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Prevalence of virulence factors in *Escherichia coli* strains isolated from the genital tract of healthy cows

[*Prevalência de fatores de virulência em cepas de* Escherichia coli *isoladas do trato genital de vacas saudáveis*]

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ABSTRACT

The prevalence of virulence genes expressing fimbriae, production of hemolysin, colicin and aerobactin, was determined in *Escherichia coli* isolates from healthy cow's genital tract not showing clinical signs of infection. The presence of fimbriae expression genes (*pap*, *sfa*, *afa*) was assayed using specific primers in a polymerase chain reaction; none were detected in any of the isolates. Yet, a prevalence of 90.4%, 69.8%, and 28.5% of virulence factors for colicin, hemolysin and aerobactin respectively, was detected in the isolates. Analysis of the bacterial pathogenicity of isolates from the bovine genital tract may contribute towards the understanding of *E. coli* behavior.

Keywords: cow, *Escherichia coli*, genital tract, virulence factors

RESUMO

Determinou-se a prevalência dos genes de virulência expressando fimbrias, produção de hemolisina, colicina e aerobactina em cepas de Escherichia coli *obtidas do trato genital de vacas saudáveis que não apresentam sinais clínicos indicativos de infecção. A presença dos genes responsáveis pela expressão de fimbrias* (pap, sfa, afa) *foi avaliada através de reação em cadeia da polimerase utilizando primers especificos para cada um dos genes, nenhum deles foi detectado em qualquer uma das cepas isoladas. A prevalência dos fatores de virulência foi de 90,4%, 69,8%, 28,5% para colicina, hemolisina e aerobactina, respectivamente. A análise da patogenicidade das cepas do trato genital pode contribuir para o entendimento do comportamento das cepas de* E. coli.

Palavras-chave: vaca, Escherichia coli, *trato genital, fatores de virulência*

INTRODUCTION

The normal microbial flora of the bovine genital tract is made up of a dynamic mixture of aerobic, facultative anaerobic and strict anaerobic micro-organisms. Under natural conditions this environment is stable, protecting the host from the setting in of pathogenic or potentially pathogenic saprophytic microorganisms. The normal microbial flora of this tract is composed by bacteria of the genera *Staphylococcus, Streptococcus* and the coliform group (Hafez, 1993). Enterobacteriaceae, especially *Escherichia coli* have been isolated from the

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urogenital tract of cattle in low numbers (Torres et al., 1994; Otero et al., 2000).

E. coli is a major cause of bacterial diarrhea and urinary tract infection (UTI) in humans (Stamm, 2002). While *E. coli* is an important urinary tract infection agent in pigs (Brito et al., 1999) and dogs (Johnson et al., 2001), it does not have this importance in cattle in which the frequency of urinary tract infection (UTI) is low (Yeruham et al., 2006). Nevertheless, *E. coli* is well known to causes endometritis and infertility in cattle (Sheldon et al., 2002).

Adhesion of *E. coli* to the uroepithelium may protect bacteria from the effect of urinary lavage, increasing their ability to multiply and invade renal tissue (Korhonen et al., 1988). The uropathogenic *E. coli* (UPEC) possess adherence factors called pilli or fimbriae, which allow them to successfully initiate infection. Specific adhesion is mediated by bacterial proteins termed adhesins which may or may not be associated with fimbriae. *Pap* (pyelonephritis-associated pilli), *sfa* (S fimbrial adhesion) and *afa* (afimbrial adhesion) operons are most commonly found to encode respectively, P, S and Afa adhesins (Le Bouguenec et al., 1992)

Besides bacterial adherence, several virulence factors may contribute to the pathogenicity of UPEC, including the production of α-hemolysin, colicin and aerobactin (Emody et al., 2003).

To the best of our knowledge, *E. coli* isolates in cattle endometritis or UTI have never had these factors analyzed. The objective of this study therefore, was to analyze adherence and virulence factors in *E. coli* isolates from genital tracts of dairy cattle.

MATERIALS AND METHODS

A herd of 15 health dairy cows free of clinical signs of urinary or uterine infections, no history of abortions and having calved at least twice, was studied during May 2004. For each animal, a transcervical-guarded swab was collected from the uterine body of each cow by the Noakes et al. (1989) validated method. The swab gear consisted of a long, stiff wire bearing a cotton wool swab, sheathed in a metal guard tube (8mm external diameter), wrapped and sterilized by autoclaving. The distal end of the guard tube was protected by a sterile aluminum paper cap that covered the outside tip of the tube until its use. The vulva of each cow was cleaned with a dry paper towel, and the tube, bearing an aluminum paper cap to prevent the swab from being contaminated during handling, was inserted through the vagina. The swab was extruded from the guard tube and rotated to permit contact with the uterine mucosa, prior to withdrawal into the guard tube and removal from the uterus. The swab was transferred to a bottle containing Stuart Transport Medium[1] and aerobically cultured as soon as possible, on MacConkey agar[1]. At least four colonies per plate were selected for further analysis. Bacteria were identified based on the colony's characteristics, Gram staining and its biochemical profile (Farmer, 1999).

Production of hemolysin was assayed by growing the isolates overnight at 37°C in LB medium (Sambrook et al., 1989), dropping 50µl of this culture on a Petri dish containing sheep blood agar, and incubating it overnight at 37°C; hemolysin production was verified by the presence of a clear hemolytic halo around the colonies.

Production of aerobactin was assayed by growing the strains isolated in a LB medium containing 200µM of α-α dipyridyl for 24 h at 37°C, without shaking. The grown mass was spun for 3min at 12000g, the supernatants filtered through a 0.22µm nitrocellulose membrane and 50µl aliquots added to orifices made in a LA medium (Sambrook et al., 1989), previously seeded with strain LG 1522 (Blanco et al., 1997). Petri dishes were kept at 37°C for 48h, and the production of aerobactin visualized by the growth of strain LG 1522 around the orifices.

Colicin production was assayed as previously described (Minshew et al., 1978; Vidotto et al., 1990). Briefly, colonies were transferred to LB plates using toothpicks, and incubated at 37°C for 18h. Cells were added to LB plates with toothpicks, and incubated at 37°C for 18h, killed by chloroform vapor and plates were overlaid with 3ml of LB soft agar (0.75% agar), containing 1ml of an overnight culture of strain MA335. Colicin-producing colonies were further typed by this specific colicin-indicator strain.

[1] Difco, Detroit - USA

E. coli strains were grown overnight in LB broth at 37°C. Bacteria were pelletized from 1.5ml of broth, suspended in 200µl sterile distilled water and boiled for 15min. Following centrifugation of the lysate, a 150µl sample of the supernatant was stored at -20°C to serve as a template DNA stock (Keskimaki et al., 2001).

Specific primers were used to amplify the sequences of the *pap*, *sfa* and *afa* genes. Primer sequences, predicted sizes of the amplified products, and specific conditions were as described by Le Bouguenec et al. (1992).

RESULTS AND DISCUSSION

A total of 71 isolates from vaginal swabs from 15 dairy cows were examined. *E. coli* was isolated from each cow swab. Eight isolates not characterized as *E. coli* by the methods used, were discarded. The prevalence of virulence factors ranged from 0.0% for *pap* to 90.4% for colicin (Table 1).

Table 1. Prevalence of virulence factor-associated genes among 63 *Escherichia coli* isolates from the genital tract of healthy cows

Virulence factor	Percentage in genital tract isolates (n=63)
Colicin	90.4
Hemolysin	69.8
Aerobactin	28.5
Pap	0.0
Sfa	0.0
Afa	0.0

n= number of isolates examined.

Bacterial adherence to host cells is a crucial step in the initiation of various infectious diseases (Korhonen et al., 1988). Adherence factors facilitate the colonization of the urinary tract and promote *E. coli* colonization and persistence in the colon or vagina where they may serve as reservoirs for ascending infection (Stamm, 2002). The frequency of the *pap* operon in *E. coli* isolates from porcine UTI was found to be similar to that found in human isolates, 54.8% (Brito et al., 1999); this gene has also been detected in dogs (Johnson et al., 2001). In the present study, PCR did not detect genes coding for *pap*, *afa*, or *sfa*, in the vaginal isolates. *Afa*-related sequences have been detected in isolates from calves (Mainil et al., 1997). The *afa*-8 operon is common among pathogenic *E. coli* strains isolated from animals and humans (Gerardin et al., 2000), and has frequently been found in animal and human isolates producing cytotoxic necrotizing factors (CNF), but has also been detected in CNF-negative strains isolated from calves and piglets (Mainil et al., 1997; Gerardin et al., 2000); specific PCR search for the *afa*-8 operon among isolates is presently underway in the laboratory.

Among the toxin-coding genes studied, colicin was the most prevalent one (90.4%), among vaginal isolates from cattle. This result is higher than that reported for humans where it ranged between 7.0% (Minshew et al., 1978) and 26.0% (Davies et al., 1981), and also for that reported for UTI isolates from pigs, (38.7%). However, Smith (1974), apud Davies et al. (1981), reported that 78.0% of *E. coli* strains responsible for infection in cattle produced colicin; however, the specific mechanisms which confer such vantage to these strains remain controversial.

Hemolysin production was found to be broadly distributed, being the second most detected virulence factor among vaginal isolates (69.8%) (Table1). This characteristic has been described as an important trait, and although hemolysin production by itself does not always lead to virulence, it may be a decisive factor for the virulence of many nephropathogenic strains (Blanco et al., 1990). Brito et al (1999) reported that 25.8% of the isolates from pig UTI produced hemolysin, a value lower than that reported in the present study (69.8%) in cattle vaginal isolates.

Forty-five percent of urinary strains isolated from pigs produced aerobactin (Brito et al., 1999), but in the present study (Table 1), only 28.5% of the vaginal isolates produced aerobactin, a factor involved in iron-binding, which confers an advantage to bacterial strains for the colonization of the urinary tract.

During parturition, the physical barriers of the cervix, vagina and vulva are compromised providing an opportunity for environmental bacteria to ascend in the genital tract causing uterine infection (Sheldon et al., 2002). This situation is in a dynamic flux, of regular contamination, followed by its clearing up and by spontaneous re-contamination by bacteria

during the first weeks following parturition (Sheldon et al., 2002). *E. coli* isolates recovered in cases of endometritis, or rarely of UTI, have traditionally been examined for their antimicrobial susceptibility, but not for their virulence factors; however, it is accepted that they come from the environment, most probably from the cattle's feces (Yeruham et al., 2006)

Taken together, the results of the virulence characteristics found in the isolates from the genital tract of healthy cows show a distinct bacterial growth pattern, difficult to be related to the non-infective *E. coli* strains present in the vagina. Nevertheless, the characteristics of the virulence factors found among genital tract isolates, reinforce the hypothesis that bacteria recovered from the vagina originate from the environment via contamination by bovine feces, but do not possess a specific mechanism for the colonization of the vaginal mucosa. Further research will be required to provide a better understanding of the behavioral properties of these isolates.

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