Full Length Article

DETECTION OF *Campylobacter coli* BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FROM BROILER CHICKENS

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ABSTRACT

Campylobacter spp. are the most common cause of foodborne illnesses and bacterial enteritis across the globe. Poultry harbours this pathogen the most and transmit them to human beings. Being the fastidious organism, isolation and detection of Campylobacter is time consuming and difficult. In this study, the most common species - Campylobacter coli was detected using Loop Mediated Isothermal Amplification assay (LAMP). A total of 191 broiler chicken caecal samples, after presumptive identification of Campylobacter, were subjected to LAMP assay with colorimetric detection. Out of 191 samples collected, 14 were found positive for C. coli. LAMP assay takes only around 45 min after extraction of DNA to detect C. coli. LAMP assay aids in rapid detection of C. coli isolates and does not require any sophisticated instruments. Thus, LAMP assay is a simple and rapid detection method for Campylobacter coli can be performed in any basic clinical laboratory.

Keywords: *Campylobacter coli*, Caecal samples, LAMP assay, Rapid colorimetric detection

Received : 21.02.2022 Revised : 29.04.2022 Accepted : 05.05.2022

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INTRODUCTION

Campylobacter spp. are the frequent cause of acute bacterial gastro-enteritis all over the world. Processing and consumption of poultry meat plays a major role in bacterial transmission (El-Shibiny *et al.,* 2009). Heavy load of *Campylobacter jejuni* and *C. coli* in broiler chickens is a major risk

factor for campylobacteriosis. Among the *Campylobacter* spp., *Campylobacter coli* accounts for about 25% of all *Campylobacter* related diarrheal diseases. The infection can lead to complications like septicemia, meningitis and abortion in humans. The frequency of antimicrobial resistance is also found to be higher in *C. coli*. (Elhadidy *et al.,* 2019). In spite of this, *C. coli* infections are often neglected, so it requires different strategy for control comparing to other species of campylobacters (Tam *et al.,* 2003).

Though *Campylobacter* spp. are common among the avian hosts, higher colonization was found in commercially reared poultry. High density of birds in commercial poultry farms are considered as the important reason behind this high colonization rate (Shane, 1992). Among the various domesticated poultry species, consumption of chicken meat contributes to 50% to 70% of the total human infections (Acheson and Allos, 2001).

Campylobacter spp. being the slow growing and fastidious organism, the conventional diagnostic procedures that detect the organism are often time consuming and laborious. One of the novel methods that overcomes these disadvantages and aids in rapid detection is Loop Mediated Isothermal Amplification (LAMP) assay. This technique was developed by Notomi *et al* in 2000, that uses four set of primers to identify six regions in target gene. In this study LAMP assay was used for the detection of *Campylobacter coli* from broiler chicken samples collected from five different districts of Tamil Nadu.

MATERIALS AND METHODS

Samples

A total of 191 chicken intestines were collected from various chicken slaughter houses of Chennai (n=47), Namakkal (n=36), Krishnagiri (n=36), Erode (n=36) and Coimbatore (n=36) districts of Tamil Nadu. Samples collected from Chennai were immediately transported to the laboratory and processed. Caecal mucosa scrapings were collected from districts other than Chennai in blood free *Campylobacter* broth and transferred to laboratory the next day.

Isolation of *Campylobacter* spp.

A loop full of caecal mucosa scraping was taken from the collected samples and streaked directly on the sterile blood free *Campylobacter* broth base with 3 % agar agar type 1, *Campylobacter* growth supplement IV and CCDA supplement and the plates were incubated at 42°C for 48 hours under microaerophilic condition. The suspected colonies were subjected to dilute carbolfuchsin staining and biochemical tests like catalase and oxidase for initial identification of *Campylobacter.*

Preparation of non-*Campylobacter* bacterial isolates

One isolate each of *Escherichia coli, Staphylococcus aureus*, *Salmonella spp.* and *Clostridium perfringens* was revived from repository of Central University Laboratory, TANUVAS, Chennai using suitable media and culture conditions. DNA from these isolates were extracted and subjected to LAMP assay specific for *Campylobacter coli* to check

the cross reactivity of primers among these common enteric pathogens.

Extraction of bacterial DNA

A loop full of pure culture colony was taken in 400 µl of nuclease free water and boiled at 100°C for 10 min in a micro centrifuge tube and immediately transferred to ice kept at -20°C. Then the suspension was centrifuged at 10000 rpm for 10 min and the supernatant was used as template in LAMP assay (Chitra *et al.,* 2015).

Loop Mediated Isothermal Amplification (LAMP) assay

The LAMP assay was performed using published primers (Yamazaki *et al*., 2008) in 10µl reaction volume containing 100-150 ng of DNA and *Campylobacter coli* specific LAMP primers containing 16 pmol each of inner primers FIP and BIP, 5 pmol of outer primers F3 and B3 and 5 pmol of loop primers F and B in 2X colorimetric LAMP master mix (New England BioLabs, London, UK, catalogue #M1800). The above mixture was incubated at 65 ^{0}C for 30 min in a water bath. Positive reactions were indicated by colour change to yellow and negative reactions were indicated by change in colour to pink (Lai *et al.,* 2020). Details of the LAMP primers are given in Table 1.

RESULTS

Bacterial isolates

Out of 191 samples streaked on to the blood free *Campylobacter* media, 61 samples showed the transparent watery colonies. On staining with dilute carbolfushsin, 48 out of 191 showed *Campylobacter* specific seagull morphology and 61 isolates were positive for biochemical tests like catalase and oxidase.

LAMP assay for *Campylobacter coli*

Out of 191 samples collected, 14 were positive for *Campylobacter coli* by LAMP assay (Figure 1). Out of 14 positive samples, 1 belonged to Namakkal and the other 13 belonged to Chennai district of Tamil Nadu. Colorimetric mastermix used in this study helps in visual detection of the LAMP results. The LAMP assay was very useful to detect the *Campylobacter coli* rapidly as well as without any use of sophisticated instruments.

Specificity of the LAMP assay

In this study, when the same LAMP assay was used for the *Escherichia coli, Salmonella* spp., *Clostridium perfringens* and *Staphylococcus aureus* isolates, none of the isolate was found to be positive (Table 2). So, this LAMP primers do not have any cross reactivity towards the most common enteric pathogens (Figure 2).

DISCUSSION

Yamazaki *et al.* (2009) used the LAMP assay to detect *C. jejuni* and *C. coli* in naturally contaminated chicken meat. Sensitivity and specificity of the assay were found to be 98.5% and 97.4%, respectively, when compared to the conventional culture test. They found that *C. jejuni* and *C. coli* were detected even in some culture negative samples by LAMP assay. Hence, this assay is very helpful in the detection of *Campylobacter coli* in contaminated chicken and its products.

Table 1. Primers used for LAMP assay of *Campylobacter coli*

Primer	Primer Sequence	Reference
CC FIP	AAGAGATAAACACCATGATCCCAG TCATGAATGAGCTTACTTTAGC	Yamazaki *et al.* (2008)
CC BIP	GCGGCAAAGACTTATGATAAAGC TACCGCCATTCCTAAAACAAG	
CCF3	TGGGAGCGTTTTTGATCT	
CCB3	AATCAAACTCACCGCCAT	
CCLF	CCACTACAGCAAAGGTGATG	
CCLB	CCACGATAGCCTTTATGGA	

Table 2. Details of non-*Campylobacter* isolates used in the LAMP assay

S.No.	Name of the organism	No. of isolated tested	No. of Positive isolates
1	*Escherichia coli*	2	0
2	*Staphylococcus aureus*	1	0
3	*Clostridium perfringens*	1	0
4	*Salmonella* spp.	1	0

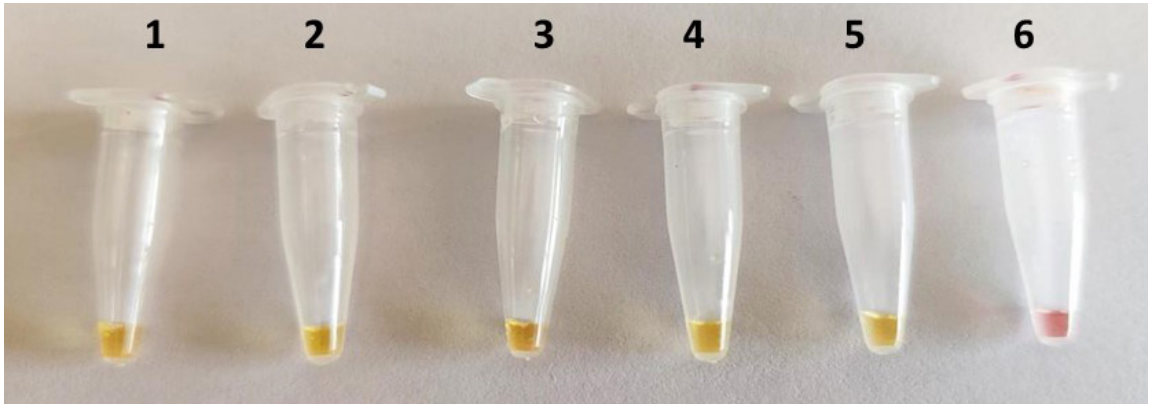

Fig. 1. Results of the Loop Mediated Isothermal Amplification assay for *Campylobacter coli*

Tubes 1 to 5: *Campylobacter coli* positive isolates; Tube 6: Negative template control
Yellow: Positive, Pink: Negative

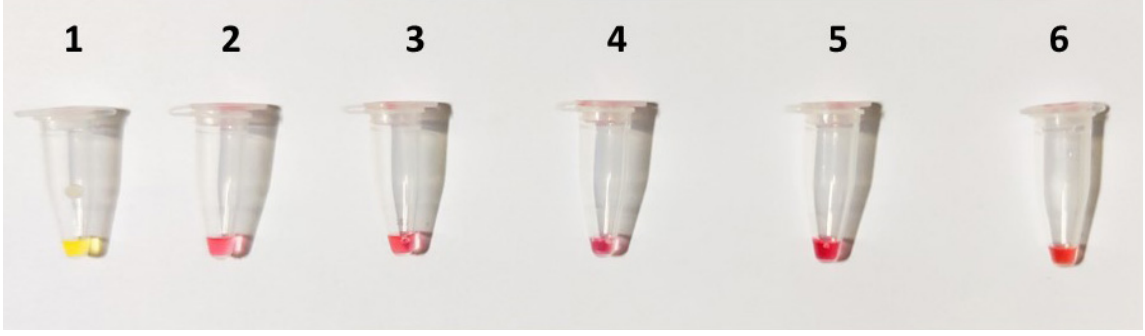

Fig. 2. Results of the LAMP assay for non-*Campylobacter* spp.

Tube 1: *Campylobacter coli*; 2:*E. coli*, 3: *Clostridium perfringens;*4: *Staphylococcus aureus;*5: *Salmonella* spp.; Tube 6: Negative template control
Yellow: Positive, Pink: Negative

Romero and Cook (2018) developed a LAMP assay targeting the 16S RNA of *Campylobacter* spp. This method was best suited for screening *Campylobacte*r at farms, poultry processing units or at slaughter house and aids in control of *Campylobacter* throughout the food supply chain. This shows that LAMP detection can be useful for screening *Campylobacter* bacteria even at field level.

Warm start colorimetric master mix was used in the present study to visualise the results which prevent aerosol contamination as there was no need to open the test reaction tube whereas Yamazaki *et al.* (2008) detected the results based on the turbidity. Colorimetric LAMP master mix was also used to diagnose malaria (Lai *et al.,* 2020) and to detect the pathogenic *Campylobacter* in human (Babu *et al.,* 2020).

Babu *et al.* (2020) designed degenerate primers against the 16S rRNA sequences for *C. jejuni, C. coli, C. lari, C. upsaliensis, C. ureolyticus, C. fetus, C. gracilis, C. rectus* and *C. concisus* for LAMP assay. The developed assay was found to be sensitive for *Campylobacter* and does not cross react with other enteric pathogens. So, this assay also helps in differentiating *Campylobacter* from other enteric pathogens that cause food poisoning.

CONCLUSION

The LAMP assay has more advantages over PCR-based techniques such as shorter reaction time, no need of sophisticated instruments, high sensitivity and specificity, and low susceptibility to inhibitors present in samples. Therefore, detection of the *Campylobacter coli* pathogens in the samples by LAMP assay will be very easy and rapid.

AKNOWLEDGEMENT

Authors thank the Vice-Chancellor, TANUVAS for providing the infrastructure to carry out this research.

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