Isolation, molecular identification and antimicrobial resistance patterns of *Campylobacter* species of dairy origin: First report from Bangladesh

S. M. Lutful Kabir,[1] Most. Mostary Lubna,[1] Md. Mehedul Islam,[1] A. K. M. Ziaul Haque,[1] Sucharit Basu Neogi,[2] Shinji Yamasaki[2]

[1]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh; [2]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan

Abstract

This study was aimed for isolation, identification and characterization of *Campylobacter* species from Bangladesh Agricultural University dairy farm during the period of January to May, 2016. A total of 80 samples (fecal samples of calves, heifers and cows; milk samples of cows) were collected from Bangladesh Agricultural University dairy farm for isolation and identification of *Campylobacter* species by using cultural, biochemical and molecular methods. Moreover, the isolated *Campylobacter* species were subjected for antimicrobial susceptibility test. *Campylobacter* like organisms were presumptively identified in 20 samples. Isolates were biochemically positive to catalase and oxidase tests and in hippurate hydrolysis test some of the isolates (n=6) shown negative that indicated the isolates were *C. coli* and some of the test isolates (n=14) shown positive that indicated the isolates were *C. jejuni*. *Campylobacter* specific 16S rRNA genes were amplified from the isolates. Out of 20 isolated *Campylobacter* 14 (17.5%) were detected as *C. jejuni* and the rest 6 (7.5%) were detected as *C. coli* by *cdtC* gene based multiplex PCR assay. *C. jejuni* were resistant to amoxicillin, erythromycin, azithromycin and susceptible to gentamicin, ciprofloxacin, norfloxacin and streptomycin. Furthermore, *C. coli* were resistant to amoxicillin and erythromycin and susceptible to gentamycin, ciproflaxacin. Out of 20 *Campylobacter* isolates, 57.14% *C. jejuni* and 33.33% *C. coli* were identified as multidrug resistant. To the best of our knowledge, this study has brought the first report on the occurrence of *Campylobacter* species with their antibiogram profiles in any dairy farm of Bangladesh.

Introduction

Campylobacter spp. are Gram negative, microaerophilic bacteria with slightly curved or spiral rods shaped under the family of Campylobacteriaceae.[1] At least a dozen of *Campylobacter* spp. has been associated with human disease and the most common are *C. jejuni* and *C. coli*.[2] Development of human infection may occur by direct contact with infected animals or by consumption of contaminated unpasteurized milk or milk products, contaminated water and raw meat and domestic birds are considered as important reservoirs of food-borne infection for humans.[3] The importance of milk for the development of human gastroenteritis due to *Campylobacter* spp. was confirmed by the summary report of European Union on food-borne disease outbreaks.[4] It is assumed that contamination of raw milk by *Campylobacter* spp. derived from secondary fecal contamination during the milking process.[5] The infection has been developed due to consumption of raw milk that is the most important source of *Campylobacter*.[6] Longer life span of dairy cattle than beef cattle can serve as a long-term reservoir of *Campylobacter* spp. in dairy cattle.[5,7] The development of environmental contamination through indirect exposure of cattle feces is considered a high risk to human infections.[7] The ideal environment for optimal growth of *Campylobacter* spp. requires an atmosphere containing approximately 5% O_2, 10% CO_2, and 85% N_2 at 37°C to 42°C.[8] The selective blood-containing agar are recommended medium that are used for culture of *Campylobacter* spp.[8] although alternative media may be used. The viability of *C. jejuni* in faces and milk may remain for up to 9 days and 3 days respectively.[9] Contamination of raw milk with *Campylobacter* spp. mainly associated with fecal contamination.[10]

The concerns for human health are the inappropriate use of antibiotics in cattle production and the development of antimicrobial resistant strains of bacteria. The increasing rate of human infections caused by antimicrobial-resistant *Campylobacter* spp. makes more difficult to clinical management of campylobacteriosis.[11] *Campylobacter* spp. with resistant to antimicrobial agent has been reported worldwide.[12-14]

In Bangladesh several studies such as occurrence, molecular detection and antibiotic sensitivity test of *Campylobacter* spp. in poultry farms have been performed.[14,15] However, there are no documented reports exist yet on the occurrence and antibiogram profiles of *Campylobacter* spp. in dairy farm where milk is widely consumed in Bangladesh. Therefore, this study was aimed to isolate and identify *Campylobacter* spp. inhabiting feces and milk originating from Bangladesh Agricultural University dairy farm and to assess antibiogram profiles of the isolated *Campylobacter* spp.

Correspondence: S. M. Lutful Kabir, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
Phone: +88-091-67401-6/Ext. 63218.
E-mail: lkabir79@gmail.com

Key words: Dairy farm; C. *jejuni; C. coli;* Molecular identification; Antibiogram profiles.

Acknowledgements: Authors gratefully acknowledge the support of Professor Dr. Shinji Yamasaki, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan who provided us microaerophilic gas packs used in this research work.

Contributions: S. M. Lutful Kabir planned and designed the study. Most. Mostary Lubna and Md. Mehedul Islam assisted in data collection, laboratory work, data analysis and drafting of the manuscript. S. M. Lutful Kabir, A.K.M. Ziaul Haque, Sucharit Basu Neogi and Shinji Yamasaki assisted in data analysis and drafting of the manuscript.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: part of this work was supported by grants from Bangladesh Agricultural University Research System (BAURES), Mymensingh, Bangladesh (Project No. 2015/102/BAU).

Received for publication: 20 August 2018.
Accepted for publication: 9 January 2019.

Veterinary Science Development 2018; 8:7838
doi:10.4081/vsd.2018.7838

Materials and Methods

Collection, transportation and processing of samples

A total of 80 samples (60 fecal samples

and 20 milk samples) were collected from Bangladesh Agricultural University (BAU) dairy farm during the period of January to May, 2016. Then the collected samples were transferred to Molecular Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh through *thermos flask*. Then the samples were processed immediately for the isolation and identification of *Campylobacter* species.

Isolation of *Campylobacter* species

Isolation of *Campylobacter* species were carried out by filtration method (0.45 µm filter; Biotech, Germany) as described by Shiramaru *et al.*[16]

Identification of *Campylobacter* spp. by biochemical tests

Differentiation of isolated *Campylobacter* spp. were performed by various biochemical tests such as catalase, oxidase and hippurate hydrolysis test according to the methods described by Foster *et al*.[17]

Preparation of DNA templates

DNA templates were prepared by boiling method according to the procedures mentioned by Hoshino *et al.*[18]

16S rRNA-gene-based PCR for identification of the genus *Campylobacter*

The 16S rRNA gene was selected for the identification of the genus *Campylobacter*. Primers (Invitrogen, USA) used for the amplification of 16S rRNA gene are shown in Table 1. The reaction mixture (20 µL) was prepared by mixing 10 µL master mixtures (Promega, USA), 1 µL forward primer (10 pmol), 1 µL reverse primer (10 pmol), 3 µL DNA template and 5 µL deionized water. The PCR reactions were carried out using a thermocycler (Astec, Japan) with the following program: initial denaturation with 1 cycle of 5 min at 94°C, 30 cycles each consisting of denaturation with 30 s at 94°C, annealing with 30 s at 47°C, extension with 1 min 30 s at 72 °C and a final extension step of 10 min at 72°C. PCR products were analyzed by 1.5% agarose (Invitrogen, USA) gel electrophoresis and the bands were visualized with UV light after staining with ethidium bromide (0.5 µg/mL) for 10 minutes in a dark place. Bands were visualized and images were captured on a UV transilluminator (Biometra, Germany).

cdtC gene based PCR for species identification

cdtC gene based multiplex PCR was used for the species identification of *C. jejuni* and *C. coli* as described by Asakura *et al.*[19] The primers (Bioneer, South Korea) used for the *cdtC* gene based PCR multiplex PCR are shown in Table 1.[19-22] The reaction mixture (20 µL) was prepared by mixing 10 µL master mixtures (Promega, USA), 1 µL forward primer (10 pmol), 1 µL reverse primer (10 pmol), 3 µL DNA template and 5 µL deionised water. The PCR reactions were carried out using a thermocycler (Astec, Japan) with the following program: initial denaturation with 1 cycle of 5 min at 94°C, 30 cycles each consisting of denaturation with 30 s at 94°C, annealing with 30 s at 55°C, extension with 30 s at 72°C and a final extension step of 5 min at 72°C. PCR products were analyzed by 2% agarose (Invitrogen, USA) gel electrophoresis. Bands were visualized and images captured as described above.

Antimicrobial susceptibility test

All *Campylobacter* spp. were tested against eight commonly used antibiotics (HiMedia, India) by the method of disk diffusion as described by Luangtongkum *et al.*[20] The zones of growth inhibition were compared with the zone size interpretative standards as described by Clinical and Laboratory Standard Institute.[21] *E. coli* ATCC 25922 was kept as a quality control bacterium in this study. At least two separate experiments were performed for confirmation of all susceptibility data.

Results

Isolation and identification of *Campylobacter* species using conventional methods

The occurrences of *Campylobacter* species available in fecal and milk samples are shown in Table 2. A total of 80 samples [fecal (60) and milk (20)] were subjected for isolation of *Campylobacter* strains by filtration method. *Campylobacter* spp. produced grey color spreading colonies on Blood agar base no. 2 media after 48 hrs of incubation at 37ºC using microaerophilic condition (5% O_2, 10% CO_2 and 85% N_2). In Gram's staining examination, the organism shown Gram negative, pink color, small

Table 1. Primers used for the various PCR.

Primer	Sequence (5'-3')	Target	Amplicon size (bp)	Reference
16S9F 16S1540R	GAGTTTGATCCTGGCTC AAGGAGGTGATCCAGCC	16S rRNA gene	1530	[22]
Cj-cdtCU1 Cj-CdtCR2	TTTAGCCTTTGCAACTCCTA AAGGGGTAGCAGCTGTTAA	Cj-cdtC	524	[19]
Cc-CdtCU1 Cc-CdtCR1	TAGGGATATGCACGCAAAG GCTTAATACAGTTACGATAG	Cc-cdtC	313	[19]
CfspCU2 CfspCR1	AAGCATAAGTTTTGCAAACG GTTTGGATTTTCAAATGTTCC	Cf-cdtC	397	[19]

Table 2. Percentages (%) of *Campylobacter* species available in fecal and milk samples.

Types of sample	Species	No. of sample	No. (%) of *Campylobacter* isolates		Overall no. (%) of *Campylobacter* species
			C. jejuni	*C. coli*	
Faecal	Calves	20	2 (10)	0 (0)	20 (25)
	Heifers	20	4 (20)	2 (10)	20 (25)
	Cows	20	5 (25)	3 (15)	20 (25)
Milk	Cows	20	3 (15)	1 (5)	20 (25)
Total		80	14 (17)	6 (7.5)	20 (25)

curved shape arranged as single or in pair under microscope (100X). Out of 60 fecal samples only 16 (26.66%) were positive for *Campylobacter* spp. and out of 20 milk samples, 4 (20%) were positive for *Campylobacter* spp. *Campylobacter* like organisms were then subjected for biochemical tests. All the isolates of *Campylobacter* spp. (n=20) produced bubbles were found positive in catalase test. All the isolates of *Campylobacter* spp. (n=20) produced deep blue color within 10 seconds were found positive in oxidase test. In hippurate hydrolysis test some of the isolates (n=6) did not develop any purple color that indicated the isolates were *C. coli* and some of the test isolates (n=14) developed purple color that indicated the isolates were *C. jejuni.*

Molecular identification of *Campylobacter* spp.

Genus specific PCR with the gene of 16S rRNA was performed. 1530 bp fragment of targeted gene was amplified successfully (Figure 1). The multiplex PCR assay targeting the *cdtC* gene was used and 14 *C. jejuni* gave specific amplification (524 bp) (Figure 2). Similarly, 6 *C. coli* gave specific amplification (313 bp) by multiplex PCR assay targeting *cdtC* gene (Figure 3). None of the tested strains produced a specific band corresponding to the gene of *cdtC* of *C. fetus* (data not shown).

Antibiogram profiles of isolated *Campylobacter* spp.

14 isolates of *C. jejuni* were subjected to antimicrobial susceptibility testing against 8 selected antibiotics (Table 3). Among all the isolates 10 (71.42%) were susceptible to gentamicin, 4 (28.57%) were susceptible to norfloxacin, 4 (28.57%) were susceptible to ciprofloxacin, 5 (35.71%) were susceptible to streptomycin and 1 (7.14%) was susceptible to azithromycin. All data of antibiogram profile shown that the isolates were resistant to amoxicillin 12 (85.71%), tetracycline 12 (85.71%), azithromycin 12 (85.71%), erythromycin 14 (100%). Furthermore, 8 (57.14%) were resistant to streptomycin, 7 (50%) were resistant to norfloxacin, 2 (14.28%) were resistant to gentamicin and 4 (28.57%) were resistant to ciprofloxacin. 6 (42.85%) isolates were intermediate resistant to ciprofloxacin, 3 (21.42%) were intermediate resistant to norfloxacin, 1 (7.14) was intermediate resistant to streptomycin, 2 (14.28) were intermediate resistant to amoxicillin. Six isolates of *C. coli* were subjected to antimicrobial susceptibility testing against 8 selected antibiotics (Table 4). Among all the isolates 4 (66.67%) were susceptible to gentamicin, 3 (50%) were susceptible to ciprofloxacin, 1 (16.67) was susceptible to norfloxacin, 1 (16.67) was susceptible to azithromycin, 2 (33.33%) were susceptible to streptomycin. Antibiogram profiles revealed that the isolates were resistant to amoxicillin 4

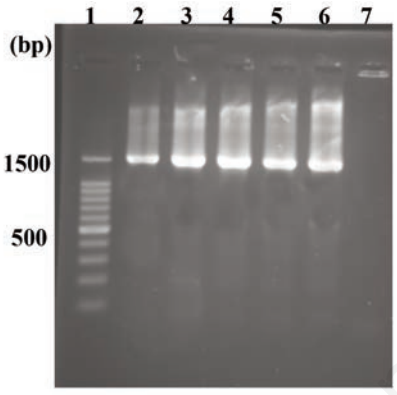

Figure 1. Detection of *Campylobacter* spp. by 16S rRNA gene based PCR. Lanes: 1, 100 bp DNA ladder (Promega, USA); 7, negative control; 2-6, tested positive samples.

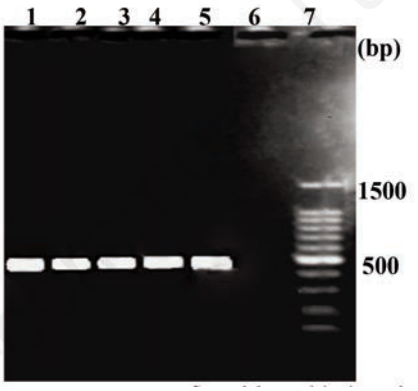

Figure 2. Detection of *Campylobacter jejuni* by *cdtC* gene based multiplex PCR assay. Lanes: 1-5, tested positive samples; 6, negative control; 7, 100 bp DNA ladder (Promega, USA).

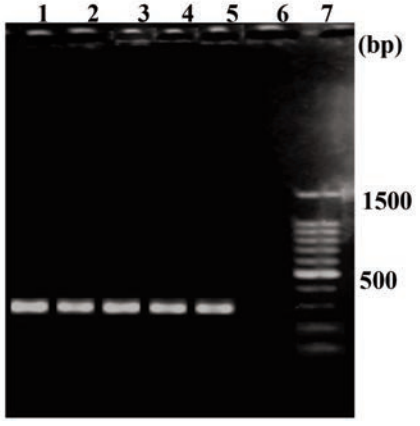

Figure 3. Detection of *Campylobacter coli* by *cdt* C gene based multiplex PCR assay. Lanes: 1-5, tested positive samples; 6, negative control; 7, 100 bp DNA ladder (Promega, USA).

Table 3. Antimicrobial susceptibility pattern of *C. jejuni* (n=14) identified by the disk diffusion method.

Antimicrobial agents	Number (%) of *C. jejuni*		
	S (%)	I (%)	R (%)
Amoxicillin	0 (0)	2 (14.28)	12 (85.71)
Tetracycline	0 (0)	2 (14.28)	12 (85.71)
Gentamicin	10 (71.42)	2 (14.28)	2 (14.28)
Erythromycin	0 (0)	0 (0)	14 (100)
Azithromycin	1 (7.14)	1 (7.14)	12 (85.71)
Ciprofloxacin	4 (28.57)	6 (42.85)	4 (28.57)
Norfloxacin	4 (28.57)	3 (21.42))	7 (50)
Streptomycin	5 (35.71)	1 (7.14)	8 (57.14)

Note: AMX, Amoxicillin (30 µg); AZM, Azithromycin (30 µg); CIP, Ciprofloxacin (5 µg); E, Erythromycin (30 µg); GEN, gentamicin (10 µg); NOR, Norfloxacin (10 µg); S, Streptomycin (10 µg); TE, Tetracycline (30 µg); b: S = Susceptible; I = Intermediate resistance; R = Resistance.

(66.67%), tetracycline 4 (66.67%), erythromycin 6 (100%), streptomycin 3 (50), norfloxacin 3 (50%), azithromycin 3 (50), gentamicin 1 (16.67) and ciprofloxacin 1 (16.67). Moreover, 2 (33.33%) were intermediate resistant to ciprofloxacin, 2 (33.33) were intermediate resistant to norfloxacin, 2 (33.33%) were intermediate resistant to azithromycin, 2 (33.33) were intermediate resistant to tetracycline and 1 (16.67%) was intermediate resistant to gentamicin. The result of resistance patterns of isolated *C. jejuni* and *C. coli* are shown in Table 5. Out of 14 C. *jejuni* isolates, 3 (21.42%) was resistant to 1 agent (AMX), 2 (14.29) were resistant to 1 agent (E), 1 (7.14%) was resistant to 1 agent (AZM). Furthermore, 1(7.14%) was resistant to 2 agents (AMX-TE), 2(14.29) were resistant to 2 agents (AMX-S) respectively. Moreover, 1 (7.14%) was resistant to 3 agents (AMX-S-TE), 2 (14.29) were resistant to 3 agents (E-S-CIP), 1 (7.14%) was resistant to 4 agent (AMX-NOR-AZM-TE) and 1 (7.14%) was resistant to 5 agents (AMX-S-E-AZM-TE) respectively. Out of 6 *C. coli* isolates, 4 (66.67%) were resistant to 1 agent (AMX), 1 (16.67) were resistant to 2 agent (AMX-E) and 1 (16.67) was resistant to 3 agents (AMX-S-TE) respectively. In this study, multidrug resistant *Campylobacter* spp. were identified by considering resistant to 2 or more drugs as described in Table 5. A total of 14 *C. jejuni* were isolated and 8 (57.14) were identified as multidrug resistant. Out of 6 *C. coli* 2 (33.33) were identified as multidrug resistant.

Discussion

To our knowledge, this study has brought the first report on investigating the prevalence of *Campylobacter* spp. in dairy farms of Bangladesh. Cultural examination, staining characteristics, biochemical tests and finally PCR were performed for the characterization of the *Campylobacter* spp. and the colony characteristics were exhibited grey color which was supported by several researchers.[15,23] The routine isolation and identification of *Campylobacter* spp. in laboratories were conducted on the basis of cultural and biochemical methods which was supported by several researchers.[24] Hippurate hydrolysis test was used for discriminating between *C. jejuni* and *C. coli* which was also used by several researchers.[13,15,16] The current study recorded 16 (26.66%) and 4 (20%) *Campylobacter* spp. from 60 fecal and 20 milk samples respectively during the study period. Out of 80 samples, 14 (17%) isolates were *C. jejuni* and the remaining 6 (7.5%) isolates were *C. coli*. Ramonaite *et al.*[24] also recorded *Campylobacter jejuni* and *Campylobacter coli* from dairy farm in Lithuania. PCR primers targeting 16S rRNA gene of *Campylobacter* spp. were amplified 1530 bp fragments of DNA confirmed the identity of *Campylobacter* spp. (Figure 1). All *Campylobacter* isolates were positive to 16S rRNA gene based PCR which is supported by Kabir *et al.*[13,14] The *cdtC* gene was amplified for detecting and discriminating between *Cj-cdtC* and *Cc-cdtC* (Figures 2 and 3) likewise several researchers.[13]

Despite of the fact that *Campylobacter* spp. is common in dairy cattle, our study revealed a moderate rate of prevalence (20%) in BAU dairy farm, Mymensingh, Bangladesh as shown in Table 2. Other researchers reported prevalence between 5% and 78.5%.[25,26] Since sampling design, cultural methods and conditions were varied among these studies, a direct comparison of the results is troublesome. However, our data contribute to previous conversation that dairy cattle are significant assortment for *Campylobacter* spp. and could be a source of infections. The present study recorded that *Campylobacter*

Table 4. Antimicrobial susceptibility pattern of *C. coli* (n=6) identified by the disk diffusion method.

Antimicrobial agents	Number of *C. coli* isolates		
	S (%)	I (%)	R (%)
Amoxicillin	0 (0)	2 (33.33)	4 (66.67)
Tetracycline	0 (0)	2 (14.28)	4 (66.67)
Gentamicin	4 (66.67)	1 (16.67)	1 (16.67)
Erythromycin	0 (0)	0 (0)	6 (100)
Azithromycin	1 (16.67)	2 (33.33)	3 (50)
Ciprofloxacin	3 (50)	2 (33.33)	1 (16.67)
Norfloxacin	1 (16.67)	2 (33.33)	3 (50)
Streptomycin	2 (33.33)	1 (16.67)	3 (50)

Table 5. Antimicrobial resistance pattern of *C. jejuni* and *C. coli*.

Species	Resistance patterns	No. of resistant isolates (%)	No. of multidrug resistant isolates (%)
C. jejuni (n=14)	No resistance demonstrated	-	8 (57.14)
	Resistant to 1 agent (AMX)	3 (21.42)	
	Resistant to 1 agent (E)	2 (14.29)	
	Resistant to 1 agent (AZM)	1 (7.14)	
	Resistant to 2 agents (AMX- TE)	1 (7.14)	
	Resistant to 2 agents (AMX-S)	2 (14.29)	
	Resistant to 3 agents (AMX-S-TE)	1 (7.14)	
	Resistant to 3 agents (E-S-CIP)	2 (14.29)	
	Resistant to 4 agents (AMX-NOR-AZM-TE)	1 (7.14)	
	Resistant to 5 agents (AMX-S-E-AZM-TE)	1 (7.14)	
	Total resistant isolates	14 (100)	
C. coli (n=6)	Resistant to 1 agent (AMX)	4 (66.67)	2 (33.33)
	Resistant to 2 agents (AMX-E)	1 (16.67)	
	Resistant to 3 agents (AMX-S-TE)	1 (16.67)	
	Total resistant isolates	6 (100)	

species may be found more frequently in fecal samples than milk samples.

In the antimicrobial susceptibility testing most of the isolates were susceptible to ciprofloxacin, gentamicin and all the isolates were resistant to amoxicillin, erythromycin. These findings are close to the findings of several researchers.[27] The current study also recorded some multidrug resistant spp. in collected samples of BAU dairy farm. Out of 20 isolates, 57.14% *C. jejuni* and 33.33% *C. coli* were detected as multidrug resistant. Resistant profiles of multidrug resistant *Campylobacter* spp. were close to the result of some researchers.[15,20] Findings of this study suggested that multidrug resistant *Campylobacter* spp. isolated from dairy farm might be an important concern for veterinary practitioners.

Conclusions

The findings of this study demonstrated the presence of multidrug resistant *C. jejuni* and *C. coli* in feces and milk samples that are not only harmful for cattle itself but also are harmful for consumers on milk consumption. Nevertheless, more studies are needed to clearly understand the genomic diversity in *C. jejuni* and *C. coli* as well as molecular mechanisms for the development of antimicrobial resistance.

References

1. Brenner DJ, Krieg NR. Bergey's Manual of Systematic Bacteriology. In: The Proteobacteria. Springer Science and Business Media 2006; pp 1147-1160.
2. Ryan KJ, Ray C. Medical Microbiology. In: An introduction to infectious diseases, 4th Ed, Mcgraw-Hill, 2004; 378-380.
3. Mezher Z, Saccares S, Marciano R, et al. Occurrence of Campylobacter species in poultry meat at retail and processing plants' levels in Central Italy. Ital J Food Saf 2016;5:47-9.
4. European Food Safety Authority. The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2013, Vol 13. EFSA J 2015;13: 1-165.
5. Oliver SP, Jayarao BM, Almeida RA, et al. Food-borne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis 2005;2:115-29.
6. Heuvelink AE, Van HC, Zwartkruis NA, et al. Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. Int J Food Microbiol 2009;134:70-4.
7. Devane ML, Nicol C, Ball A, et al. The occurrence of Campylobacter subtypes in environmental reservoirs and potential transmission routes. J Appl Microbiol 2005;98:980-90.
8. Corry JE, Atabay HI, Forsythe SJ, et al. Culture media for the isolation of Campylobacters, Helicobacters and Arcobacters. Prog Ind Microbiol 2003;37:271-316.
9. Silva J, Leite D, Fernandes M, et al. Campylobacter spp. as a food-borne pathogen: a review. Front Microbiol 2011;2:1-12.
10. Park SF. The physiology of Campylobacter spp. and its relevance to their role as food borne pathogens. Int J Food Microbiol 2002;74:177-88.
11. Levin RE. Campylobacter jejuni: a review of its characteristics, pathogenicity, ecology, distribution, subspecies characterization and molecular methods of detection. Food Biotechnol 2007;21:271-347.
12. Chen X, Naren GW, Wu CM, et al. Prevalence and antimicrobial resistance of Campylobacter isolates in broilers from China. Vet Microbiol 2010;144:133-9.
13. Kabir SML, Kikuchi K, Asakura M, et al. Evaluation of a cytolethal distending toxin (cdt) gene-based species-specific multiplex PCR assay for the identification of Campylobacter strains isolated from diarrheal patients in Japan. Jpn J Infect Dis 2011;64:19-27.
14. Kabir SML, Asakura M, Shiramaru S, et al. Molecular identification and antimicrobial resistance profiles of Campylobacter strains of poultry origin in India with special emphasis on fluoroquinolone resistance. Asian J Med Biol Res 2015;1:1-8.
15. Kabir SML, Sumon MH, Amin MM, et al. Isolation, identification and antimicrobial resistance patterns of Campylobacter Species from broiler meat sold at KR market of Bangladesh Agricultural University campus. J Agric Food Tech 2014;4:1-7.
16. Shiramaru S, Asakura M, Inoue H, et al. A cytolethal distending toxin gene-based multiplex PCR assay for detection of Campylobacter spp. in stool specimens and comparison with culture method. J Vet Med Sci 2012;74:857-62.
17. Foster G, Holmes B, Steigerwalt AG, et al. Campylobacter insulaenigrae sp. nov., isolated from marine mammals. Int J Syst Evol Microbiol 2004;54:2369-73.
18. Hoshino K, Yamasaki S, Mukhopadhyay AK, et al. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic Vibrio cholerae O1 and O139. FEMS Immunol Med Microbiol 1998;20:201-7.
19. Asakura M, Samosornsuk W, Hinenoya A, et al. Development of a cytolethal distending toxin (cdt) gene-based species-specific multiplex PCR assay for the detection and identification of Campylobacter jejuni, Campylobacter coli and Campylobacter fetus. FEMS Immunol Med Microbiol 2008;52:260-6.
20. Luangtongkum T, Jeon B, Han J, et al. Antibiotic resistance in Campylobacter: emergence, transmission and persistence. Future Microbiol 2009;4:189-200.
21. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, twenty-fifth informational supplements, Vol 35, CLSI document M100-S25, 2015; 1–231.
22. Samosornsuk W, Hinenoya A, Misawa N, et al. Development of a cytolethal distending toxin (cdt) gene-based species-specific multiplex PCR assay for the detection and identification of Campylobacter jejuni, Campylobacter coli and Campylobacter fetus. FEMS Immunol Med Microbiol 2007;52:260-6.
23. Doyle MP. Campylobacter jejuni, In: D.O. Cliver Ed, Academic Press, Food diseases Inc., Boston. 1990; 217-222.
24. Jamshidi A, Bassami MR, Farkhondeh T. Isolation and identification of Campylobacter spp. and Campylobacter coli from poultry carcasses by conventional culture method and multiplex PCR in Mashhad, Iran. Iran J Vet Res 2008;9:132-7.
25. Ramonaite S, Rokaityte A, Tamuleviciene E, et al. Prevalence, quantitative load and genetic diversity of Campylobacter spp. in dairy cattle herds in Lithuania. Acta Vet Scand 2013;55:1-6.
26. Sasaki Y, Murakami M, Haruna M, et al. Prevalence and characterization of food-borne pathogens in dairy cattle in the eastern part of Japan. J Vet Med Sci 2013;75:543-6.
27. Butzler JP. Campylobacter from obscurity to celebrity. Clin Microbiol Infect 2004;10:868-76.