Isolation and phenotypic identification of *Campylobacter* in dairy supply chain

S. Bade¹, D. Bhardwaj¹, A. Jaswal¹, H. M. Rashmi¹, N. Kumar¹ and H. V. Raghu^{1*}

¹Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal – 132 001, Haryana, India

Abstract

A total of 175 samples comprising of raw milk (30), freshwater (30), dairy wastewater (30), Human handler swab (30), Milk filter swab (30), and faecal swab (25) samples were collected from respective sources for the isolation of *Campylobacter* species. Among 175 samples, 53 samples were selected based on its positive growth in selective enrichment and characteristics colonies on mCCDA media. From 53 positive samples, 91 colonies showed characteristics purple to mauve colour colonies on Hi-Chrome media. Among 91 isolates selected, 41 isolates have shown pink-coloured curved rods/s-shaped rods/cocci's by Gram's staining (G –ve) and 3 isolates among these were identified as *Campylobacter* based on biochemical tests such as oxidase, catalase, and microaerobic growth at 25°C. Later, all 3 isolates were evaluated for the H₂S production and Hippurate hydrolysis test wherein 2 isolates showed positive hippurate hydrolysis test and negative H₂S production and in another isolate has shown negative Hippurate hydrolysis test and positive H₂S production. Based on the Hippurate hydrolysis test and H₂S production these isolates were tentatively considered as *C. jejuni* (2 nos) and *C. coli* (1 no). These isolates were mainly originated from raw milk and faecal swab samples and none of the other samples have shown any presence of *Campylobacter* spp. among the samples screened. The occurrence rate of *Campylobacter* spp. was found more in faecal swab samples as compared to raw milk samples and it was mainly due to its origin of its presence in faecal samples.

Key words: *Campylobacter*, Dairy, Identification, Isolation, Supply chain

Highlights

- In total, 175 samples from different dairy supply chains were used to isolate *Campylobacter* species.
- About 91 isolates grew on mCCDA and Hi-Chrome media, showing purple to mauve colored colonies.
- Based on a Hippurate hydrolysis test and H₂S production, 3 isolates were tentatively confirmed to be *Campylobacter* species
- These isolates originated mainly from raw milk and fecal swabs.

INTRODUCTION

Like food safety, consumers are now looking for quality too. The food-borne infection which caused due to microorganisms increasing day by day and the public health burden due to diseases caused by them also increasing since perishable foods such as milk and milk products are susceptible to microbial contamination under the unfavourable conditions (hot, humid) mainly during the processing and marketing operations in

developing countries like India. Raw milk may be consumed at the farm or it may be sold directly to the market. In Poland, the selling of raw milk is legal as in the other EU countries. Besides, the hygiene regulations applicable to food of animal origin permit the selling of only the milk containing no more than 100,000 microorganisms per 1 mL originating from farms under veterinary supervision and meeting the systemic sanitary criteria in Poland (MARD,

*Corresponding Author, E Mail: Raghu.V@icar.gov.in , 4rvsy.dmnndri@gmail.com

2007). As we see the above requirements, we can't assume or do not guarantee the safety for the consumption of raw milk, it may be hazardous when consumed without pasteurization or any processing.

Raw milk is a source of food for various microorganisms because it contains a lot of nutrients. The pathogens like *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., *Enterobacter* spp., *E. coli*, etc. has been found in raw milk. Among this *Campylobacter* species outbreaks in raw milk have been increasing, mainly in European Countries (Bintsis, 2017). Diseases like gastritis and enteritis are caused due to the consumption of raw milk (Dhanashekar *et al.*, 2012). The *Campylobacter* spp. are micro-aerophilic, spiral in shape, Gram-negative bacteria, the primary cause of bacterial diarrhea in humans in the United States, reported at 1.3 million cases, and 120 deaths per year (Kaakoush *et al.*, 2015). In humans, *Campylobacter* gastroenteritis varies from moderate to serious diarrheal disease (Kaakoush *et al.*, 2015). The infection caused due to *Campylobacter jejuni* is called campylobacteriosis, or *Campylobacter jejuni* gastroenteritis. Infection primarily occurs in old people, infants, and immunocompromised patients (Same and Tamma, 2018). The person who has been infected with the organism from contaminated food or water is at risk of contracting the disease (Bintsis, 2017). The individuals who are immunocompromised or sick are mostly at higher risk of contracting campylobacteriosis and the infection is also more severe in those individuals. Campylobacteriosis is usually considered as a self-limiting disease, and there is usually no evidence of antimicrobial therapy for treating this infection (Barr and Smith, 2014). However, if it is started early in the course of infection on very sick or immunocompromised patients, the treatment can delay or decrease the length and impact or severity of the illness. Complications are rare, but *Campylobacter jejuni* infection can cause serious sequelae such as reactive arthritis, meningitis, recurrent colitis, acute cholecystitis,

haemolytic uremic syndrome, pancreatitis, cystitis, and Guillain-Barre syndrome. From a food safety point of view, *Campylobacter jejuni* and *Campylobacter coli* are most significant and cause enteritis in human beings as well as livestock (Facciola *et al.*, 2017).

Campylobacters are the intestinal inhabitants of a wide variety of wild and domestic animals, primarily birds. Insufficiently cooked meat, particularly poultry, unpasteurized milk, contaminated drinking water, direct contact with animals, fecal contamination of domestic animals, and surface water contaminating birds act as the primary source of the organism (Igwaran and Okoh, 2019). However, there is also over contamination of milk as a result of mastitis. It causes animals to suffer from diarrhoea and abortion. The occurrence of these *Campylobacter* species has been identified in bulk tank milk, dairy filters, manure, and animal faeces. In India there is very little information available on the occurrence of *Campylobacter* mainly in the dairy supply chain as a source of the pathogen, due to fewer detection practices (Sippy *et al.*, 2012). As no systematic monitoring of *Campylobacter* in milk, as well as milk products is conducted, the public health burden due to campylobacteriosis is also growing today (Facciola *et al.*, 2017). So there is a need for the routine analysis of this pathogen in the dairy supply chain to know the chances of infection of *Campylobacter* by consumption of milk and milk products. Therefore this study was conducted to determine the occurrence of *Campylobacter* in the study area by isolating *Campylobacter* from dairy supply chain.

MATERIALS AND METHODS

Procurement and maintenance of culture: The reference cultures required for our study *C. coli* ATCC 33559 and *C. jejuni* ATCC 29428 were procured from American type culture collection and checked for purity. Cultures were activated in Brucella broth (M348, Himedia, Mumbai, India) followed by streaking on modified

charcoal cefoperazone deoxycholate agar (mCCDA, M8871, Himedia, Mumbai, India), and *Campylobacter* agar base (CAB, M994, Mumbai, India), followed by incubation at 37°C for 24-48 h. A single pure colony from respective selective media after microscopic examination was picked up and maintained on Brucella broth or Bolton broth by routine sub-culturing after every 15th day. All the experiments were conducted using overnight grown cultures.

Sampling: For the collection of samples from the cattle-yard, initially, the hands of the milking man were sanitized using 70% alcohol. Several streams of milk were discarded from all four teats as foremilk. After that samples were collected in 100 mL sterile sample bottle directly from all quarters of the teats. There was a total of 175 samples among which 30 raw milk, 30 fresh water, 30 dairy waste water, 30 human handler swab samples, 30 milk filter swab, and 25 faecal swab samples were collected from Live stock research centre (LRC) and Experimental dairy plant, ICAR-NDRI, Karnal, Haryana. After aseptic collection, samples were labelled and packed with sterile bottles and transported with an ice box to the microbiology section of National Referral Centre for Milk Quality and Safety, ICAR-NDRI, Karnal for bacterial isolation. Samples were processed immediately for bacterial identification to species level using culture media and then isolates were kept in refrigerator at 4°C until microbial characterization with regular sub-culturing. Sampling of human handler and faecal swab was collected by swab techniques followed by evaluation using conventional technique.

Isolation of *Campylobacter* species from milk and water samples: Initially, the ATCC culture of *Campylobacter jejuni* and *Campylobacter coli* were evaluated. Samples were processed to isolate the *Campylobacter* spp. as per the ISO (10272-1-2006) protocol. Bolton broth was used for the selective enrichment of *Campylobacter* species. Before use, the Bolton

broth was autoclaved at 121°C at 15 lbs. pressure for 15 minutes. After cooling to 50°C, the rehydrated contents of Bolton selective supplement was added followed by 5% defibrinated sheep blood, mixed well separately and aseptically dispensed into the tubes. The tubes were marked respective to their sample numbers and 1 mL of samples (milk and water) inoculated in 9 mL of Bolton broth. The inoculated tubes were incubated at 37°C for 4-6 h and then at 41.5°C for 44 h in the microaerophilic condition which was created using 'CampyGen' gas pack in an anaerobic jar. The supplement added to the medium contains different antibiotics such as vancomycin, cefoperazone, and trimethoprim for better selectivity. Modified charcoal cefoperazone deoxycholate agar base (mCCDA) was recommended for selective detection and enumeration of *Campylobacter* species (Smith *et al.*, 2015). Further, enrichment was done by plating on blood free modified charcoal cefoperazone deoxycholate agar (mCCDA). The inoculated plates were incubated at a microaerophilic environment (85% N₂, 5% O₂ and 10% CO₂) at 41.5°C for 24-48 h. The typical dewdrop like, greyish, flat colonies on this blood-free agar were isolated. The microaerophilic environment was created in an anaerobic jar by using the 'CampyGen' gas pack and the CCDA supplement containing antibiotics was also used with mCCDA to increase the selectivity of media. The characteristics colonies were further transferred into Brucella broth and incubated at 37°C for 24 h. subsequently, these isolates were stored in 40% glycerol and kept at -20 °C until further investigation.

Isolation of *Campylobacter* isolates from human handlers, dairy milk filters, and faecal swab samples: Ten mL of ringer's solution tubes were prepared and autoclaved at 121°C for 15 mins at 15 psi pressure. The sterile cotton swab was dipped into the ringers solution tubes so that it will get wet. After that swabs were taken out from the tubes and rubbed on the hands of humans working in the dairy chain and a similar

procedure was followed for dairy milk filters and faecal swab samples, and transferred back into the tubes. One mL of sample from the ringer's tube was transferred into an autoclaved 9 mL of Bolton broth tube. All the tubes were marked for their identification and kept at 37°C for 4-6 h and 41.5°C for 44 h in microaerophilic conditions. Tubes which were showed turbidity were streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) plates followed by incubation at 41.5°C for 24-48 h in microaerophilic condition. The characteristics of greyish, flat colonies are selected and picked for further biochemical confirmation. In the case of dairy milk filters and faecal samples, the samples were taken from different locations.

Identification of positive *Campylobacter* isolates by phenotypic methods: The characteristics of greyish, flat, and dewdrop like colonies were further evaluated for its confirmation using different identification tests such as Grams staining, oxidase test, catalase test, micro-aerobic growth at 25°C & 42°C and H₂S test, etc. as per ISO 10272-1-2006 method.

Hippurate hydrolysis test: The sodium hippurate broth was prepared and autoclaved at 121°C at 15 lbs pressure for 15 minutes and inoculated with overnight grown culture. The sample was then incubated at 41.5°C for 24 h under microaerophilic conditions. The broth was centrifuged and the precipitate was removed. The prepared ferric chloride reagent was added to the supernatant. If the precipitate remains after 10 minutes, benzoic acid is present and the test was considered positive for hippurate hydrolysis (Mitruka, 2017).

RESULTS

Selection of selective media for isolation of *Campylobacter*: During the selection of selective media for the isolation of *Campylobacter*, two different selective media such as blood containing agar CAB (*Campylobacter* agar base) and blood-free agar modified charcoal cefoperazone deoxycholate

agar (mCCDA) were screened using standard ATCC cultures of *Campylobacter coli* (33559) and *Campylobacter jejuni* (29428) for better isolation of *Campylobacter* species. As the mCCDA contained activated charcoal and was blood-free, it was shown better selectivity and the chances of contamination were also less than blood containing media 'CAB'. The characteristics of colonies of *C. coli* and *C. jejuni* are shown in Fig. 1.

Isolation of *Campylobacter* from dairy supply chain: Out of 175 total samples, 30 samples of raw milk including 12 buffalo milk and 18 cow milk samples were processed for isolation of *Campylobacter* species. Out of 30 raw milk samples, 12 samples showed positive growth in selective enrichment, and characteristics of greyish colonies were also observed on mCCDA. The characteristics colonies from these 12 samples were further streaked on HiChrome *Campylobacter* agar base and 25 colonies had shown characteristic mauve to purple-colored colonies. In case of freshwater and dairy wastewater, thirty samples each from freshwater and dairy wastewater were processed for isolation of *Campylobacter* species. Thirty samples from freshwater and 9 from dairy wastewater were showing positive growth on selective enrichment and characteristics growth on mCCDA. Further, these samples were streaked on HiChrome CAB and 6 characteristic colonies from freshwater and 13 from dairy wastewater were selected for further confirmation. Human handler swab samples, 10 among 30 samples had shown positive growth in selective enrichment, and characteristics of greyish colonies on mCCDA medium. The characteristics colonies on these 10 samples were further streaked on HiChrome *Campylobacter* agar base, only 2 isolates had shown characteristic mauve to purple-colored colonies. Five samples among milk filter swabs had shown positive growth in selective enrichment, and characteristic greyish colonies on mCCDA medium. The characteristics colonies on these 05 samples were further streaked on HiChrome *Campylobacter* agar

base and 10 isolates had shown characteristic mauve to purple-colored colonies. Fourteen samples among 25 samples of faecal swab samples had shown positive growth in selective enrichment, and characteristic greyish colonies on mCCDA medium. All the characteristics colonies isolated from 175 numbers of samples were further streaked on HiChrome *Campylobacter* agar base and 91 isolates showed characteristic mauve to purple-colored colonies from 53 samples (Fig. 2).

Identification of positive *Campylobacter* isolates by phenotypic methods: All 91 isolates were further screened by Grams staining. Out of 91 isolates, 41 were observed as Gram-negative curved rods/s-shaped/coccoid in shape (Fig. 3A). Out of 25 raw milk isolates, 13 were confirmed as gram-negative rods. Similarly, 2 isolates from freshwater, 5 from dairy wastewater, 17 from dairy milk filter, and 17 from faecal swab sample were confirmed by Gram's staining. A study carried out by Fridrich *et al.* (2019) observed that *Campylobacter jejuni* can change its shape from helical to coccoid when there was an unfavourable condition to survive. That's why coccoid shaped isolates are also screened in this present study. All 54 isolates confirmed as G-ve curved rods/s-shaped/coccoid in shape were initially checked for the oxidase test to screen oxidase-positive isolates and 23 isolates had shown oxidase-positive reaction (Fig. 3B). Out of which 8 raw milk isolates, 9 faecal swab isolates, 3 isolates each from dairy waste water and dairy milk filters had shown oxidase-positive reaction. In case of catalase test, only 15 isolates had shown catalase enzyme activity through the production of efferve scence in the presence of Hydrogen peroxide (Fig. 3C). These isolates were from different sources, such as 5 each from raw milk and faecal swab samples followed by 3 isolates from dairy wastewater and 2 isolates from dairy milk filter. None of the isolates from freshwater samples showed catalase test positive reaction. Total of 15 catalase positive isolates were further evaluated formicro-aerobic growth at 25°C wherein only

3 isolates showed no growth on Hi-Chrome CAB and suspected as *Campylobacter* isolates (Fig. 3D). Among three isolates, one isolate from raw milk and two from the faecal swab sample were suspected as *Campylobacter* species. While, none of the samples from freshwater, dairy wastewater, human handler swab, and dairy milk filter were confirmed by this test. Other 51 isolates among 54 isolates from raw milk and faecal swab samples such as freshwater, dairy wastewater, human handler swab, and milk filter swab which were confirmed based on Grams reaction, none of them showed characteristic results of catalase, oxidase and micro-aerobic growth at 25°C. All 3 isolates were further evaluated for H₂S production and hippurate hydrolysis for tentative species identification. Wherein, 2 of the isolates showed hippurate hydrolysis with no H₂S production and was considered as *C. jejuni*. The remaining one isolate showed no hippurate hydrolysis along with H₂S production and it was tentatively indicated as *C. coli*. From the hippurate hydrolysis and H₂S production test (Fig. 3E and Fig. 3F), it was evident that among three isolates, 2 isolates might be *C. jejuni* and third isolate might be *C. coli*. The suspected isolates were mainly isolated from raw milk and faecal swab sample and they showed H₂S production test negative and hippurate hydrolysis test positive. In this study, out of 91 isolates selected initially, two were tentatively identified as *C. jejuni* (2.19 %) while one was identified as *Campylobacter coli* (1.0 %). With respect to the total no. of samples screened, 1.71% overall occurrence of *Campylobacter* species were observed including 1.14% of *C. jejuni* and 0.57% of *C. coli*. In case of individual samples types, the occurrence rate of *C. jejuni* in raw milk was found to be 3.33% (1/30) and 4% (1/25) for faecal swab samples. Similarly, a 4% (1/25) occurrence rate of *C. coli* was observed in the faecal swab samples but there was a zero percentage (0 /30) occurrence rate was observed in raw milk. While in freshwater, dairy wastewater, human handler swab, and dairy milk filter, zero percent occurrence was found for both *C. jejuni* and *C. coli*.

Campylobacter in dairy supply chain

Fig. 1. The growth of *Campylobacter* species on mCCDA and CAB medium. The greyish, flat, characteristics colonies of *Campylobacter* on black colored mCCDA media and small, grayish in coloration, flat with irregular edges, non-hemolytic, mucoid colonies on blood containing CAB

Campylobacter in dairy supply chain

Fig. 2. Suspected isolates/colonies selected for biochemical confirmation

Fig. 3. Identification of *Campylobacter* species by phenotypic methods A. Grams staining - Gram-negative curved rods/s-shaped/cocci in shape, B. Oxidase test, C. Catalase test, D. Micro-aerobic growth at 25°C, E. Hippurate hydrolysis test, F. H₂S production test

DISCUSSION

To our acquaintance, this research work has given the very information regarding the occurrence of *Campylobacter* species in dairy supply chain. Initially, selective medium was selected based the characteristic growth of *Campylobacter* on commonly available selective medium based on their characteristic growth and convince of use in the laboratory. Therefore, the mCCDA media was selected for the further isolation of *Campylobacter* from the dairy supply chain. In a study by Jones (2003), two selective media were compared for isolation of *Campylobacter* species from stool samples which included blood containing medium (CampyBAP) and the other was blood-free medium (CCDA) and they found CCDA would be a superior medium as compared to the blood containing CampyBAP. The superiority of mCCDA media may be due to supplementation of medium with polymixin B and it has yielded higher isolation rate and greater selectivity as compared to *Campylobacter* isolation agar (Chon *et al.*, 2012). Isolation and identification of *Campylobacter* was done on the basis of cultural and biochemical methods. About 91 characteristic colonies were selected from the mCCDA medium followed biochemical confirmation isolates colonies based on Grams reaction, catalase oxidase test followed by hippurate hydrolysis test and H₂S production. Finally, three isolates have phenotypically confirmed as *Campylobacter* spp. which were catalase-positive, oxidase-positive and no growth at 25°C under micro-aerobic conditions. Later, all 3 isolates were further evaluated for the H₂S production and Hippurate hydrolysis test to differentiate between *C. jejuni* and *C. coli*. Two isolates showed positive hippurate hydrolysis test and negative H₂S production, while one isolate had shown negative hippurate hydrolysis test and positive H₂S production. Based on the hippurate hydrolysis test and H₂S production, it was confirmed that two isolates as *C. jejuni* and one *C. coli*. The overall, 1.71 percent presence of *Campylobacter* was observed in 175 samples from the dairy supply chain. The occurrence rate of *Campylobacter*

species in raw milk and fecal swab samples was found to be 3.33% (1/30) and 4% (2/25), respectively. Hence the occurrence of *Campylobacter* was found to be more in the fecal swab as compared to the raw milk sample. Except for raw milk and fecal swab sample, none of the samples had shown the occurrence of *Campylobacter*. Out of three biochemically confirmed *Campylobacter* isolates, two confirmed as *Campylobacter jejuni* and one as *Campylobacter coli* by using H₂S and hippurate hydrolysis test. From the two biochemically confirmed isolates of fecal swab samples, one was tentatively identified as *C. jejuni* and other *Campylobacter coli*, while the one positive isolate from raw milk was tentatively identified as *C. jejuni*. While in freshwater, dairy wastewater, human handler swab, and dairy milk filter, zero percent occurrence was found for both *C. jejuni* and *C. coli*. These results are very well supported by Wysok *et al.* (2011) who reported the presence of *Campylobacter* in milk from the selected regions of Poland. Out of 150 bulk milk samples, 7 samples were found positive. The occurrence rate of *Campylobacter* in milk samples found 4.6% which was more than the occurrence rate in milk in the present study (1.71%). A total of 62 raw bulk tank milk samples were processed and only a single sample was found positive. The occurrence rate of *Campylobacter* was found at 1.6% which was lower than the occurrence rate in our current study. The occurrence rate of the present study was lower than the reports of Modi *et al.* (2015), who reported 2.91% occurrence rate in raw milk samples and the positive isolates are found to be *C. jejuni*. Hussain *et al.* (2007) reported an occurrence rate of *Campylobacter* in raw milk samples collected from three big cities of Pakistan with 10.2% prevalence which was higher than the prevalence rate in the current study results. Elango *et al.* (2009) reported 1.44% prevalence in raw milk samples. From 2008 to 2011, Giacometti *et al.* (2012) presented a prevalence data by monitoring four selected foodborne pathogens such as *Salmonella*, *L.monocytogenes*, *E.coli*, and *C. jejuni* from official microbiological records, these were

detected by Real-time PCR in raw milk sold by self machines in seven Italian regions (60,907 samples from 1,239 vending machines). *Campylobacter jejuni* was the second most prevalent pathogen with 0.09% prevalence rate in milk samples with a significant increase in *C. jejuni* prevalence was observed over the years of the study. In Nigeria, Salihu *et al.* (2010) reported 12.9% prevalence rate of *Campylobacter* spp. in dairy faecal swab samples of cattle.

Based on the above finding, it is concluded that the prevalence of *Campylobacter* was more observed in the faecal swab samples than raw milk samples based on phenotypic methods.

Conflict of interest: Authors have no conflict of interest in this study.

REFERENCES

- Barr W and Smith A, 2014. Acute diarrheal in adults. *Am Fam Physician*, 89(3): 180-189
- Bintsis T, 2017. Food-borne pathogens. *AIMS Microbiol*, 3(3): 529-563, doi: 10.3934/microbiol.2017.3.529
- Chon JW, Hyeon JY, Yim JH, Kim JH, Song KY *et al.*, 2012. Improvement of modified charcoal-cefoperazone-deoxycholate agar by supplementation with a high concentration of polymyxin B for detection of *Campylobacter jejuni* and *C. coli* in chicken carcass rinses. *Appl Environ Microbiol*, 78(5): 1624-1626, doi: 10.1128/AEM.07180-11
- Dhanashekar R, Akkinepalli S and Nellutla A, 2012. Milk-borne infections. An analysis of their potential effect on the milk industry. *Germs*, 2(3): 101-109, doi: 10.11599/germs.2012.1020
- Elango A, Dhanalakshmi B, Pugazhenthir TR, Jayalalitha V, Kumar CN *et al.*, 2009. PCR-restriction fragment length polymorphism of *Campylobacter jejuni* isolates. *Egypt J Dairy Sci*, 37(2): 69-173
- Facciola A, Riso R, Avventuroso E, Visalli G, Delia SA *et al.*, 2017. *Campylobacter*: from microbiology to prevention. *J Prev Med Hyg*, 58(2): E79-E92
- Frirdich E, Biboy J, Pryjma M, Lee J, Huynh S *et al.*, 2019. The *Campylobacter jejuni* helical to coccoid transition involves changes to peptidoglycan and the ability to elicit an immune response. *Mol Microbiol*, 112(1): 280-301, doi: 10.1111/mmi.14269
- Giacometti F, Serraino A, Finazzi G, Daminelli P, Losio MN *et al.*, 2012. Sale of raw milk in northern Italy: food safety implications and comparison of different analytical methodologies for detection of foodborne pathogens. *Foodborne Pathog Dis*, 9(4): 293-297, doi:10.1089/fpd.2011.1052
- Hussain I, Mahmood MS, Akhtar M and Khan A, 2007. Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. *Food Microbiol*, 24(3): 219-222, doi: 10.1016/j.fm.2006.06.001
- Igwaran A and Okoh AI, 2019. Human campylobacteriosis: A public health concern of global importance. *Heliyon*, 5(11): e02814, doi: 10.1016/j.heliyon.2019.e02814
- ISO 10272-1, 2006. Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: Detection method
- Kaakoush NO, Castaño-Rodríguez N, Mitchell HM and Man SM, 2015. Global epidemiology of *Campylobacter* infection. *Clin Microbiol Rev*, 28(3): 687-720, doi:10.1128/CMR.00006-15
- MARD (Minister of Agriculture and Rural Development), 2007. Regulation of the Minister of Agriculture and Rural Development from 29

Author's contribution: SB, DB, and HVR: Conceived and design the study, carried out the sampling and worked on the isolation and phenotypic identification of *Campylobacter* in dairy supply chain; AJ, HVR, NK, and HMR: Performed the data analysis and prepared the manuscript.

ACKNOWLEDGEMENTS

This work was carried out under the Early Career Research Scheme funded by Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Ministry of Science and Technology, Govt. of India. The Authors gratefully acknowledge the Director, ICAR-NDRI for providing the necessary infrastructure facility to carry out this research.

- December 2006- On veterinary requirements at the production of products of the animal origin intended direct sales. Journal of Laws, No 5, item 38, Government Legislation Centre, Warsaw, Poland
- Mitruka BM, 2017. Methods of Detection and Identification of Bacteria (1977). CRC Press, Taylor & Francis Group, USA
- Modi S, Brahmabhatt MN, Chatur YA and Nayak JB, 2015. Prevalence of *Campylobacter* species in milk and milk products, their virulence gene profile and anti-bio gram. Vet World, 8(1): 1-8, doi: 10.14202/vetworld.2015.1-8
- Salihu MD, Junaidu AU, Magaji AA and Rabiu ZM, 2010. Study of *Campylobacter* in raw cow milk in Sokoto State, Nigeria. Br J Dairy Sci, 1(1): 1-5
- Same RG and Tamma PD, 2018. *Campylobacter* infections in children. Pediatr Rev, 39(11): 533-541, doi: 10.1542/pir.2017-0285
- Sippy R, Sandoval-Green CM, Sahin O, Plummer P, Fairbanks WS *et al.*, 2012. Occurrence and molecular analysis of *Campylobacter* in wildlife on livestock farms. Vet Microbiol, 157(3-4): 369-375, doi: 10.1016/j.vetmic.2011.12.026
- Smith S, Meade J, McGill K, Gibbons J, Bolton D *et al.*, 2015. Restoring the selectivity of modified charcoal cefoperazone deoxycholate agar for the isolation of *Campylobacter* species using tazobactam, a β -lactamase inhibitor. Int J Food Microbiol, 210: 131-135, doi: 10.1016/j.ijfoodmicro.2015.06.014
- Wysok B, Wiszniewska-Łaszczych A, Uradziński J and Sztejn J, 2011. Prevalence and antimicrobial resistance of *Campylobacter* in raw milk in the selected areas of Poland. Pol J Vet Sci, 14(3): 473-477, doi:10.2478/v10181-011-0070-3