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Short Communication

Phylogenetic analysis of *Escherichia Coli* isolated from mastitis milk

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ABSTRACT

Pathogenic *Escherichia coli*, when colonize the bovine mammary glands, results in the cell damage as well as changes in the composition of the milk. A total 50 milk samples of Frieswal (HF x Sahiwal) cow herd maintained at Military Farm, Meerut were collected from mastitis (n= 35) and normal (n=15). *Escherichia coli* were detected through simplex PCR using published primers of 23s rRNA. All the 35 mastitis milk samples were positive for *Escherichia coli*, which was further confirmed by sequencing and phylogenetic analysis by using MEGA 5.2 software.The Phylogenetic analysis of our isolate (KF278640) with other*Escherichia coli* fall in a clade with (AM158281.1) *Escherichia coli* 16S rRNAtRNA– Glu and 23S rRNA.

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Mastitis is a disease of dairy cattle that results in losses in milk production. Among the major bacterial pathogenic, *Escherichia coli* is one of the important cause of bovine mastitis (Sayed et al., 2008). Rapid and early detection of the pathogens in mastitic milk samples are very much essential for monitoring and controlling the infections in the dairy industry (Deb et al., 2013). Though, conventional microbiological procedures could identify the potential cause; nevertheless, they are time consuming, laborious and not highly specific (Phuektes et al., 2001). Due to these limitations of the conventional approaches, the development of PCR–based methods and phylogenetic analysis provides an alternate option for the rapid identification of bacterial type. The aim of the current study was detection of *Escherichia coli* from mastitis milk and its subsequent phylogeny with its close relatives using NCBI database.

CMT positive (n = 56) and normal (n = 20) were collected from Frieswal (HF X Sahiwal) cow herd maintained in Military Farm, Meerut, Uttar Pradesh, India. Atotal 50 sampling of milk was conducted as per the standard protocol described earlier (Watts, 1990). All collected samples were stored immediately in ice pack and transported to laboratory.300 µL of milk sample were added to 300 µL of lysis buffer contains 0.1M NaCl, 20 mMTris–HCl, 1mM EDTA, 0.5% SDS and 100 µg of proteinase K/ml. Samples were incubated at 37°C for 4 h. Than DNA was extracted by phenol–chloroform method (Sambrook et al.1989). DNA pellet was dried and dissolved in 50µL of TE buffer. DNA samples were stored at –20°C until further used. Primers used for the present study (Forward: 5` ATC AAC CGA GAT TCCCCC AGT 3` and reverse: 5` TCA CTA TCG GTC AGTCAG GAG 3`) was obtained from literature (Riffon et al., 2001) The polymerase chain reaction (PCR) was carried out with standard protocol. Briefly, a total 25µL solution was prepared with 50 ng/µL of template DNA, 1X PCR buffer (Tris–HCl 100 mmol/l, pH 8.3; KCl 500 mmol/l), 0.25 µmol/l primers, 2.0 mmol/l $MgCl_2$, 0.25 mmol/l dNTPs, and 0.5U Taq DNA polymerase (Sigma–aldrich, USA). Program for PCR reaction followed was 94°C for 5 min, followed by 35 cycles of 94°C for 3seconds, annealing at at 55°C for 30 seconds and 72°C for 30 seconds, and a final extension at 72°C for 10 min. A 232 base pair PCR products were separated on 1.0% agarose gel with 0.5 µg/ml of ethidium bromide and photographed under Gel Documentation system (Alpha imager® EP).

The PCR product were send for sequencing and the sequence were edited as well as aligned using BioEdit software and submitted to NCBI (Accession number: KF278640). In the present study the 23S rRNA sequence obtained were compare with other known *Escherichia coli* by BLAST analysis at NCBI (http://ncbi.nlm.nih.gov/BLAST). In this study the presence of *Escherichia coli* in mastitis milk samples was confirmed by PCR (Figure 1). Further, the sequenced PCR products, after analysis and homology testing, matched with other *Enterobacter* species (91–95%). We have received the gene bank accession number (KF278640).

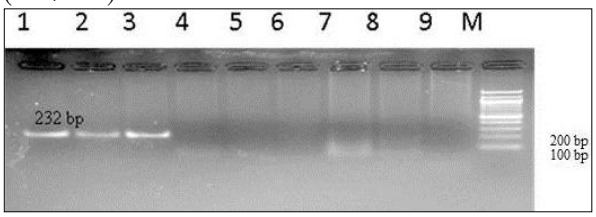

Figure 1: PCR products (232 bp) amplified from *Escherichia coli* DNA (1–3) (Accession number KF278640); no amplification for (4–9); Normal Milk samples (8–9); 100bp DNA marker (M)

Mastitis with *Escherichia coli* affecting other food samples were assessed using sequences of 23S rRNA genes available in GenBank. BLAST pairwise alignment was done to view the phylogenetic tree (Figure 2) by using MEGA 5.2 software using

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Figure 2: Phylogenetic analysis of *Escherichia coli* isolate (Accession number KF278640) from mastitis milk samples using BLAST pair wise analysis and MEGA 5.2 software.

the neighbour–joining method at a boot strap value more than 95%.

PCR based detection and identification of mastitis pathogens are a specific, sensitive and economical (Brikenmeyer and Mushahwar, 1991).PCR approach in mastitis diagnosis can thus noticeably perk up dairy cow health programs.

The high incidence of *Escherichia coli* in clinical mastitis milk samples which encountered in the present study agrees with the findings of many other researchers who considered *Escherichia coli* organisms as one of the major etiological agents for clinical mastitis(Dopfer et al., 1999,Gonzalez et al., 1990). *Escherichia coli* are enteric and or opportunistic environmental organisms which can infect udder and thus could possibly serve as a reservoir for recurrent episodes of clinical mastitis (Gonzalez et al., 1990, Miltenburg et al., 1996, Dopfer et al., 1999). The Phylogenetic analysis from the p[resent study revealed that, the isolated 23S rRNA gene of other *Escherichia coli* (KF278640) from the mastitis milk fall in a clade with *Escherichia coli* 16S rRNAtRNA– Glu and 23S rRNA(AM158281.1).

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