Isolate and Molecular Identification of Bundle Forming Pilus Gene for Escherichia coli Caused Caw Mastitis

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ABSTRACT
Mastitis is one of the disease infected Caws and effect of their milk product lead economic loss. The aim of this study isolated and molecular identification Bundle Forming Pilus Gene for Escherichia coli caused Caw mastitis. Samples were collected from Caws infected with Mastitis at different farm in Iraq, then applied for cultivation on the suitable media for isolated of E. coli. The E.coli isolates was confirmed by VITEK II automated microbial identification system method and identification by PCR method for expression of bundle forming pilus gene. The result was shown that (9 from 100) from mastitis cases give E. coli positive. All E.coli isolates were confirmed by VITEK method. All E.coli isolates were expressed of bundle forming pilus gene. Conclusion: E.coli caused severe mastitis in Caw and the severity of infection come from express of bundle forming pilus gene that increases the virulence of E.coli be more resistance to antibiotics and unsuitable conditions that may be lead to many serious diseases if dietary contaminated with mastitis E.coli .

1. Introduction

The most prevalent diseases of caws is acute mastitis is poses a risk for caws behaviors become lying very harmful and effect on their producing [1]. The causative of mastitis many species of gram positive and negative bacteria, one of these is E. coli a gram negative bacilli [2]. Many researchers center on the implement the mechanisms for the action of pathogens virulence as gram negative bacteria endotoxin, which effects on the enhanced many diseases on the different organs in the body like as; ovary, hypothalamus, and endometrium [3-5]. Infection of intramammary related with pathogens production of proinflammatory interleukins and cytokines as TNF- alpha and IL-2 caused abortion by induced fever [6]. The farm and domestic animals infected with a lot of disease through different causative pathogens like viral and candida that also effect on the animal behavior and their production and the disease may be transmitted to owner leads to serious infection and effect on immune response because the modulation in the virulence genetic mutation [7-9]. Microorganisms depend on their virulence factors to invade the host then colonized and cause disease. Modulation or genetic mutant that happened in the virulence is the power for the pathogen to continue in the environment. One of the virulence factor of pathogenic E coli is bundle forming pilus that modulated formation of microcolony on the epithelial cells then make E. coli to adherent and could to penetrate through the cells [10]. The bundle forming pilus responsible for the tighten of biofilm forming enterophathogenic E. coli, the mutant strain do not expressed bundle forming pilus forming more spread biofilm than the strain expressed bundle forming pilus that be more adhesins biofilm [11]. So that, bundle forming pilus are an important virulence for enterophathogenic E. coli that need for localization and adherent to epithelial cells [12]. In this study investigated the expression of E.coli bundle forming pilus that caused caw mastitis.

2. Methodology

Sample collection and Identification of *Esherichia coli*

A swab sample was collected from a Caw suffering from mastitis (100 samples were collected from different farm in Iraq). The swabs were striking on the selective and differential media for growing

and isolated the *E. coli*, and then the isolates were verified by VITEK II automated microbial identification system.

Bundle Forming Pilus Gene for *E. coli*

For investigated the expression of Bundle Forming Pilus Gene for *E. coli*, all positive isolates were applied for PCR method to investigated the expression of Bundle Forming Pilus Gene by using PCR kits from Promega, USA. The primer was design by Primer 3 software version 4.1.0, as describe in (Table 1).

Table 1: Primer sequence for Bundle Forming Pilus Gene for *E. coli*

Gene	Sequences (5'_3')	Product size/bp
Forward	CTTCACCGCTTGTTTCAGCC	**540**
Reverse	CGCGCTGCGATTTATTGTGA	

3. Results and discussion

From 100 mastitis samples, 9 isolated were confirm as *E. coli* as showed in the Figure 1 the colony of isolate appear pink on the Macconkey agar and green metallic shine on the EMB agar. All nine *E. coli* isolates were confirmed by VITEK II system and then applied for PCR method to investigated the expression of Bundle Forming Pilus Gene for *E. coli*, the result was confirmed the expression of Bundle Forming Pilus Gene for all nine *E. coli* Caw mastitis isolates (Figure 2).

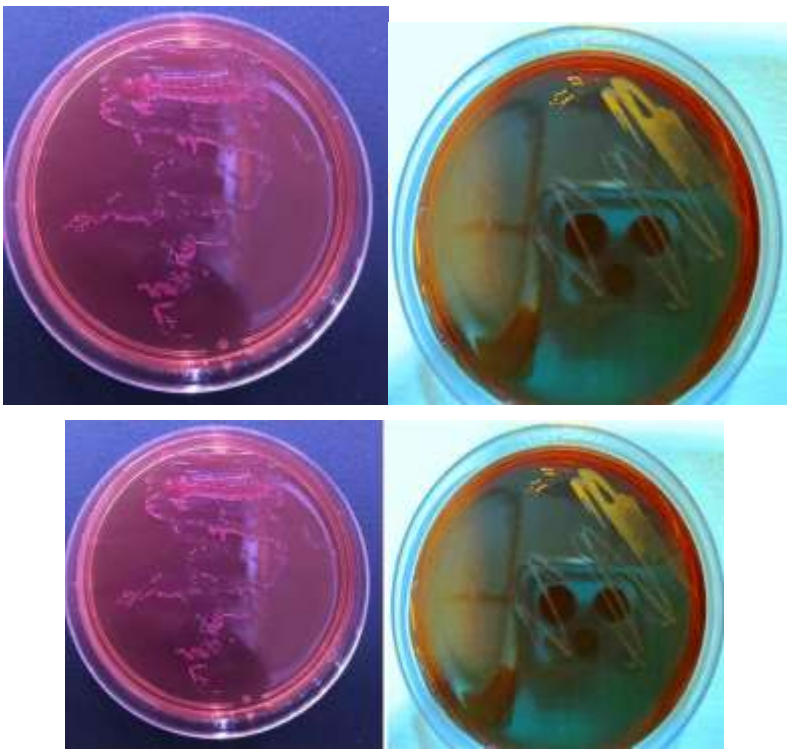

Figure 1: *Escherichia coli* colony appearance from Caw mastitis Isolate on Macconkey agar on the left and EMB on the right

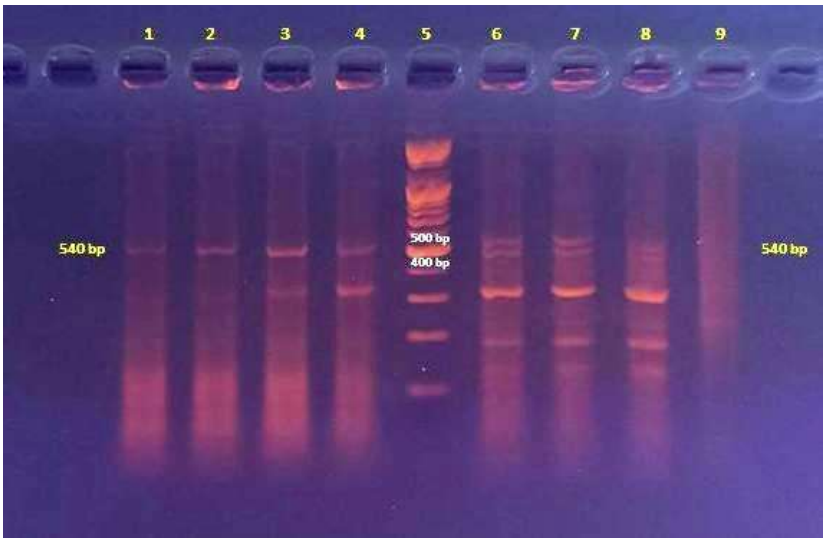

Figure 2: Agarose gel electrophoresis of Bundle Forming Pilus Gene *Escherichia coli* (1.5% agarose,7v/cm2 for 60 min) for Gene gene (540 bp amplicon), lane 5 represent M100bp DNA Ladder.

Discussion

E. coli is one of the pathogen infected caws and causes mastitis the serious disease that effect on the behavior, reproduction and production of the caws. The LPS from *E.coli* associated with rise the production of prostaglandin E2 caused elongated the death phase and lowing the ovulation of caws and conception [3]. In the previous study was found that intrauterine of LPS injection to caws were led to suppressed ovarian cells role [13]. Infection of intramammary related with pathogens production of proinflammatory interleukins and cytokines as TNF- alpha and IL-2 [6] caused abortion by induced fever which inhibited growth of embryo through production of prostaglandin [3]. One of *E. coli* mechanisms for resist the bad condition such as environment factors or antibiotics and could in the growing is forming biofilm. The bundle forming pilus gene is the gene responsible for modified microcolony and enable them for colonization and adherent to the epithelial cells, also has a role in the biofilm formation and be localized not aggregation that give the new strain nor virulence and caused severe inflammation [14]. In this study all *E. coli* isolated from caw mastitis are expressed bundle forming pilus gene. In previous study was found that all 44 *E. coli* strain caused diarrheal infection expressed bundle forming pilus gene [15]. Enteropathogenic E. coli found the expressed the protein of bundle forming pilus in the inner cytoplasmic membrane [16]. Bundle forming pilus is associated with bacterial auto- aggregation and mediating in the starting adherence of the *E. coli* with the host cell, and the regression of bundle forming pilus leads to pili regression to enteropathogenic *E. coli* and contributed to its pathogenesis, while the expression of bundle forming pilus leads to generation of pili and promoting of efficient movement of bacterial protein and permits nearer sites between host cell surface and bacterial pathogen [17].

4. Conclusion and future scope

The results of this study was concluded that E. coli caused Caw mastitis was expressed bundle forming pilus gene, that give the bacterial more virulence and be more resistance to antibiotics and unsuitable conditions

Conflict of interest: No conflict of interest

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