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DETECTION OF *ESCHERICHIA COLI* O157:H7 AND *STAPHYLOCOCCUS AUREUS* IN BROILER MEAT AVAILABLE IN LOCAL MARKETS OF WAYANAD, KERALA

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Abstract: Poultry has been playing very important role for the human food supply throughout the globe. Current study was undertaken for a period of four months from August 2015 to October 2015 to detect the presence of *Escherichia coli* O157:H7 and *Staphylococcus aureus* in broiler meat available in local markets of Wayanad byusing conventional cultural and biochemical identification and standardized molecular detection protocols using PCR technique. A total of 40 broiler meat samples from 20 different local markets of Wayanad district, Kerala were analysed. The overall incidence of *E. coli* was 55%, of which incidence of pathogenicstrain *E. coli* 0157 was nil. *S. aureus* was isolated from 90% samples. High incidence of *Staphylococcus aureus* in meat suggests the need for adopting strict hygienic measures in its production to prevent public health hazards.
Keywords: Poultry, wayanad, *E. coli O157:H7, S. aureus.*

Introduction

Poultry is a major fast growing source of meat in the world today (Kearney, 2010). Because the poultry meat and its products are cheaper than other meatsthey are widely accepted and consumed in all parts of the world (Guerrero-Legarreta, 2010). Poultry meat is also one of the cheapest available sources of animal protein for urban Indian consumers because large commercial integrators. Chicken can make many positive contributions to the diet of those on low incomes (David Farrell, 2015). The magnitude ofthe public health burden due to resistant foodborne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water and proper cooking and home hygiene, among others. *Escherichia coli* O157:H7 are mostly associated to contaminated food materials. The low infectious dose and life-threatening complication of these organisms in humans has made *Escherichia coli* O157:H7an important pathogen and serious threat to public health (Akkaya *et al*., 2006). *Staphylococcus aureus* is one of the leading microorganisms associated with

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food poisoning causing outbreaks (Altabari and Al-Dughaym, 2002). The presence of *S. aureus* in meat is often attributed to inadequate hygiene during handling by the individuals involved in the production of meat (Hatakka *et al.,* 2000).

Hence present study was designed to analyse the presence of *E. coli* and its pathogenic strain O157: H7 and *Staphylococcus aureus* in broiler meat available in local markets of Wayanad district, Kerala which will further help in designing future food safety control strategies.

Material and Methods

Sample collection

A total of 40 broiler meat samples were collected and transported to laboratory under chilled condition and were immediately processed.

Detection of *E. coli* and *S. aureus*

The standard protocol described by Meng *et al*. (2001) was used for the isolation of *E. coli* with slight modifications from poultry meat samples. On EMB agar, the colonies with characteristic blue black colour with a distinct green metallic sheen were inoculated onto nutrient agar slants and incubated at 37^0C overnight. The single pure colony of the recovered isolates were further subjected to biochemical characterization, for *E. coli* O157:H7 procedure described by Fujisawa *et al*. (2000) was used and PCR confirmation was carried out.Standard protocol described by Lancette and Bennett (2001) was used for the isolation of *S. aureus.*

Details PCR

The VTEC and *S. aureus* isolated from retail poultry meat samples were further subjected to PCR confirmation as per the procedure described by Brakstad *et al.* (1992) and Paton and Paton (1994) with slight modifications. Details of PCR were mentioned in Table 1 and 2.

Agarose gel electrophoresis and visualization

Gel electrophoresis was carried out using 1.5 per cent agarose (GeNei™, Mumbai) gel containing ethidium bromide with a submarine gel electrophoresis system (Chromous biotech Pvt. Ltd).

Results and Discussion

From 40 broiler samples evaluated from local markets 55% (n=22) were positive for *E. coli* by culture and after biochemically confirmation however on PCR amplificationnone showed the presence of genes (*eaeA* and *stx1*) specific for Verotoxic *E. coli.* All *E. coli* isolates showed fluorescence under long wavelength UV light when cultured on MUG agar (Fig. 01).Absence of *E. coli*O157 in the study correlates with the study conducted by Rathore *et al.*

(2010) at IVRI on beef and beef products. Kumar *et al.* (2011) at UP, India observed 38. 18% (42/110) chicken meat samples were contaminated with *E. coli*which was similar to the present findings.

A total of 40 broiler meat samples were analysed for detecting *S. aureus* from different local markets and the occurrence rate was found to be 90% (n=36). PCR assay for the confirmation of *S. aureus* from broiler meat was standardized employing a set of primers of *nucA* gene and the electrophoretic analysis of the PCR product revealed the specific amplification of a 278 bp (Fig.02).

Table 1. Components of PCR reaction mixture

Reagent	**Concentration**	**Quantity**
Template DNA	1 µg	~2.50µl*
PCR buffer (10X)	1 X	2.00 µl
$MgCl_2$	1.5mM	1.5 µl
Taq DNA polymerase (1U/ µl)	1 U	1.00 µl
dNTP Mix (10 µM)	0.2 µM	0.50 µl
Forward primer (10 µM)	0.2 µM	0.50 µl
Reverse primer(10 µM)	0.2 µM	0.50 µl
Nuclease free water	To make total volume	
Final reaction volume	25	25 µl

* Volume varies according to template concentration

Table 2. Cycling conditions used for various gene amplification in PCR

Process	**Temperature**		**Duration**
Initial denaturation	94 ^{0}C		5 min
Denaturation	94 ^{0}C		50 sec
Annealing	*S. aureus*	*nuc*:58^{0}C	50 sec
	E. coli O157:H7	*eae*A: 61^{0}C	
		*stx*1: 58 ^{0}C	
Extension	72 ^{0}C		50 sec
Repeat the cycles from denaturation to extension for 35 times			
Final extension	72 ^{0}C		5 min
Hold	4 ^{0}C		Infinity

Table 3. Occurrence of *E. coli*,*E. coli* O157:H7 and *S. aureus* in different market

Sl. No	Market Location	No. of samples collected	No. of samples positive for *E. coli*	No. of samples positive for *E. coli O157:H7*	No. of samples positive for *S. aureus*
1	Chundale	08	03	ND	07
2	Kalpetta	06	02	ND	04
3	Kovoor	04	02	ND	03
4	Meenangadi	08	07	ND	08
5	Mepadi	06	02	ND	06
6	Vythiri	08	06	ND	08
Total		**40**	**22 (55%)**	ND	**36 (90%)**

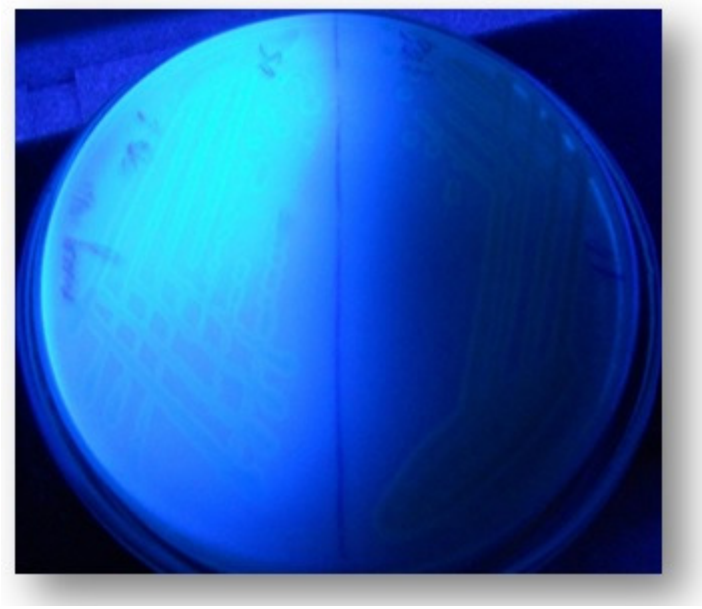

Fig 1: MUG agar inoculated with O157(Nonfluorescent colonies) and Non O157 (Fluorescent colonies) *Escherichia coli* and exposed under low wave length UV light

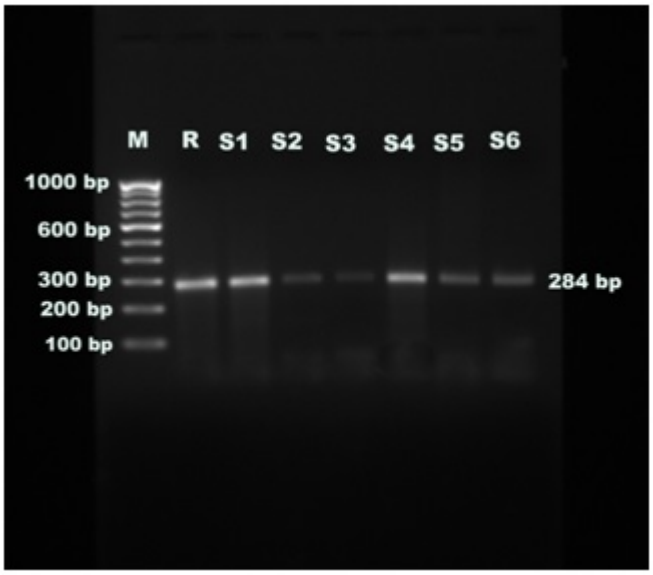

Fig 2. Gel electrophoresis of *S. aureus nucA* gene.
M: Marker, R: Reference, S1-S6: Positive isolates

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