ducks (Nonga & Muhairwa, 2009) with *Campylobacter* organisms in Tanzania (Table 2). In all the studies, the subjects were found to be positive for *Campylobacter*; and *C. jejuni* accounted for the majority of *Campylobacter* detected followed by *C. coli*. The findings of these few studies indicate a need for increased surveillance and *Campylobacter* screening including at the slaughter facility and sales levels.

Table 2: Isolation of *Campylobacter* in different studies on poultry in Tanzania

Poultry species	Number of samples	Prev (%)	Species prevalence (%)			References
			<i>C. jejuni</i>	<i>C. coli</i>	others	
Chickens	1338	56.35	85.2	13.8	1	Kazwala <i>et al.</i> , 1993; Mdegela <i>et al.</i> , 2006; Chuma, 2009; Jacob <i>et al.</i> , 2011
Ducks	90	80	81.9	18.1	0	Nonga and Muhairwa, 2009

Key: Prev = prevalence

***Campylobacter* colonization in livestock**

Many different animal species maintain *Campylobacter* species with no clinical signs. There do not appear to be significantly different colonization levels of *Campylobacter* in food animals between developed and developing countries (Padungton & Kaneene, 2003) and the role of commonly found *C. jejuni* as a primary pathogen in farm animals is uncertain (Padungton & Kaneene, 2003). *C. jejuni* can be found in faeces of diarrhoeic and healthy calves and piglets. Although these organisms are not a problem in animal health terms, they are of major importance to veterinary public health professionals (Moore *et al.*, 2005). Of the farm animals sampled in the reviewed studies goats had the lowest prevalence and pigs the highest (table 3). However, in both studies, *C. jejuni* accounted for the majority of *Campylobacter* detected in cattle and pigs; while *C. coli* was the only species detected in goats. In a study in goats by Jiwa *et al.* (1994a) it was found that only study subjects in households keeping other animals particularly pigs and poultry were positive carrying isolates similar to those found in these other animals. They concluded that goats are not natural hosts for *Campylobacter*; and that pigs and poultry serve as sources of infection.

Table 3: Species specific prevalence of *Campylobacter* in different studies on farm animals in Tanzania

Animal species	Number of samples	Prev (%)	Species prevalence (%)			References
			<i>C. jejuni</i>	<i>C. coli</i>	Others	
Goats	168	1.8	0	100	0	Jiwa <i>et al.</i> , 1994a
Cattle	1049	2.67	88.9	11.1	0	Kusiluka <i>et al.</i> , 2005; Nonga & Karimuribo 2009
Pigs	66	66.7	74	26	0	Mdegela <i>et al.</i> , 2011

Key: Prev = prevalence

Campylobacter colonization in wild birds and mice

Campylobacter species colonize a range of hosts, including domestic animals and wild birds. Playing a reservoir role, wild birds have higher chances of contaminating water sources, environment and food; and in turn transmitting the pathogens to humans and poultry (Altekruse et al., 1999; Luechtefeld et al., 1982). Several studies in developed and developing countries have reported occurrence of *Campylobacter*, particularly *C. jejuni*, in wild birds including crows (Ito et al., 1989; Adegbola et al., 1990; Waldenstrom et al., 2002; Saleha, 2004; Molina-Lopez et al., 2011). A study by Adegbola et al. (1990) in Nigeria revealed phenotypical and genotypical similarities among *C. jejuni* isolates from free flying birds and humans. The present review includes a study that isolated thermophilic *Campylobacter* from crows at a prevalence of 72.8% (Mdegela et al., 2006). The obtained high prevalence is of epidemiological and public health significance, as it highlights on the possibility that crows are among sources of thermophilic *Campylobacter* to humans and chickens in Tanzania. It has been suggested that, free flying birds including crows around poultry farms may transmit thermophilic *Campylobacter* to chickens if they get access to the rearing houses (Genigeorgis et al., 1986; Altekruse et al., 1999).

In a pilot study conducted by Jiwa et al. (1994b) *C. jejuni* was isolated from 40% (n=20) of field mice (*Mastomys nataliensis*) screened. The authors claimed conformity of biotype profiles of the mice isolates with profiles of human isolates obtained in the same study locality.

Campylobacter colonization in non-human (wild) primates

As humans are coming into closer proximity with wild primates for a variety of reasons, surveillance and reporting of infectious agents in wild primate populations are increasingly important (Kaur et al., 2011). Previously campylobacteriosis, salmonellosis, and shigellosis in free-ranging human-habituated mountain gorillas were reported in Uganda (Nizeyi et al., 2001). In this review one of the studies was conducted to investigate on colonization of human-habituated chimpanzees (*Pan troglodytes schweinfurthii*) with *Campylobacter* organisms. The authors (Kaur et al., 2011) isolated *Campylobacter* organisms from the faeces of about one-third of the sampled study subjects. Following characterization of the organisms by phenotypic, genotypic and phylogenetic analyses, the authors proposed to classify the obtained isolates into a single novel nomenclature, *Campylobacter troglodytis*. The authors recommended further studies to determine whether the organism is pathogenic to chimpanzees and whether this novel *Campylobacter* colonizes humans and causes enteric disease.

Contamination of meat products with Campylobacter

Poor slaughter methods and unhygienic meat handling may constitute a potential risk of infections to humans (Steinhauserova et al., 2005). As *Campylobacter* are enteric organisms and stay in the intestinal contents, cross-contamination of meat can originate from the faeces of the same animal or different animals through the slaughterhouse environment or equipment especially during flaying, evisceration or from cross contamination from hide to carcass (Gannon, 1999; Hakkinen et al., 2007). However, if contaminated water is used to wash carcasses may also be a source of contamination. The present review identified two studies which assessed contamination levels of animal meat products, particularly beef (Nonga et al., 2009) and pork (Nonga et al., 2011).

A survey on cattle carcasses revealed a contamination level of 9.3%. This was fairly high as compared to a carcass contamination level of 2% previously reported in a neighbouring country of

Kenya (Osano & Arimi, 1999). Many more studies elsewhere have reported variable levels of cattle carcasses contamination with *Campylobacter* organisms (Ono & Yamamoto 1999; Beach *et al.*, 2002; Hakkinen *et al.* 2007; Valnegri *et al.*, 2008). On the other hand a study conducted on pork revealed *Campylobacter* carcass contamination level of 10.6% which was comparable to findings obtained elsewhere (Aquino *et al.*, 2002). The rate was however fairly low when compared to studies conducted by Steinhäuserova *et al.* (2005) and Malakauskas *et al.* (2006) who reported higher pig carcass contamination levels ranging from 34-63.6%. In contrast, studies in developed countries including Poland (Kwiątek *et al.*, 1990), Belgium (Ghafir *et al.*, 2007), and Sweden (Lindblad *et al.*, 2007) reported low carcass contamination levels. Differences in levels of *Campylobacter* isolation from carcasses may be influenced by the levels of colonization of slaughter animals, abattoir hygiene, slaughter and dressing methods, sampling and analysis methods and sampling plan. The findings of both studies on contamination levels of animal products suggest a need for increased surveillance and *Campylobacter* screening in food of animal origin so as to better protect consumers.

Seasonal patterns of *Campylobacter* infections

In several the United Kingdom, Nordic countries and New Zealand, seasonal peaks in human *Campylobacter* infections have been observed (Hudson *et al.*, 1999; Nylen *et al.*, 2002). Several other studies have investigated the seasonality of *Campylobacter* colonization in poultry (Bang *et al.*, 2003; Wilson, 2002; Evans *et al.*, 2002), and attempted to correlate data on human and chicken isolates (Evans *et al.*, 2002). In this review, two studies conducted in Tanzania tried to investigate on seasonal variation on human infections with *Campylobacter* organisms (Lindblom *et al.*, 1995; Vargas *et al.*, 2004), but didn't find any significant difference. On the other hand studies on *Campylobacter* colonization in animals were conducted in different seasons and yet produced more or less similar results. Previously a study in the Democratic Republic of Congo, had reported high prevalence of *Campylobacter* during the wet season (Blasser & Barth, 1981).

Resistance of *Campylobacter* isolates to antimicrobials

While most cases of *Campylobacter* enteritis are self-limiting, in situations where antibiotic therapy is indicated either erythromycin or ciprofloxacin are the drugs of choice (Tadesse *et al.*, 2011). However data indicate an upward trend of *Campylobacter* resistance to antibiotics with varying patterns being seen in different countries and regions (Moore *et al.*, 2005; Sack *et al.*, 2001). *Campylobacter* with resistance to antimicrobial agents have been reported in both developed and developing countries (Ruiz *et al.*, 1998; Saenz *et al.*, 2000; Gaudreau & Gilbert, 2003; Avrain *et al.*, 2004; Chu *et al.*, 2004; Jain *et al.*, 2005; Moore *et al.*, 2005; Senok *et al.*, 2007; Mazi *et al.*, 2008), and the situation seems to deteriorate more rapidly in developing countries, where there is widespread and uncontrolled use of antibiotics. Studies suggested an association between antimicrobial use in food animals and the development of resistance in human isolates (Padungton & Kaneene, 2003).

In this review, only a single study on ducks addressed the issue of antimicrobial resistance of the *Campylobacter* isolates (Nonga & Muhairwa, 2009), in which fifty isolates of *C. jejuni*, the best characterized and most clinically relevant species in this genus, were tested for sensitivity to twelve antimicrobials. The authors reported that all the tested isolates were susceptible to streptomycin, nitrofurantoin and amikacin; and at least half of them were resistant to cefuroxime sodium, tetracycline and ampicillin. They further indicated that between 20–50% of isolates were resistant to erythromycin, gentamicin, cloxacillin and amoxicillin; and 10% and 16% of the *C. jejuni* were resistant to norfloxacin and ciprofloxacin respectively. The study observed that isolates from adult ducks showed significantly higher levels of resistance to most antibiotics than did duckling isolates.

Previous reports indicate that *C. jejuni* has been found to be sensitive to several classes of antibiotics, including macrolides (especially erythromycin), which have been traditionally utilized as first-line therapy, and quinolones such as ciprofloxacin (Moore *et al.*, 2005). However, high levels of quinolone resistance in *Campylobacter* have been documented in Europe and Asia (Ruiz *et al.*, 1998; Saenz *et al.*, 2000; Chu *et al.*, 2004; Jain *et al.*, 2005). *Campylobacter* resistance to this group of antibiotics is currently deemed a rapidly emerging global problem; and the widespread use of fluoroquinolones in clinical practice and utilization in veterinary practice are thought to be contributing factors for these high levels of resistance. Increasing levels of *Campylobacter* resistance to tetracycline have also been reported (Mazi *et al.*, 2008; Senok *et al.*, 2007; Gaudreau & Gilbert, 2003), some studies attributing the pattern to its persistent use in animal husbandry. A described natural horizontal transfer of tetracycline resistance gene (*tet*(O) gene) without antimicrobial selection pressure between *C. jejuni* in the digestive tract of chickens (Avrain *et al.*, 2004) may also explain these high rates of tetracycline resistance.

Conclusion

In the present review summarized reports provide evidence of *Campylobacter* infections both in humans and animals (including the wild), as well as contamination of meat products in Tanzania. One of the reports also informs of the existence of antibiotic resistant *Campylobacter* isolates in the country. This has an implication on public health as the organism is zoonotic. We recommend collaborative efforts from veterinary and human medicine, as promoted by the “one health” approach, to allow for efficient and more effective strategies for prevention and control of infections both in human and animal populations. One of the challenges for researchers in the country would be to conduct further work using DNA based techniques for determination of relatedness of the isolates circulating in animals and humans. There is also much to be done in the country in order to understand the pattern and trends of antibiotic resistance in both human and animal derived *Campylobacter* isolates. Furthermore, environmental reservoirs of *Campylobacter* and risk factors for human infections with *Campylobacter* need to be investigated.

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