Haryana Vet. (June, 2023) **62(1),** 26-29 ISSN 0033-4359 (P) **Research Article**

MATRIX ASSISTED LASER DESORPTON IONIZATION TIME OF FLIGHT MASS SPECTROMETRY (MALDI TOF MS) FOR STRAIN OF PATHOGENIC *E. coli* FROM FRESH MEATS

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ABSTRACT

The current study was undertaken to target the most common food pathogen, *Escherichia coli* from fresh meats. The organism was isolated, identified and characterized following standard isolation protocol from a total of 425 samples comprising chicken (120), mutton (100), chevon (105), and carabeef (100) procured from Udgir city and nearby villages/towns revealing the overall occurrence of 9.17% while, sample group-wise occurrence of *E. coli* in chicken, mutton, chevon, and carabeef samples was in the tune of 12.50%, 09.00%, 09.52%, and 05.00%, respectively. The PCR analysis of *E. coli* isolates for virulence markers was carried out by targeting two virulence-associated genes *viz*. temperature-sensitive haemagglutinin (*tsh*) and colicin V plasmid operon gene (*cvi*) with a product size of 824 bp and 1181 bp, respectively. A total of 25 (64.10%) isolates were positive for both virulence-associated genes, 4 (10.25%) were positive for only *cvi* and 2 (5.13%) isolates were positive for *tsh* only. Among chicken isolates, 12/15 (80.00%) harboured both *cvi* and *tsh* genes. Application of Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) revealed confirmation of 17 presumptive *E. coli* isolates up to species level and 22 isolates at genus level with probable species identification as *E. coli* out of 39 phenotypically characterized isolates. According to a database of standard strains of MALDI Biotyper, *E. coli* were classified as DSM 1576 DSM (22) followed by DH5 BRL (6), RV412_A1_2010_06_aLBK (4), DSM 682 DSM (3), MB11464_1 CHB (2), ESBL (1) and B421 UFL (1). The MALDI-TOF MS provided precise genus level confirmation of all 39 phenotypically characterized isolates demonstrating its superiority over the PCR technique.

Keywords: *E. coli*, MALDI-TOF MS, PCR, Strain typing

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Food-borne diseases, caused by agents that enter the body through the intake of contaminated food materials are one of the primary public health concerns (Tan *et al.*, 2013). Food-borne infections caused by germs such as *Escherichia coli* place a significant strain on healthcare systems in many developing nations, and can significantly diminish the countries' economic productivity. *E. coli* is a faecal contamination indicator, and the presence of *E. coli* isolates in food implies the presence of other enteric pathogens. This bacterium can be spread to humans via contaminated food, water, direct contact with animals, and human-to-human transfer, and it can cause serious illnesses (Chinen *et al.*, 2009; Karmali *et al.*, 2010; Baker *et al.*, 2016). Animal-derived foods (meat and meat products) have played a key role in the spread of *E. coli* infections in humans. For example, beef, chevon, pork, chicken, and/or their products, have been linked to foodborne illness in the United States and Europe (CDC, 2010, CDC, 2014, EFSA, 2018).

The present investigation was carried out with the objective to assess the occurrence of *E. coli* organisms in fresh meats sold in local markets of Udgir city of Maharashtra. The recovered isolates were characterized by employing polymerase chain reaction (PCR) for confirmation and further Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) for strain typing.

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MATERIALS AND METHODS

Isolation and *in-vitro* pathogenicity test of *E. coli*: In this investigation, isolation and identification of *E. coli* was carried out as per the FDA-BAM method described by Rivas *et al.* (2015). The organism was isolated, identified, and characterized following standard isolation protocol from a total of 425 samples comprising of chicken (120), mutton (100), chevon (105), and carabeef (100) collected from Udgir city and nearby villages/towns. All *E. coli* isolates were further characterized by *in-vitro* pathogenicity tests and PCR for virulence-associated genes. Pathogenicity characteristics of the *E. coli* isolates in the form of the ability to produce haemolysin tested on Congo red agar, as per the technique suggested by Berkhoff and Vinal, (1986) and 5% defibrinated sheep blood agar, as per the protocol described by Beutin *et al.* (1989) revealing exhibition of positive reaction by 28 isolates (71.79%).

Polymerase Chain Reaction: The duplex PCR reaction for characterization of two virulence genes *viz*. temperature-sensitive haemagglutinin (*tsh*) and colicin V plasmid operon gene (*cvi*) with a product size of 824bp and 1181bp, respectively were standardized using earlier published primers described by Ewers *et al.* (2005), (as shown in table 2). The PCR reaction was attempted for standardization for field isolates of *E. coli* using a MTCC culture of *E. coli* as a

positive control. The protocol earlier standardized in the laboratory was followed with little modification as per the annealing conditions of primers used. The optimal amount of template decided by Nanodrop was added to the reaction mixture containing 2.0 l of each primer pair (20 pmol each) and 12.5 l of 2X Taq green master (New England Biolabs inc.). The remaining portion was filled with auto-claved DW up to 25 l. The cycling conditions of PCR targeting both the genes were as follows: 30 cycles of 940 for 5 mins, 940 for 30 sec, 600 for 1 min 30 sec, and extension at 720 for 3 mins followed by a final extension at 720 for 10 mins.

MALDI-TOF MS: Presumptive one or two pure colonies of *E. coli* isolates directly picked up from freshly grown Brain Heart Infusion (BHI) agar were suspended in 300 l of molecular grade nuclease-free water (Sigma-Aldrich Inc.) and vortexed briefly. Then, 900 l of absolute ethanol was added, vortexed, and centrifuged at 20,800×g for 3 minutes. The supernatant was decanted and the pellet was dried at room temperature. Further, 50 l of 70% formic acid and 50 l of acetonitrile were added and mixed by pipetting followed by centrifugation at 20,800×g for 2 minutes. An amount of 2 l of supernatant was applied to the 24-spot plate and allowed to dry at room temperature followed by the addition of 2 l of MALDI matrix (a saturated solution of cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). For each plate, a bacterial test standard provided by Bruker Daltonics was included to calibrate the instrument and validate the run. Spectra were analyzed using MALDI Biotyper automation control and the Bruker Biotyper 2.0 software and library (version 2.0, 3,740 entries; Bruker Daltonics). The identification score criteria were performed as recommended by Bruker Daltonics which evaluated as per the protocol described by Shell *et al.* (2017), wherein a score of 2.000 indicated species-level identification, a score of 1.700-1.999 indicated identification to the genus level, and a score of 1.700 was interpreted as no identification.

RESULTS AND DISCUSSION

On screening of 425 samples, of fresh meats comprising chicken (120), mutton (100), chevon (105), and carabeef (100) collected from Udgir city and nearby villages/towns. The overall occurrence of *E. coli* was depicted to the tune of 9.17% and corresponding sample category wise occurrence 12.50, 09.00, 09.52 and 05.00% in chicken, mutton, chevon, and carabeef samples, respectively indicating maximum occurrence in chicken samples and minimum in carabeef samples (as shown in table 1). Higher occurrence of *E. coli* in poultry can be corroborated with the results of Zende *et al.* (2013) who reported 16.6% positivity of *E. coli* in retailer's shop

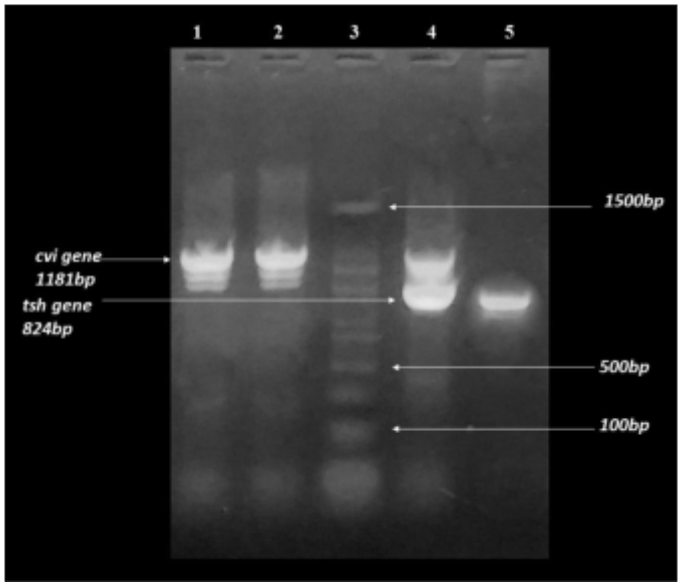

Fig. 1. PCR of *tsh* and *cvi* for *E. coli* ; 1: amplication of *cvi* gene, 2: amplication of *cvi* gene, 3: 100bp ladder, 4: amplication of *cvi* and *tsh* genes, 5: amplication of *tsh* gene

samples in freshly slaughtered chicken meat from retail outlets in Mumbai, and Ema *et al.* (2022) who observed 20% prevalence of *E. coli* in chicken meat samples collected from a different restaurant and fast-food shops of BAU campus and Mymensingh city of Bangladesh. Similarly, Saad *et al.* (2011) also reported the presence of *E. coli* in 12% and 16% of chicken thigh and chicken breast samples collected from different poulterer's and butcher's shops at Cairo, El- Kalyobia, and El-Gharbia governorates of Egypt, respectively. However, the results of lower occurrence of *E. coli* with carabeef samples contradict with reports of Abdel-Rhman *et al.* (2015) who screened a total of 250 meat samples comprising 80 fresh beef, 85 ground beef, and 85 beef burgers collected from supermarkets and butchers' shops in Egypt for *E. coli*, wherein out of 250 samples, 25(10%) were found positive for *E. coli*. The results also disagree with the reports of Adzitey *et al.* (2020) who screened beef samples collected at the Tamale metropolis of Ghana depicting an 86.67% of prevalence of *E. coli* organisms. Further, Nehoya *et al.* (2020) also reported a significant presence of *E. coli* in 41.66% raw beef samples from Windhoek city of Namibia.

E. coli is a bacterium that indicates poor sanitation condition. Moreover, cross-contamination from the environment and other sources, such as improperly cleaned and sterilized cutting boards, post-processing handling equipment, packing material, or harsh storage conditions, may have also contributed to the higher positivity of *E. coli* in poultry meat. On contrary, better hygiene followed at carabeef selling places might have resulted in lower recovery of isolates.

In the present study, on PCR analysis of all 39

Table 1. The overall occurrence of *E. coli*

Sr. No.	Type of sample	Total	Positive isolates	% Positivity	Occurrence of both genes *tsh* & *cvi*	Occurrence of only *tsh* gene	Occurrence of only *cvi* gene
1.	Chicken	120	15	12.50%	12 (80.00%)	01 (06.66%)	01 (06.66%)
2.	Mutton	100	09	09%	05 (55.55%)	—	01(10.11%)
3.	Chevon	105	10	09.52%	06 (60.00%)	—	02 (20.00%)
4.	Carabeef	100	05	05%	02 (40.00%)	01(20.00%)	—
	Total	425	39	9.17%	25 (64.10%)	02 (5.12%)	04 (10.26%)

Table 2. Details of the primers used in the study

Target Gene	PrimerSequence (5' to 3')	Size(bp)	Reference
tsh	F- ACTATTCTCTGCAGGAAGTCR- CTTCCGATGTTCTGAACGT	824	Ewers *et al.* (2005)
cvi	F-TGGTAGAATGTGCCAGAGCAAGR- GAGCTGTTTGTAGCGAAGCC	1181	

Table 3. Classification of *E. coli* isolates resembling standard strains as per the database of MALDI-TOF M

Source	Type of strains						
	DSM1576 DSM	MB11464_1 CHB	RV412_A1_ 2010_06 aLBK	DH5 BRL	DSM 682 DSM	B421 UFL	ESBL
Chicken	10	1	1	-	2	-	1
Mutton	5	1	1	2	-	-	-
Chevon	6	-	-	2	1	1	-
Carabeef	1	-	2	2	-	-	-
Total	22	2	4	6	3	1	1

recovered *E. coli* isolates targeting haemagglutinin (*tsh*) and colicin V plasmid operon gene (*cvi*) with a product size of 824bp and 1181bp, respectively (as shown in Fig. 1), 25 (64.10%) isolates were found positive for both virulence-associated genes, 4 (10.25%) for only *cvi* and 2 (5.13%) isolates were positive for tsh only.The overall distribution of the virulence-associated genes was variable amongst the isolates recovered from different sources of meat. Among chickens, 12 (80.00%) isolates harbored both *cvi* and *tsh* genes and 1 (06.66%) isolate each carried only *cvi* and only *tsh* genes. A total of 05 (55.55%) mutton isolates were positive for both *cvi* and *tsh* genes, while 01(10.11%) was observed positive for *cvi* gene only. Similarly, 06 (60.00%) chevon isolates carried both genes and 02 (20.00%) were positive for *cvi* only. The carabeef isolates harbored both the genes in 02 (40.00%) isolates and 01 (20.00%) for *tsh* gene only.

These results resemble to the findings of Kwon *et al.* (2008), wherein researchers screened 216 field isolates of *E. coli* revealing significant detection rates of virulence-associated genes including *tsh* (94%) and *cvi* (89%) in broilers. They also reported that *tsh* and *cvi* could be considered the essential virulence traits of avian pathogenic *E. coli*.

Pathogenicity characteristics of the *E. coli* isolates in the form of the ability to produce haemolysin tested on congo red agar and 5% defibrinated sheep blood agar revealed that 28 isolates (71.79%) showed positive reaction. Between the groups of samples, maximum pathogenicity was exhibited by isolates recovered from chicken samples (80.00%) followed by mutton samples (77.77%), chevon samples (70.00%), and least by isolates from carabeef samples (40.00%). On correlating these results of *in vitro* pathogenicity assays with that observed in PCR assay, it was noted that 25/28 (89.29%) isolates showed the presence of both *tsh* and *cvi* virulence-associated genes.

Application of MALDI-TOF MS revealed confirmation of 17 presumptive *E. coli* isolates up to species level and 22 isolates at genus level with probable species identification as *E. coli* on screening all 39 phenotypically and genotypically characterized isolates in the present investigation. According to the database of standard strains of MALDI Biotyper, *E. coli* were classified as DSM 1576 DSM (22) followed by DH5 BRL (6), RV412_A1_ 2010_06_aLBK (4), DSM 682 DSM (3), MB11464_1 CHB (2), ESBL (1) and B421 UFL (1)(as shown in table 3). A similar technique was used by Christner *et al.* (2014) for a real-time outbreak investigation comprising screening of 293 clinical *E. coli* isolates. Dallagassa *et al.* (2014) evaluated 180 commensal and pathogenic strains of *E. Coli* by MALDI-TOF MS, suggesting that MALDI-TOF MS has the potential to distinguish commensal and

uropathogenic *E. coli* strains. The studies reported by Karger *et al.* (2011) and Rohde *et al.* (2011) also depict the use of MALDI-TOF MS technique for the characterization of *E. coli* organisms.

CONCLUSIONS

MALDI-TOF MS has emerged as a promising technique for sensitive and rapid microbial detection and diagnosis. The technique provided precise genus-level confirmation of all 39 phenotypically characterized isolates of *E. coli* recovered from meats in this investigation, signifying its advantage over the PCR technique.

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