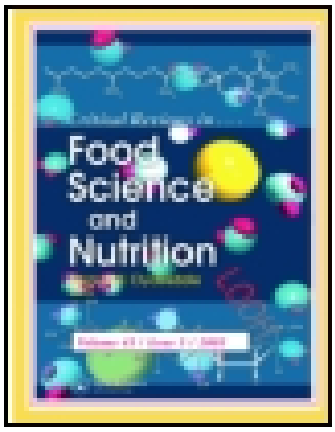

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Control of Campylobacter in Poultry Industry from Farm to Poultry Processing Unit-a Review

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Control of *Campylobacter* in Poultry Industry from Farm to Poultry Processing Unit-a Review

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Abstract

Campylobacter is an emerging zoonotic bacterial threat in the poultry industry. Most of the human cases of campylobacteriosis recorded have revealed their poultry origins. Various control measures have been employed both at the farm and processing levels to combat with it. The antibiotic treatment, phage therapy, competitive exclusion and vaccination have been adapted at the farm level to reduce colonization of *Campylobacter* in poultry gut. While prevention of intestinal spillage, scheduled slaughter, logistic slaughter, chemical decontamination of carcasses are recommended to reduce contamination during processing. The post harvest interventions such as heat treatment, freezing, irradiation of contaminated carcass

can effectively reduce *Campylobacter* contamination. Thus, integrated approaches are required to tackle infection of *Campylobacter* in humans.

Keywords: *Campylobacter*, poultry industry, postharverst interventions, processing

Introduction:

Campylobacter is one of the major cause of bacterial food borne diarrheal diseases worldwide. The campylobacteriosis ranks fourth among diarrheal diseases most prevelant in the world and accounts for 8.4% of all the diarrheal diseases expressed as 7.5 million DALY (Disability Adjusted Life Years). In later stages the disease, can be manifested in various forms such as Guillain-Barré syndrome, reactive arthritis (ReA), and irritable bowel syndrome (Murray et al., 2012; Havelaar et al., 2012). The prevelance of *Campylobacter* in broiler flocks in EU were as high as 60 -80% (Reich et al., 2008; European Food Safety Authority, 2010). In India the status of *Campylobacter* is anonymus. Reports from Bareilly (12.79% prevelance) and Varanasi in Uttar Pradesh, and Hisar in Haryana have revealed prevelance of this bacteria in poultry meat in Indian subcontinent (Singh et al., 2009).

The poultry carcasses and products are mostly contaminated by its intestinal content where the bacteria is colonized leading to spread of campylobacteriosis in humans, which is the most common cause of bacterial gastroenteritis (Samuel et al., 2004). It has been documented that poultry is frequently colonized with *C. jejuni* without apparent symptoms and handling and consumption of infected poultry meat is one of the most important causes of human campylobacteriosis (Friedman et al., 2004). Nowadays risk assessment of *Campylobacter* in poultry plants is used as a tool for prevention of hunan zoonotic diseases (Nauta et al., 2009).

***Campylobacter*: bacteria**

Campylobacter occurs primarily as commensal in humans and domestic animals. The genus *Campylobacter* contains small (0.2ï 0.8 ε m × 0.5ï 5 ε m) Gram-negative, slender spirally

curved rods. When the bacterial cells are grouped together, they form an ñSò or a ñVò shape of gull-wing. Species have single polar unsheathed flagellum at one or both ends of the cell and show a corkscrew-like motion. The oxidase activity is present in all species except for *C. gracilis*. They obtain energy from amino acids, or a tricarboxylic acid cycle intermediate. They neither ferment nor oxidize carbohydrates. *C. jejuni* reduces nitrate and hydrolyzes hippurate and indoxyl acetate.

Thermophilic *Campylobacter* species are able to grow between 37 and 42°C with an optimum temperature of 41.5°C. This thermophilic nature could be the probable reason for its higher rate of prevelance in poultry. Absence of cold shock protein genes makes them susceptible to grow below 30°C (Levin, 2007). These are non-spore-forming and fastidious bacteria. They grow best at low oxygen tension (5% O_2, 10% CO_2 and 85% N_2) i.e are microaerophilic in nature (Garénaux et al., 2008). Several selective broths such as Bolton broth (BB), *Campylobacter* enrichment broth (CEB), and Preston broth (PB), have been used for their isolation.

Poultry: source of *Campylobacter*

Studies have revealed that about 50 ï 70% of human campylobacteriosis can be attributed to the consumption of poultry and poultry products (Allos, 2001). Thus, poultry is now regarded as one of the most important reservoirs for *Campylobacter* and constitutes a very significant vehicle for its transmission to humans (Humphrey et al., 2007) leading to intestinal disorders (Health Protection Agency, 2010). Various studies have demonstrated high levels of *Campylobacter* in the broilers, on the broiler carcasses and retail chickens (Zhao et al., 2001;

European Food Safety Authority, 2010). The *C. jejuni* has been found to be the major causative agent contributing 85% of illness followed by the *C. coli* (Friedmanet al., 2000). The organism can be transmitted via both horizontal and vertical transmission, of which horizontal transmission is the major route of infection to humans.

Campylobacter exsists as a commensal organism colonized in the intestinal tract of poultry. The intestinal tract (cecum and colon) of such birds, can harbor a large number of *Campylobacter* spp. which may leak or rupture during processing causing carcass contamination (Berrang et al., 2001). *Campylobacter* spp. is often entrapped in the cervices and channels of skin which protects them and provides a favorable environment for growth. Under microenvironment of skin *Campylobacter* are able to survive even under frozen conditions (Chantarapanont et al., 2003).

The consumption of chicken and chicken meat products has also been associated with a large number of outbreaks of acute campylobacteriosis in human populations worldwide, both in developed and developing countries. *Campylobacter* mostly affects children, old and immuno-suppressed patients (Corry and Atabay, 2001). Most of the cases of campylobacterosis are associated with handling and eating raw poultry or undercooked poultry meat or cross-contamination of cooked foods with raw chicken (Nadeau et al., 2002). According to a surveillance study conducted in England and Wales, *C. jejuni* was reported to be 12 times more prevalent in human campylobacteriosis than *C. coli* (Friedman et al., 2000).

Campylobacteriosis in human:

Foodborne zoonoses are the most important cause of morbidity and mortality worldwide in human. The World Health Organization (WHO) estimates that over two million people die each year from diarrheal diseases mainly caused by the ingestion of contaminated foods (World Health Organization, 2012; European Food Safety Authority, 2007). Campylobacteriosis has been the most commonly reported zoonosis in the European countries followed by Salmonellosis and Yersiniosis (European Food Safety Authority, 2007; European Food Safety Authority, 2010b). It has been documented that 50-70% cases of human campylobacteriosis have been attributed to consumption of poultry and poutry products (Allos, 2001). The post harvest interventions such as handling, preparation and consumption of broiler meat may account for 20ï 30% of human cases of campylobacteriosis, while 50ï 80% may be attributed to the chicken reservoir as a whole (European Food Safety Authority, 2010a).

Campylobacter is a common cause of diarrhea among infants and children in the developing world. Children below one year, young individuals between the age group of 15-25 years and the immunosuppressed persons are more prone to the development of chronic campylobacteriosis and doses as low as 500 organisms can cause illness (Friedman et al., 2000). The various risk factors associated are poor sanitation and close contact with animals. *C. jejuni* followed by *C. coli* has been the most frequently isolated bacterial organisms from diarrheal cases repoted. *Campylobacter*-associated diarrhea is generally an acute but self-limiting (Janssen et al., 2008) in nature. It is frequently isolated from stools up two weeks following an episode, with 14 days mean excretion times following diarrhea (Rao et al., 2001). *Campylobacter* infection may have an enduring impact on childhood growth. In addition it also produces immune-mediated long term sequelae including reactive arthritis and Guillain-Barré syndrome. It

is also a risk factor for post-infectious inflammatory bowel syndrome and probably, inflammatory bowel disease (Gradel et al., 2009; Kalischuk and Buret, 2010). The *Campylobacter* infection has been found to affect the epithelial barrier integrity (Chen et al., 2006), suggesting that prolonged excretion may be associated with persistent mucosal injury. Several incidences of campylobacteriosis have also been reported from almost all parts of India (Naik and Jayaraj, 1998).

Poultry industry:

India is the biggest consumer market in the world of poutry, egg and meat and has great opportunities for expansion and growth as there is a large gap in consumption (52 Eggs & 2.5 kg of poultry meat) and recommended pattern (180 eggs & 11 kg; National Institute on Nutrition, India). In India eggs are the most economical source of animal protein without any religious taboos attached to it. The changing consumption patterns especially amongst the youths is intriguing egg and poultry meat on account of their high nutritional value. The fast paced growth (at the rate of 6% per annum) of organized retail sector in India is expected to grow to USD 239 billion by 2015. This is mainly due to the participation of many large scale private Indian as well as foreign investors into the processing sector. Inspite of the great potential and opportunities there are still many challenges to be confronted by the Indian poultry industry. *Campylobacter* is one of them, challenging the poultry industry at the farm, transport, slaughter, processing and retail levels.

The reduction of *Campylobacter* in chicken is a priority because hazards arise across dressing, processing and packaging chain that can result in the introduction of *Campylobacter*

into food. A survey carried out in UK by the Food Standards Agency (FSA) on the prevelance of *Campylobacter* in retail chicken was done between May 2007 and September 2008. Its reports evinced that *Campylobacter* was present in 65% of the fresh chicken samples tested. An EU baseline survey carried out in 2008 and published by EFSA4 in March 2010 showed that in UK estimated prevalence of *Campylobacter* in broiler batches (cecal contents) was 75% and 86% broiler carcasses (skin samples). These results were above the weighted EU mean prevalence of 71% and 77% respectively. There was a wide range of *Campylobacter* prevalence across members states. In UK alone 42% of samples contained less that 100 *Campylobacter* per gram (cfu/g) and 27% contained more than 1,000 *Campylobacter* per gram (cfu/g).

Prevention and control strategy:

The control strategies of *Campylobacter* needs to be multi-tiered and goal orientated providing flexibility to producers and processors. For this purpose three tiered approach has been proposed by the Codex. The first tier deals with hygiene measures to be adopted followed by the hazard specific measures and finally the risk based measures.

Poultry industry is largely censured of being the major source of human campylobactreiosis. Control interventions for its prevention can be widely segregated into

A. Preharvest Interventions

B. Postharvest Interventions

Preharvest Interventions

These are the methods that can be utilized for preventing colonization of poultry by bacteria on farm. In absence of any vertical transmission newly hatched chicks are free from *Campylobacter*. It has been observed that the poultry show a lag phase of 2-3 week to *Campylobacter* colonization. But once a bird gets colonized, the whole flock gets affected within few days as the organism is easily transmitted through feco-oral transmission as well as through feed and water (Lee and Newell, 2006; Ridley et al., 2011). Thus, appropriate interventions would avert colonization and consequent amplification.

Three general strategies have been proposed to control *Campylobacter* on the poultry farm:

(1) Reduction of the environmental exposure (Biosecurity measures),

(2) An increase in the poultry's host resistance to reduce *Campylobacter* carriage in the gut (e.g., competitive exclusion, vaccination, and host genetics selection), and

(3) Use of antimicrobial alternatives to reduce and even eliminate *Campylobacter* from colonized chickens (Lin, 2009).

Biosecurity Measures: There should be limited access to the personnels with restricted entries. The poultry shed should have protected entrances. Farm workers can also be a source of entry of infections. Facilities like provision of gumboots, overalls, double footdips, hand sanitisers and changing rooms can be beneficial (European Poultry meat Industry Guide, 2010; Ridley et al., 2011). Rodents, vermins and insects play an vital role in the horizontal transmission of the disease. An effective intervention at this stage would minimize spread (Newell et al., 2011). The litter used should be obtained from reliable sources and should be disinfected properly before

use. The used litter should be promptly removed and disposed off. Water sanitation is also must to prevent dissemination of the infection (Messens et al., 2009). The addition of organic acids such as lactic acid in drinking water can effectively prevent transmission (Chaveerach et al., 2002). The use of chlorinated water can also be an effective measure (Ellis-Iversen et al., 2009). The feed provided must be properly treated i.e. either heat treated or mixed with proprietary product containing formic and/or propionic acid or formaldehyde before feeding the flock. Skanseng et al., (2010) have reported a synergistic response of formic acid with sorbates in controlling gut colonization of *C. jejuni*. Regular sampling and testing of feed and water must be done. The equipments used at farm must be cleaned and disinfected before reuse. In between flocks the entire poultry house must be cleaned and disinfected (Newell et al., 2011).

Bacteriocin: Bacteriocins are low molecular weight peptides that are produced in bacterial ribosomes and possess antimicrobial properties. They are mainly cationic, hydrophobic, or amphiphilic peptides, with molecular weights of 5 to 6 kDa. Mature peptides usually carry 20 to 60 amino acids. It has been observed that supplementation of purified bacteriocin in feed or water, reduced *C. jejuni* from infected chicks. Bacteriocins are effective against antibiotic-resistant pathogens. Svetoch et al., (2005) first reported the identification and characterization of novel anti-*Campylobacter* bacteriocins from bacteria isolated from chicken intestine. A clinical study conducted on chicken and turkey poult showed that the bacteriocins are highly effective to reduce the colonization of *Campylobacter* (Coe et al., 2006). Till date four different bacteriocins have been isolated and successfully purified and characterized. Oral administration of these bacteriocins dramatically reduced *C. jejuni* colonization in chicken intestine (Svetoch et al.,

2008). *Bifidobacterium longum* PCB 133 has been shown to effectively reduce *C. jejuni* (Santini et al., 2010).

Vaccination: Using genetics to develop a live attenuated *Campylobacter* vaccine is an attractive approach to control the *Campylobacter*. Mutants of *C. jejuni*, which are non virulent in the ferret model have been found to be protective in animal models (Levine and Kaper, 1993).

Subunit vaccines: Two *Campylobacter* antigens, þagellin and a protein called PEB1, have been suggested as a subunit vaccine candidates for use either as puriýed recombinant proteins or by expression in carrier vaccine strains, such as live attenuated *Salmonella* or *Shigella* species (Martin et al., 1989).

Killed whole cell vaccines: The inactivated microorganisms offer several advantages as potential vaccines for mucosal immunization. As vaccines, they are inexpensive to produce and possess multiple antigens that can be important for protection. A killed whole cell vaccine against *Campylobacter* species could be safe, immunogenic, and protect against disease, particularly if combined with an effective mucosal adjuvant, such as *E. coli* heat-labile toxin (LT) (Walker and Clements, 1993). Some recent works using a mixture of heat- and formalin-killed *C. jejuni* in mice has shown that LT enhances the mucosal immune response over a wide range of vaccine doses (Baqar et al., 1995).

Competitive exclusion: This technique has been successfully used to control *Salmonella*, but competitive exclusion trials with *Campylobacter* have produced mixed results (Newell and Wagenaar, 2000). In-vitro control of *Campylobacter* have been observed by use of competitive microflora of organisms like *Bacillus*, *Paenibacillus*, *Lactobacillus*, *Streptococcus*,

Enterococcus and *Escherichia* (Svetoch and Stern, 2010). Laisney et al., 2004 have shown that the protection by competitive exclusion is strongly dependent on bird variety to which it is applied.

Phage therapy: Bacteriophages are naturally occurring predators of bacteria that are ubiquitous in the environment (Hagens and Loessner, 2010). *Campylobacter* specific bacteriophages have been isolated from various sources such as manure, sewage, abattoir effluents, and broiler chickens. Bacteriophages have been applied as a decontamination technique to reduce *Campylobacter* on poultry meat under experimental conditions (El-Shibiny et al., 2009). The use of bacteriophages to reduce the amount of *Campylobacter* entering the food chain at farm level is a potentially useful intervention strategy , where reductions in numbers of *Campylobacter* in chickens could lead to a measurable reduction in carcass contamination (El-Shibiny et al., 2005). Oral administration of bacteriophages has been investigated to reduce *C. jejuni* load (Carvalho et al., 2010). Bacteriophages are highly speciýc for bacterial species, and multiply at the expense of the cell, eventually reducing the number of viable bacterial cells. Due to their host speciýcity, effects on other microbial populations are minimal.

Antibiotic treatment: Antibiotics are used in human and veterinary medicine for treatment and prevention of infections and as growth promoters in food animals. But their increased use has resulted in the increased incidence and level antibiotic resistance to many enteric bacteria and encouraged the persistence and transfer of antibiotic-resistance determinants in microbial genomes. Some antibiotics have been found to effectively reduce *C. jejuni* in poultry GI tract (Hermans et al., 2010).

Use of Fatty acids and Essential oils: Feeding of medium chain fatty acids have shown to reduce the gut colonization of broilers by *Campylobacter* (Van Gerwe et al., 2010; Solis de los Santos et al., 2010). Studies regarding use of essential oils to prevent or reduce colonization are still in progress (Hermans et al., 2010).

Postharvest Interventions:

Hauling and Transportation: Studies have revealed that *Campylobacter* colonization increases due to defecation onto crates and birds in crates below, during transportation and holding before slaughter owing to disturbance of intestinal functions and reduced immune under stress factors of transportation (Hastings et al., 2010; Patriarchi et al., 2011; Wesley et al., 2009). Feaces, feathers and skin are the main sources of contamination during transportation.

Scheduled slaughter : Scheduled slaughter means identifying flocks positive for *Campylobacter* before they are slaughtered and subjecting carcasses from these flocks to special treatment such as freezing, heat treatment or other *Campylobacter*-reducing measures. But the draw back with this system is that it requires specific and rapid testing short time before slaughter.

Logistic slaughter : Logistic slaughter means slaughtering positive flocks after negative flocks to avoid cross contamination. Many slaughterhouses perform logistic slaughter based on samples investigated for *Salmonella*. These samples are taken two or three weeks before slaughter, much too early to be of any use regarding *Campylobacter*. Additionally, there could be a conflict between logistic slaughter with respect to *Salmonella* and *Campylobacter* status.

At processing level:

At Poultry Processing Plant :

Scalding: Scalding is the immersion of carcasses in scalding water tanks. The scalding temperatures used vary from 50-52°C (soft scald) to 56-68°C (hard scald). Soft scalding is used more suitable for the fresh poultry meat production. For reduction of the contamination during scalding, following measures are recommended.

Counter-current scald tanks: This is a system whereby water in the tank should move through the system flowing against incoming carcasses. This flow creates a ódirty to cleanô gradient and Carcasses moving through the tank are washed by even cleaner water.

High water flow rates in the tanks: High flow rates of water and adequate agitation dilutes dry matter and bacterial load in the tank (Cason et al., 2001).

Multi-stage scalds tanks: Multiple stage tanks are better than single stage tanks because they create more opportunities to clean the carcasses. When combined with a counter-current water flow system, the multi-stage scald tank provides a dilution effect. Consequently, there is a marked reduction in carcass contamination (3.0-3.9 log10 reduction per carcass) even at ósoft scaldô temperatures. With such a system, Berrang and Dickens, 2000 obtained almost a thousandï fold reduction in *Campylobacter* contamination of carcasses. Hinton et al., (2004) showed that significantly less *Campylobacter* was recovered from the final tank of a multiple-tank counter-flow scald system than from the first tank.

Defeathering: Cross-contamination of carcasses by the machinery may occur at this step. The feather follicles in the skin at this stage are open and may lead to movement of *Campylobacter* cells inside the follicles, which may decrease the efficacy of subsequent carcass rinses (Cason et al., 2004). Guerin et al., (2010) and Son et al., (2007) found that in contrast to scalding, the defeathering step consistently increased the prevalence and level of campylobacter contamination. It is generally well accepted that contamination increases largely due to the escape of faecal material through the cloaca by the action of the picker fingers pressing on the abdomen (Allen et al., 2008).

Evisceration: The evisceration process involves removal of the feet, head and viscera of the birds and the harvesting of edible offal. Several studies show that *Campylobacter* contamination levels increase during evisceration. Generally, carcasses visibly contaminated with faecal material have significantly higher *Campylobacter* counts than carcasses without contamination (Allen et al., 2008; Boysen and Rosenquist, 2009).

Prevention of spillage and intestinal contents: During the evisceration spillage of cecal content is an important source of contamination of the carcasses. Especially, if the machines used for evisceration are not adapted to the variation of carcass sizes within a batch. At this stage, the rupture of the viscera may occur and release of intestinal contents causing carcass contamination.

Chilling: Studies have found that air chilling results in a decrease in *Campylobacter* levels on the carcass (Boysen and Rosenquist, 2009).

Sanitation and Process Hygiene: Peyrat et al., (2008) reported that *C. jejuni* is able to survive overnight on food processing equipment surfaces after cleaning and disinfection procedures and

that it may contaminate carcasses during the slaughter process. Segregation of *Campylobacter*-positive flocks from negative flocks at the slaughter house, followed by slaughtering with special attention to the positive flocks, has proved to be an effective to reduce the spread of contamination (Wagenaar et al., 2006). Strict cleaning practices after processing positive poultry is essential for prevention and certification. In most of the cases carcasses gets contaminated with rupture of gut along the processing line. There is a gradual reduction in the levels of *Campylobacter* on the meat as a result of washing, de-feathering, submersion chilling, etc.

In house practices: Since *Campylobacter* spp. is heat sensitive, cooking temperatures and times are sufficient to eliminate the organisms. Routinely, hot water is used to wash working surfaces and utensils in order to control the presence of *Campylobacter* spp. in the food processing plant. Cowan, (1999) however, reported that washing with hot water and with the addition of hypochlorite enhances significantly the reduction of contaminated sites.

Chemical decontamination : Organic acids (lactic and acetic acids), chlorine, chlorine dioxide, acidified sodium chlorite, trisodiumphosphate and peroxyacids have been selected as being likely to give rise to negligible toxic residues. Another aspect is that for some chemical decontaminants, the beneficial effect increases during chill storage. Few studies have investigated this aspect (Cox and Pavic, 2010).

Physical decontamination treatments :

Irradiation: Application of radiations for preservation of meat has the benefit that it leaves the meat essentially unchanged in appearance. Another advantage is that they can be generated using relatively inexpensive machines. Application of irradiation to inactivate *Campylobacter* within

the meat as well as on the outside has also been studied. The irradiation D value for *C. jejuni* is 0.19kGy (Collins et al., 1996), whereas the maximum permissible level of irradiation of fresh poultry is 7 kGy as recommended by WHO. Gamman irradiation has been approve by USDA, FDA and CAC for decontamination of poultry carcasses (Cox and Pavic, 2010).

Freezing: Application of freezing to some -20^{o}C for few weeks is already being practiced for treating carcasses from *Campylobacter*-colonized flocks without affecting the general appearance and quality of meat. Stern and Kotula (1982) have reported of inactivation of *Campylobacter* at -15^{o}C in as minimum as 3 days but complete elimination by freezing has not yet been observed (Lee et al., 1998).

Heat treatment: Cooking of poultry at 70^{o} C can minimise the risk of illness. Heat treatments other than cooking could be added to the processing line in the slaughterhouse to control dissemination of organism.

Biosecurity: Biosecurity measures have shown to effectively reduce campylobacteriosis in chickens (Gibbens et al., 2010) and these are equally essential and applicable during postharvest intervention.

Application of Good Manfacturing Practice and HACCP: All the factors essential for production of safe and wholesome poultry meat must be complied by the processing plants. These are infrastructure; machineries, equipments and utensils; hygiene and sanitation of the plant (Allen et al., 2011), maintenance, repair and disposal and human resources involved in the

production system. The reduction in enteropathogens entering the processing plant, at critical control points, reduces the exposure of consumers to these organisms (Cox and Pavic, 2010).

Conclusion:

Attempts to prevent *Campylobacter* colonization of chickens by Biosecurity measures have proven quite difýcult. At best, colonization can be delayed, but not prevented. The ubiquitous presence of *C. jejuni* in the environment and in warm-blooded animals including pets, rodents, and wild birds is probably the major factor responsible for this lack of success. The effect of experimental vaccination of birds was assessed, but the vaccination was hampered by large serotypic diversity of *C. jejuni* strains. Probiotic treatment with lactic acid bacteria and competitive exclusion with beneýcient microþora was only partially effective. Treatment of drinking water with acids or acidic feed additives has a limited beneýcial effect. When a *Campylobacter* positive þock enters the slaughter house cross-contamination of meat is highly likely. Thus, there is an urgent need to control measures that can be used on the ýeld and that are equally acceptable to consumers. There is a need in the poultry industry for intervention strategies that predictably reduce *Campylobacter* levels in preharvest poultry and poultry carcasses. It the responsibility of regulatory agencies and the processors to promote a safer poultry product through control of *Campylobacter*.

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