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CONTAMINATION LEVELS OF *Campylobacter* spp. IN CHICKEN MEAT

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Abstract: The present study was devised to investigate the contamination levels of *Campylobacter* spp. in poultry meat collected at retail outlets of Chennai city as this pathogen is a leading cause of enteric diseases throughout the world. A total of 327 samples from chicken meat consisting of various portions like breast, thigh and bone marrow were screened by conventional plating in Park and Sanders agar with incubation at 42°C for 48 hours under microaerophilic conditions. The characteristic colonies were confirmed by Polymerase chain reaction targeting *fla* and *ceu*E gene for *Campylobacter jejuni* and *Campylobacter coli* respectively. Out of 327 chicken meat samples, 218 showed typical phenotypic characteristics of *Campylobacter* species and 83 of them were found to be positive by PCR. *Campylobacter jejuni* was present in 72 (86.74%) and *Campylobacter coli* in 59 (71.08%) out of the 83 *Campylobacter* isolates. Breast meat showed higher prevalence (68.67%) than thigh meat (31.32%) where as none were isolated from bone marrow.
Keywords: *Campylobacter* spp., Prevalence, chicken meat, Chennai.

INTRODUCTION

Campylobacter spp. are among the most common causes of enteric bacterial diseases in humans throughout the world (Taylor and Blaser, 1991). *Campylobacter jejuni* and *Campylobacter coli* are the two species most frequently involved in human infections. Campylobacteriosis is characterized by diarrhea, fever and abdominal cramps which may sometimes proceed towards debilitating sequelae with reactive arthritis and Guillain–Barre' syndrome (Yuki *et al*., 2004). Poultry are considered as the major reservoirs of *Campylobacter* spp. thus, most of the infections are acquired by the handling and consumption of contaminated poultry (Acheson and Allos, 2001). Keeping the above facts in view, the present study was undertaken to determine the prevalence of *C. jejuni* and *C. coli* among broilers post slaughter in chennai, India.

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MATERIALS AND METHODS

A total of 327 chicken meat portions which included 138breast, 99thigh and 90bone marrow were aseptically collected and *Campylobacter* spp. was isolated by inoculating them in Park and Sanders agar following the protocol of Indumathi, (2016). Characteristic *Campylobacter* colonies *i.e*. small, mucoid, greyish translucent types were picked up and confirmed by PCR. Isolates were then species identified using the oligo nucleotide primers given in Table 1. Multiplex PCR assay followed by Al Amri *et al*. (2007) was also employed for further confirmation of the isolates along with little modifications.

Table1: Oligo nucleotide primers used in the identification of Campylobacter spp.

Target organism	Target gene	Primer Sequence	Product size	Reference
Campylobacter spp	16S rRNA	GGATGACACTTTTCGGAGC CATTGTAGCACGTGTGTC	816bp	Linton *et al.*,1996
Campylobacter jejuni	*fla*	TCTGCTAAGGCTCCAAGT CTCAAGCGGCTCAAGATG	367bp	Dhanalakshmi,2011
Campylobacter coli	*ceu* E	AATTGAAAATTGCTCCAACTATG TGATTTTATTATTTGTAGCAGCG	462bp	Begum *et al*., 2015

RESULTS AND DISCUSSION

A total of 218 samples showed characteristic greyish translucent colonies on the Park and Sanders growth media (Fig. 1). The overall prevalence of *Campylobacter* spp. using the Gold standard conventional isolation (Hindiyeh, 2000) was found to be 66.67% with breast meat having a high prevalence of 61.47% followed by thigh meat (34.4%) and bone marrow 4.12%. The contamination levels of this study (66.67%) are in accordance with the studies conducted by Kottawatta *et al*. (2017) who showed prevalence of *Campylobacter* spp. to be 59%.

Fig.1: Presumptive *Campylobacter* spp. colonies in Park and Sanders Agar

Tang *et al*. (2009) has stated that such high occurrence might be due to improper handling, contaminated water and cross contamination in various stages of chicken processing as well as packaging. Isolation rates from breast meat (61.47%) are similar to the study done by Luber and Bartelt (2007) which showed that 87% of the breast fillets were contaminated and that was attributed to cross contamination from contaminated chicken. Thigh muscle contamination levels were estimated to be 34.4% where as a study conducted by Sallam in 2007 has stated that the prevalence was around 70% in chicken thighs which could probably due to their anatomical proximity to the final part of the digestive tract (Mezher *et al*.,2011). Of all the 218 presumptive isolates, 83 samples (38.07 percent) were found positive by 16S rRNA (Fig. 2) however this is in contrast to the statement by Mateo *et al*. (2005) who regards PCR to be an accurate and sensitive molecular method for detection and confirmation of *Campylobacter* in food samples with their study proving to have 100percent of positive coincidence.

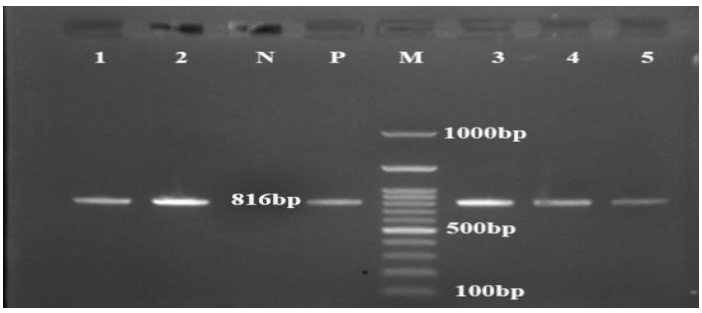

Fig. 2: Molecular characterization of *Campylobacter* spp. by PCR targeting 16srRNAgene

M- 100bp ladder, P-Positive control, N-Negative control, Samples: Lane 1-5

Polymerase Chain Reaction further showed that 22.01% of isolates being positive for *C.jejuni* (Fig. 3) and 18.07% samples positive for *C.coli* (Fig. 4). In the multiplex PCR (Fig. 5), most of the *Campylobacter* isolates (67.47%) obtained were found to be *C.jejuni* (56 out of 83) where as *C.coli* in only 7 out of the 83 isolates(8.43%). A mixed population of *C.jejuni* and *C.coli* was identified in 17 isolates.

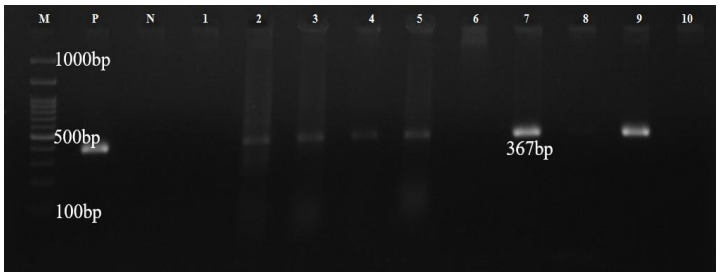

Fig. 3: Screening of *C.jejuni* targeting *fla*A gene
Product size -367bp; P-Positive control, N-Negative control, Positive samples-2, 3, 4, 5, 7 and 9, M-100bp ladder

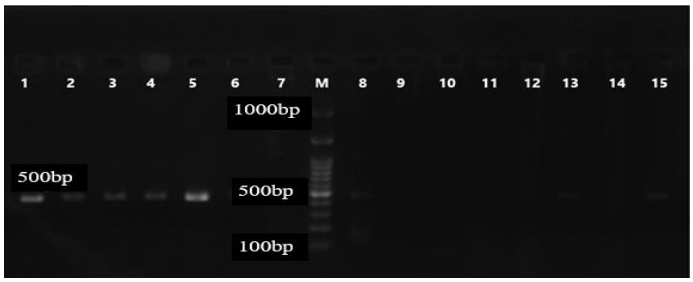

Fig. 4:Screening of *C.coli* using *ceu*E gene

Product size-500bp;M-100bp ladder,**1,2,3,4,5, 8,13,15– Positive samples**

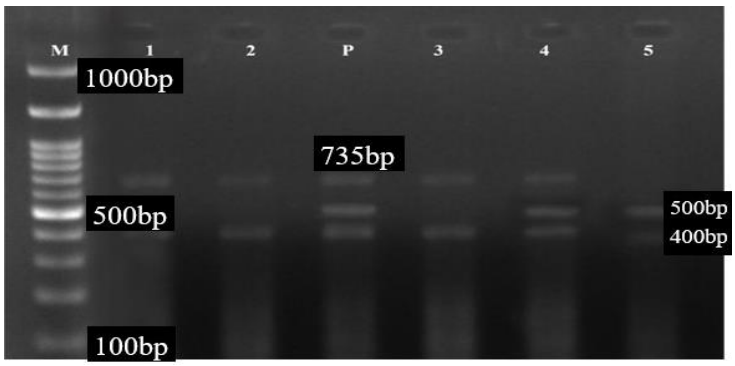

Fig. 5: Differentiation of *Campylobacter* spp. by Multiplex PCR
P-positive control, Samples –Lane 1 to 5, M- 100bp Ladder
C.jejuni –Lane 1,2,3,4; *C.coli* –Lane 4,5 and *Campylobacter* spp.-Lane 1 to 5

The prevalence recorded by PCR is lesser than that recorded by Culture Techniques. It could be due to the fact that all the colonies observed in conventional culture method are only presumptive and not confirmatory. Even in Polymerase Chain reaction, simplex PCR is found to be more sensitive than multiplex PCR which again concludes the fact that multiplex PCR can only be used for convenience and rapid detection and the Simplex PCR gives the true picture on the confirmation of an organism.

CONCLUSION

The routes in which the enteric microflora *Campylobacter* is introduced and spread between poultry and its meat are still largely uncertain. This can present a problem for poultry meat producers in terms of how to minimise early contamination. Though producers and retailers can be made to take steps to safeguard public health by introducing their own checks for *Campylobacter* along their production processes, their approach should include the entire supply chain, to work against the spread of *Campylobacter* from farms right through to the consumer.

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