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Research Article

PREVALENCE OF THERMOPHILIC *CAMPYLOBACTER JEJUNI* ISOLATED FROM CLOACAL SAMPLES OF POULTRY

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ABSTRACT

In the present study, 43 thermophilic *Campylobacter* species were isolated from 370 cloacal samples of poultry. All the 43 isolates were identified as *Campylobacter* species on the basis of biochemical characteristics and 16S rRNA based PCR ribotyping using genus specific primers. These isolates were further characterized using species specific PCR by targeting 735bp and 500bp amplicon of *hip*O and *asp* gene for *C. jejuni* and *C. coli,* respectively. All the 43 isolates were confirmed to be *Campylobacter jejuni* by PCR.

Key words: *Campylobacter* species, *C. jejuni*, *C. coli*, poultry, molecular investigation, PCR

Thermophilic *Campylobacters* are ranked fourth among top five pathogens in causing food borne infections (CDC, 2010) and second most emerging zoonotic pathogens after *Salmonella* (Epps *et al.,*2013). Out of the four most common species i.e., *C. jejuni*, *C. coli*, *C. lari* and *C. upsalinesis*; *C. jejuni* is responsible for majority (80-90%) of these infections and is a leading food borne pathogen followed by *C. coli* (Biswas *et al.,* 2011). *C. jejuni* are able to colonize intestinal tract of vast variety of mammals and birds. Poultry is considered to be its major reservoir and the organism colonizes caecal mucosal crypts of the gastrointestinal tract of chicken's very efficiently (Coward *et al.,* 2008). Chicken remain asymptomatic carriers until they reach slaughter age in broilers and layers (Bolton, 2015).

Discrimination between thermophilic *Campylobacters* is difficult as these fastidious organisms poorly hydrolyse a range of sugars and possess few biochemical characteristics that can reliably be used to distinguish between species (Lastovica and Allos, 2008). Differentiation between *C. jejuni* and *C. coli* biochemically is based on a single test *i.e.,* hippurate hydrolysis with 90% accuracy (El-Baky *et al.*, 2014). Therefore polymerase chain reactions (PCR) based assays have been developed to discriminate between these *Campylobacters* (Burnett *et al.,* 2002; Ertas *et al.,* 2004; Tang *et al.,* 2009).

Although *Campylobacter* species have been isolated from all food-producing animals and pets, poultry products contaminated with *C. jejuni* remain the main source for human infection (Epps *et al.,* 2013). Therefore, the present study focused on studying the prevalence of *C. jejuni* and *C. coli;* the two most prevalent thermophilic *Campylobacter* in poultry in the Bikaner region.

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MATERIALS AND METHODS

Sample Collection: A total of 370 cloacal swab samples were collected from local poultry farms in and around Bikaner in sterilized container with ice pack and processed within 1h. In the 1st phase of sampling from October 2014 to March 2015, 220 cloacal swabs and in the 2nd phase from October 2015 to February 2016, 150 cloacal swabs were analyzed for thermotolerant *Campylobacter spp. i.e., C. jejuni* and *C. coli*.

Isolation and Identification of *Campylobacters*: *Campylobacter* enrichment broth base (Preston enrichment broth base, Himedia) supplemented with *Campylobacter* supplement IV (Himedia) and 7% lysed horse blood were used for pre-enrichment. The swabs were inoculated in Preston enrichment broth and incubated under micro-aerophillic conditions (reduced O_2 level and 5% CO_2) at 42ºC for 48 h. A loopful of inoculated broth was streaked onto the modified charcoal cefoperazone desoxycholate agar (mCCDA) having *Campylobacter* supplement V (Himedia) and the plates were incubated under same micro-aerophillic conditions. Presumptive colonies with consistent morphology of *Campylobacter spp*. were subcultured, Gram-stained, examined for

motility and also subjected to further biochemical tests such as catalase, oxidase, urease production, nitrate reduction, hippurate hydrolysis, H_2S production in triple sugar iron (TSI) agar, and growth at 25ºC and 42ºC (Ertas *et al.,*2004; Lastovica and Allos, 2008). All the isolates were stored by dispensing lawn of colonies from selective agar to 1ml brain heart infusion broth with 15-20% glycerol in 1.5 ml cryo-vials and stored at -80ºC till further use.

DNA Extraction from Isolates: DNA extraction was done as per the protocol of Ertas *et al.* (2004) with some modifications. Briefly, lawn of culture from agar plate was dispensed in 400 µl phosphate-buffered saline (PBS), vortexed and centrifuged at 12000rpm for 5 min. Supernatant was discarded and the pellet was resuspended in 375 µl Salt–Tris EDTA (STE) buffer and incubated at 55ºC for 4 h with intermittent vortexing every 30 min. After incubation, equal volume of phenol was added and shaken vigorously and centrifuged at 12000rpm for 10 min. The upper phase was transferred into another Eppendorf tube. Genomic DNA was precipitated with absolute ethanol and 0.3 M sodium acetate at -20ºC for 1 h or 4ºC overnight, centrifuged at 12000rpm for 10 min and pellet was washed twice with 90% and 70% ethanol, respectively; each step was followed by 5 min of centrifugation. DNA pellet was suspended in 100µl sterile distilled water or Tris-EDTA buffer and stored at -20^{0}C till used.

Genotypic Confirmation: The genus *Campylobacter* was confirmed on the basis of a 16S rRNA gene based PCR (Linton *et al.,* 1996; Tang *et al.,* 2009). Identification of species was done by PCR based amplification of *hip*O gene and *asp* gene for *C. jejuni* and *C. coli*, respectively (Table 1) using standard conditions (Linton *et al.,* 1997; Amri *et al.,* 2007). The primer pairs used and the PCR conditions used for amplification of *Campylobacter* are given in Table 1.

RESULTS AND DISCUSSION

A total of 370 cloacal swab samples from poultry were collected and processed for isolation of thermotolerant *Campylobacter* in two phases. Out of these, 75 isolates (35 in Phase I and 40 isolates in Phase II), were presumed to be thermotolerant *Campylobacter* on the basis of greyish dew drop colonies along the streak on mCCDA, Gram's negative character with spiral or 'S' shaped curved bacilli and corkscrew type motility. All these isolates were oxidase and catalase positive, hydrolysed hippurate and reduced nitrate; the basic biochemical tests used for the confirmation of *Campylobacter spp.* None of the isolates was positive for urease and H_2S production. All the isolates failed to grow at 25°C.

Out of 75 isolates, subjected to genus specific 16S rRNA based PCR confirmation, only 43 (21 from phase I and 22 from phase II) isolates produced an amplicon of 816bp. These 43 *Campylobacter spp*. were further subjected to species specific PCR by targeting hippurase (*hip*O) gene for *C. jejuni* and asperkinase (*asp*) gene for *C. coli.* All the 43 isolates were confirmed to be *C. jejuni* as a band of 735bp specific for *C. jejuni* was obtained in all isolates (Fig 1). *hip*O gene is highly conserved in *C. jejuni* and is absent in other *Campylobacter* species (Amri *et al.,* 2007). Similarly, *asp* gene is only present in *C. coli* (Linton *et al.,* 1997). None of the isolates from this study showed a band specific for *C. coli*. These genes are most widely used genes for the identification of *C. jejuni* and *C. coli,* respectively. Similar to our study, Modi *et al.* (2015) also didn't confirm any of the isolate to be *C. coli.* Vaishnavi *et al.* (2015) also detected lower prevalence of *C. coli.* However, Marinou *et al.* (2012) isolated *C. coli* (14/860) from chicken carcass and did not identify any *C. jejuni*.

Thus, the overall prevalence of *Campylobacter spp*. was 11.23% with all the isolates being *Campylobacter jejuni* in the present study. Similarly, low prevalence (4.9-13.9%) of *C. jejuni* was previously reported by Shweta *et al.* (2009), Rizal *et al.* (2010) and Cortez *et al.* (2006) from poultry and human. On the contrary, Zhao *et al.* (2001) and Marotta *et al.* (2015) detected comparatively higher prevalence (65.7-70.7%)

Table 1
Primers used for amplification of *Campylobacter* spp., *C. jejuni* and *C. coli*

Targeted gene	Primer Sequence 5'-3'	Tm (°C)	Target size (bp)
Campylobacter genus specific gene	F: GGATGACACTTTTCGGAGC	55	816
	R: CATTGTAGCACGTGTGTC		
C. jejuni specific (hipO) gene	F: GAAGAGGGTTTGGGTGGTG	64	735
	R: AGCTAGCTTCGCATAATAACTTG		
C. coli specific (asp) gene	F: GGTATGATTTCTACAAAGCGAG	60	500
	R: ATAAAAAGACTATCGTCGCGT		

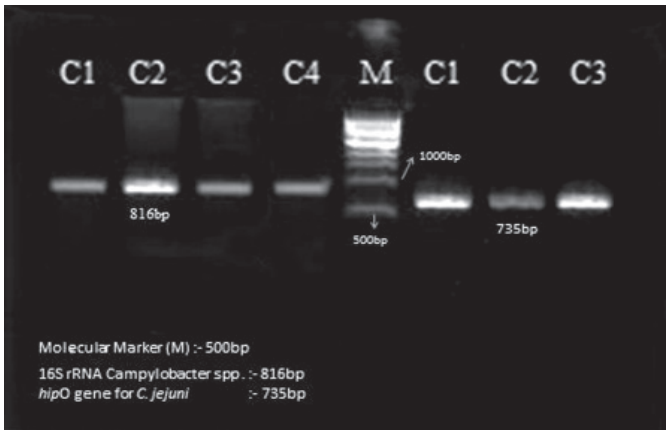

Fig 1. PCR amplification of 16S rRNA *Campylobacter* spp. specific gene and *C. jejuni* species specific (*hip*O) gene.

of *C. jejuni* from various poultry samples. None of the isolates in this study belonged to *C. coli*, further study with more number of samples are required to ascertain its presence.

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