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Gholam Ali Moradli, Tagi Zahraei Salehii, Mahmod Jamshidian & Farhad Mosakhani

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Molecular identification virulence genes of *Escherichia coli* isolated from bovine clinical mastitis

Gholam Ali Moradli ¹, Tagi Zahraei Salehii ¹,* , Mahmood Jamshidian ¹, and Farhad Mosakhani ²

¹ Department of Microbiology, Faculty of Specialized of Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, IR Iran. 
² Department of Microbiology, Faculty of Veterinary Medicine, Karaj Islamic Azad University, Karaj, IR Iran.

**ABSTRACT**

The aims of this study were molecular identification some of virulence genes in *Escherichia coli* isolated from milk of bovines with clinical mastitis. (*n* = 60) *E. coli* isolates from acute clinical mastitis were examined for detect the presence of the genes encoding shigatoxin1 (*stx1*), intimin (*eaeA*), cytotoxic necrotizing factor 2 (*cnf2*), aerobactin (*iucD*) and *P. fimbriae* (*pap*). The majors finding in the PCR assays were: 8 isolates (13.33%) had at least one virulence gene. None of isolates contained the genes for *stx1*, *eaeA*, the most common gene in the examined isolate was *iucD* which was positive in 6 isolates (10%). One isolate (1.66%) was positive for both *iucD* and *pap* genes and one isolate (1.66%) for *cnf2* gene. In this study similar to previous investigations indicated that prevalence virulence genes in *E. coli* isolated of bovine mastitis is deferent. The results of this investigate similar to previous studies indicated none of the potential virulence genes or specific pathotype was observed in *E. coli* isolates from bovine clinical mastitis.

**INTRODUCTION**

Gram negative bacteria are the etiological agents most often isolated from acute clinical cases of mastitis. *Escherichia coli* are among the most common infectious agents isolated from severe mastitis cases (Hogan et al., 2003). Escherichia coli mastitis has increased in many countries and herds (kaipainen et al., 2002). There are few reports on the role of *E. coli* in bovine mastitis in Iranian dairy farm (Ghanbarpour et al., 2010). The pathogenesis of coliform mastitis is completely different from disease such as colibacillosis in calves (Burvenich et al., 2003). *E. coli* is considered to be an opportunistic pathogen and originate from a contaminated environment. The severity and course of the disease vary greatly and mainly depend on cow’s response. However, the virulence of the bacterial strain involved may also play a role. Bacteria require virulence factors to colonize, multiply and survive in adder. These include toxins, adhesions, invasions, capsule production and the ability to resist serum complement and scavenge iron (lehtolatinen et al., 2003). Dominant virulence factors have not been identified in mastitis derived *E. coli* isolate (kaipainen et al., 2002).

*P. fimbriae*, aerobactin coded by *pap*, *iucD* genes are produced by human urophathogenic *E. coli* strain (UPEC) (Yamamoto et al., 1995). *P. fimbriae* of *E. coli* isolates from cow mastitis were detected (kaipainen et al., 2002), (Ghanbarpour et al., 2010). Investigations indicate that in 20% of strains isolated from mastitis were positive for aerobactin production which was significantly higher than fecal isolates (kaipainen et al., 2002). A chromosomal gene, *eaeA*, encodes the protein intimin, which is involved in attaching and effacing (AE) activity which causes disease because of their intimate attachment to the entrocyte and effacement of the microvillus border (AEEC). STEC produces two type of shigatoxin (*STX1, STX2*). STEC produce two type of *E. coli* Shiga toxins, those that are immunologically similar to the Shiga toxin produced by Shigella dysenteriae (*STX1*) and those that are immunologically distinct from Shigella dysenteriae Shiga toxin (*STX2*). Bovine STEC produces either *STX1, STX2*, or both (Frank et al., 1995). The presence of *eaeA*, *Stx1* genes were detected in *E. coli* isolates from bovine mastitis (Bean et al., 2004), (Mottaz, 2010). The cytotoxic necrotizing factor (CNF) toxins (*cnf1, cnf2* genes) are associated with damage to vascular endothelial cells and thrombotic microangiopathy (Wenz et al., 2006). Whereas *cnf2* commonly was found in fecal strains from cattle, lambs and goat but also mastitis (Ghanbarpour et al., 2010). The aims and objectives of this study were molecular identification some of virulence genes in *Escherichia coli* isolated from milk of bovines with clinical mastitis that is include *eaeA*, *stx1, cnf2, iucD*, *pap*. 

* Correspondence to: Tagi Zahraei Salehii, Department of Microbiology, Faculty of Specialized of Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, IR Iran. 
Email: tsalehi@ut.ac.ir; Phone No: 098-216-5295115, Fax No: 098-216-5295115 

**Key words:** *Escherichia coli*, Virulence genes, Bovine Mastitis
MATERIALS & METHODS

Bacterial isolates and reference strains

E. coli isolates (n = 60) from clinical cases of bovine mastitis from dairy farms in Karaj province, Iran. Milk samples from individual cows with clinical mastitis were collected in sterile vials. Samples were cultured on 5% sheep blood agar and Mac Conkey agar. The isolates were identified as E. coli based on colony morphology and IMViC biochemical tests (Ghanbarpour et al., 2010 and Bean et al., 2004). Sakai E. coli strain was used as positive control for intimin, STX1 E. coli S5 for cnf2 E. coli isolate (1.66%) for cnf2 gene (table 2), (fig 2).

Extraction of genomic DNA

All of the E. coli isolates were cultured in luria Bertani agar for overnight growth. DNA was extracted from E. coli isolates by Fermentase kit.

PCR Protocols

Two multiplex PCR and one simplex PCR to detect the genes encoding shigatoxin 1 (stx1), intimine (eaeA), cytotoxic necrotising factor 2 (cnf2), aerobactin (iucD) and p fimbriae (pap) were performed with using of primers in table 1. MXP1 to detect the genes encoding shiga toxin (stx1), intimin (eaeA) was performed in a total volume of 50 μL containing 1.5 mM of MgCl2, 250 μL each of the deoxyribonucleoside triphosphates, 0.5 μM each of the virulence gene specific primers, 1.25 U of Taq polymerase and 5 μL of template DNA. The amplification condition included 25 cycles of a denaturation step at 94 °C for 30 s, primer annealing at 50 °C for 45 s and extension at 70 °C for 90 s and final extension step of 10 min at 70 °C was performed. PCR products were analyzed by electrophoresis through 1.5% agarose gel, after which the gel was stained with ethidium bromide and photographed (Leyla Guler et al., 2006). MXP2 that detects the genes encoding intimin and p fimbriae (iucD) was positive in 6 isolates (10%). One isolate (1.66%) was positive for both iucD and pap genes (fig 1) and one isolate (1.66%) for cnf2 gene (table 2), (fig 2).

RESULTS

DNA from the 60 E. coli isolated from bovine clinical mastitis was amplified for the genes eaeA, stx1, iucD, pap and cnf2 (fig 1, 2). The majors finding in the PCR assays were: 8 isolates (13.33%) had at least one virulence gene, None of isolates contained the genes for stx1, eaeA (fig 2), and the most common gene in the examined isolate was iucD which was positive in 6 isolates (10%). One isolate (1.66%) was positive for both iucD and pap genes (fig 1) and one isolate (1.66%) for cnf2 gene (table 2), (fig 2).

DISCUSSION

In most E. coli infections, the pathogenicity of the bacterial strain mainly depends on the expression of particular bacterial virulence factors (Lehtolainen, 2004). E. coli mastitis differs from other E. coli infection because the clinical signs are primarily caused by the host response and not by the pathological changes derived from bacterial toxins and other damaging factors (Burrenich et al., 2003). Previous studies indicated that E. coli mastitis is not caused by especial strains with certain specific virulence factor but by a great variety of strains originated from the environment of the cow (Lipman et al., 1995, Sanchez-Carlo et al., 1984). Adhesion and invasion of bacteria to the host epithelia is considered the first step in bacterial infections. Different fimbriae and adhesions are necessary to the pathogenicity of E. coli strains, causing diarrhea and urinary infections (Lehtolainen., 2004). Afimbrial adhesins are found in E. coli isolated from intestinal and extraintestinal Sources of cattle (Lehtolainen et al., 2004). p fimbriae are important in adhesion of uropathogenic E. coli strains (Soto and Hultgren., 1999). Adherence or attachment of E. coli to epithelial tissue dose not play a major role in the pathogenesis of bovine mastitis (Hogan and Smith., 2002). However, studies performed by Kaipainen et al. (2002) showed 7% of finish isolates were positive for pap gene and did not detected in Israli isolates and investigation of Ghanbarpour et al. (2010) showed none of the isolates contained the genes for intimine and p fimbriae. Our findings support previous studies since 1.66% of all isolates were positive to p fimbriae and none isolates were not positive for eaeA gene.
Toxin production is another common virulence factor of E. coli. After colonization, some strains produce toxins that have a deleterious effect on host cells, disturbing their functions. Production of cnf2 is common among bovine E. coli strains. The prevalence of cnf2 positive E. coli strains among isolates from cattle with mastitis was 7.5%, 9.4%, and 9.44%, respectively (Bean et al., 2004; Wenz et al., 2006; Ghanbarpour et al., 2010). The stx2 gene in the finish and Israeli isolates was 14%, 3% respectively (Kaipainen et al., 2002). In this study, only one cnf2 producing E. coli strain has detected relation mastitis. The other toxin produced by E. coli is STX1. This toxin act by inhibiting protein synthesis and are lethal for in vitro cultured (Frank et al., 1998).

The most common virulence gene detected was stx1, with a prevalence of 31% (Bean et al., 2002). Momtaz (2010) showed that virulence gene of stx1, stx2 was 23.8%. In our material similar to previous study (Guler et al., 2006) did not detected stx1 gene. Different siderophores are important for E. coli, chelating iron subsequent bacterial infection. In mammary infections, this ability is thought to be more important than in other infections since milk contains lactoferrin which also chelates iron and efficiently inhibits the growth of gram-negative bacterial (Lehtolainen, 2004). In study performed by Kaipainen et al. (2002) 11% finish isolates and 4% Israeli isolate were positive for are gene, in other study performed by Ghanbarpour et al. (2010).

CONCLUSION

The results of this study similar to previous investigations indicated that prevalence virulence genes in E. coli isolated of bovine mastitis is deferent, this investigate similar to previous studies indicated none of the potential virulence genes or specific pathotype was observed in E. coli isolates from bovine clinical mastitis and to infect the bovine adder, specific virulence genes do not seem to be necessary for E. coli bacteria.

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REFERENCES


Lehtolainen T2004. Escherichia coli mastitis Bacterial factors and host response. Thesis of Ph.D.Faculty of Veterinary Medicine University of Helsinki Finland.


