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Determination of P (F11) and F1 fimbriae of *Escherichia coli* isolated from avian cellulitis

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ABSTRACT

Avian cellulitis has been induced by virulent strains of *E. coli*. The purpose of this study was to determine P (F11) and F1 fimbriae of 90 *E. coli* isolates of avian cellulitis. Isolates were subjected to six consecutive passages on solid and static broth for expression of fimbriae. Five (5.5%) isolates from O1 serogroup, showed a mannose-resistant haemagglutination MRHA pattern when grown on solid medium. In the SDS-PAGE, crude fimbrial extracts of MRHA strain showed a major fimbrial subunit of 18 kDa. This band was also reacted with anti F11 serum on immunoblotting. Sixty-nine (76.6%) *E. coli* isolates from different serogroups showed mannose-sensitive hemagglutinating (MSHA) pattern when grown on static broth medium. In immunoblotting test, crude fimbrial extracts of MSHA isolates demonstrated a single band with 17 to 17.5 kDa apparent molecular weight as revealed by absorbed anti-F1A serum. It would appear that avian cellulitis *E. coli* isolates have F1 and P fimbriae similar to those of colisepticemic *E. coli* isolates.

Key words: cellulitis, chicken, broiler, *E.coli*, F1 fimbriae, P fimbriae

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Introduction

Avian cellulitis in broiler chickens is characterized by a diffuse inflammatory reaction in the subcutaneous tissue that results in the complete or partial condemnation of the carcass at processing (SINGER et al., 1999). The lesions are characterized by thickened and brown discoloration of the skin, together with fibrinopurulent exudate, some casseation, hyperkeratosis and infiltration of mononuclear cells and heterophils (DERAKHSHANFAR and GHANBARPOUR, 2002). Although many bacterial species, such as *Pasteurella multocida, Pseudomonas aeruginosa, Enterobacter agglomerans, Proteus vulgaris, Streptococcus dysgalactiae* and *Arcanobacterium (Actinomyces, Corynebacterium) pyogenes* have been associated with cellulitis, *Escherichia coli* remains the most commonly isolated (JOHNSON et al., 2001; DERAKHSHANFAR and GHANBARPOUR, 2002). Different studies were designed to compare the virulence factors of cellulitis-derived *E. coli* to colisepticemic *E. coli*, in order to clarify whether isolates associated with cellulitis comprise a unique subset of pathogenic *E. coli* (JEFFREY et al., 1999; JEFFREY et al., 2002; NGELEKA et al., 1996; PEIGHAMBARI et al., 1995a).

Bacterial adhesins play an important role in adherence of bacteria to host epithelial cells (NGELEKA et al., 1996). Avian pathogenic *E. coli* (APEC) may express two main groups of fimbriae, F1 (type 1) and P, which mediate the bacterial colonization (DOHO-MOULIN and FAIRBROTHER, 1999; DOZOIS et al., 1995; POURBAKHSH et al., 1997a). F1 fimbriae are termed mannose-sensitive hemagglutinating fimbriae (MSHA), and P are termed mannose-resistant hemagglutination (MRHA) fimbriae (DOZOIS et al., 1995; POURBAKHSH et al., 1997b). Results of previous studies suggest that cellulitis isolates may express F1A and/or MRHA fimbriae (NGELEKA et al., 1996). The present study was designed to determine whether *E.coli* isolates from avian cellulitis express the P (F11) and F1 fimbriae same as the colisepticemic isolates of *E. coli*.

Materials and methods

Bacterial strains. Ninety *E. coli* isolates from chickens with cellulitis were examined (Table 1). O serotyping of *E. coli* isolates was done by using Mast diagnostics Kit (Mast Group Ltd., Merseyside, U.K.) in slide

agglutination technique. Reference strains of *E. coli* C1976 (O1:K1:H7: F11) and BAM (rough, F1BAM) were used for production of fimbrial antisera.

Culture conditions. Isolates were subjected to six consecutive passages for 24 h on tryptic soy agar (TSA) (Biolife Laboratories, Italy) at 37 °C. Isolates were also subjected to six passages for 48 h in static tryptic soy broth (TSB) (Biolife Laboratories, Italy), at 37 °C.

Hemagglutination. Isolates were tested for hemagglutination of human OP1 and chicken erythrocytes in the presence and the absence of 2.5% D-mannose, as described by DOZOIS et al. (1994).

Antisera. Antisera against partially purified F1A and F11 fimbriae of reference strains were prepared in New Zealand white rabbit according to standard procedures (EDWARDS and EWING, 1972; VAN DEN BOSCH et al., 1993). The purified fimbriae, emulsified in Freund´s complete adjuvant and then in incomplete Freund´s adjuvant, were injected at three–week intervals. Six weeks later a booster injection of fimbriae in Freund´s incomplete adjuvant was given. The rabbit was bled two weeks after booster injection.

Absorbed antisera. Absorption to remove antibody to non-specific or common antigens was performed with homologous strain grown for two days at 16 °C on TSA, as described by EDWARDS and EWING (1972).

Preparation of crude fimbrial extracts. For preparation of crude fimbrial extracts, isolates were grown on TSA (for maximal production of P fimbriae); bacterial cells were harvested using Tris buffer (10 mM Tris-HCL pH 7.4). The bacteria, which were grown in TSB, were centrifuged, washed and re-suspended in PBS. Samples were heated at 56 °C for 20 minutes and were homogenized for two 5-minute periods in Sorvall Omnimixer (Omni Corporation International, Waterby, CT, U.S.A.) to remove the fimbriae. Samples were centrifuged twice for 20 minutes at 8500 rpm and supernatant was retained for SDS-PAGE and immunoblotting.

Immunoblotting. SDS-PAGE was performed as described by LAEMMLI (1970). Separated bacterial components were transferred to nitrocellulose membranes by electrophoretic blotting (Uniform Electro Transfer, Paya Pajoohesh Co., Mashhad, Iran) at 100V for 2 h in 20mM Tris, 150 mM

Glycine, and 20% methanol (pH 8.6). Nitrocellulose membranes were incubated with absorbed antiserum as described by DOZOIS et al. (1995).

Results

Expression of P (F11) fimbriae. When isolates were grown on solid medium (TSA) at 37 °C, only five O1 isolates showed MRHA with human OP1 erythrocytes. In the SDS-AGE, the partially purified fimbriae from reference strain C1976 and five MRHA isolates demonstrated a major subunit of 18 kDa band. This band was also observed in immunoblotting using prepared anti-F11 serum. The18 kDa major fimbrial subunit of the avian P fimbriae reacted with the absorbed antiserum against F11 fimbriae (Fig. 1).

Expression of F1 (type 1) fimbriae. Among the 90 *E. coli* isolates, sixty-nine (76.6%) showed MSHA pattern when grown on static broth medium (TSB) at 37 °C. Examination of the partially purified fimbriae from reference strain F1ABAM and crude fimbrial extracts of MSHA isolates on SDS-PAGE demonstrated a 17 to 17.5 kDa subunit. Also in immunoblotting, hydrolysed crude fimbrial extracts of these 69 isolates demonstrated a single band with 17 to 17.5 kDa apparent molecular weight, as revealed by absorbed anti-F1ABAM serum (Fig. 2). F1 fimbriated isolates belong to different O serogroups, and some of them were untypable. The number and serogroups of MRHA and MSHA *E. coli* isolates are presented in Table 1.

Table 1. Number and serogroups of MRHA and MSHA *E.coli* isolates

O groups	N^o of isolates	N^o of MRHA isolates	N^o of MSHA isolates
O1	5	5	-
O2	13	-	11
O20	3	-	1
O36	2	-	-
O78	47	-	39
O115	1	-	1
Untypable	19	-	17
Total	90	5	69

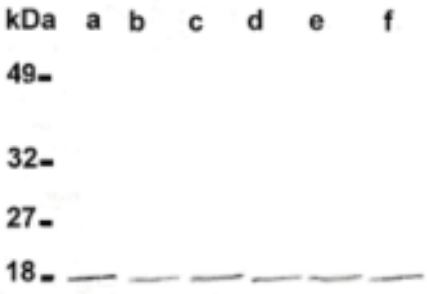

Fig. 1. Immunoblotting of crude fimbrial extracts of reference strain C1976 (a) and MRHA isolates (b, c, d, e and f) with anti-F11 serum

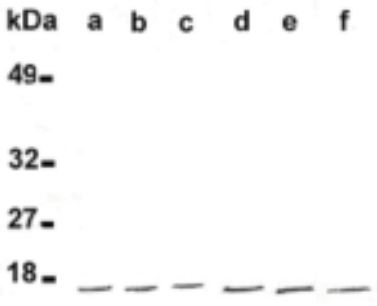

Fig. 2. Immunoblotting of crude fimbrial extracts of MSHA isolates (a, b, c, d and e) and crude fimbrial extract of reference strain BAM (f) with anti-F1A serum

Discussion

Although, scratches, injuries and trauma to the skin increase in bird density, litter and chick quality are predisposing factors for cellulitis. Nevertheless, *E. coli* is the primary causative agent of avian cellulitis (PEIGHAMBARI et al., 1995b; JEFFREY et al., 1999). Gross and microscopic findings showed that cellulitis isolates induced more severe lesions than airsacculitis and faecal isolates (PEIGHAMBARI et al., 1995b). Different O

serogroups of *E. coli* were isolated from avian cellulitis, but *E. coli* serotype was not a determining factor for cellulitis-type pathogenicity (JEFFREY et al., 1999). NGELEKA et al. (1996) examined *E. coli* isolates from avian cellulitis for the presence of DNA sequence related to F1A (fim) and P (pap) (pyelonephritis associated pili) fimbrae. They found that all isolates contain the fim gene but only 57% of these isolates exhibited MSHA of guinea pig erythrocytes. On the other hand, 51% of isolates contained pap-related DNA sequences associated with Pap fimbriae of the P family. However, only 50% of pap+ isolates exhibited MRHA of human erythrocytes, indicating that in many cases F1 and P fimbriae were not expressed (NGELEKA et al., 1996). In the present study, five (5.5%) of 90 *E. coli* isolates expressed P (F11) fimbriae, all of them belonging to O1 serogroup. P fimbriae encoded by pap gene cluster have been associated with *E. coli* isolates, causing urinary tract infections (UTI) in humans and dogs. P fimbriae may also be expressed by *E. coli* of avian origin and are closely related to P fimbriae of serotype F11 (DOZOIS et al., 1995; VAN DEN BOSCH et al., 1993). P fimbriae were expressed *in vivo* by bacteria colonizing air sacs, lungs, kidney and pericardial fluid of chickens, suggesting that P fimbriae may be involved in the colonization of systemic organs and development of septicaemia (POURBAKHSH et al., 1997a). In immunoblotting, bands of P (MRHA) fimbriae from avian *E. coli*, cross-reacted with anti-serum F11 fimbriae completely. Similarly, VAN DEN BOSCH et al. (1993) showed that P fimbriae produced by avian pathogenic *E. coli* isolates were identical to F11, as demonstrated by Western blotting and ELISA. VAN DEN BOSCH et al. (1993) reported that F11 fimbriae were expressed on 96% of serotypes most commonly encountered in avian colibacillosis isolates. DOZOIS et al. (1995) found that only a low proportion of avian pap+ *E. coli* isolates appeared to express an adhesin, with an MRHA pattern similar to that of the pap encoded P fimbriae. They have confirmed that only 5 of 14 isolates showed serological or functional evidence of production of P fimbriae (DOZOIS et al., 1995). POURBAKHSH and FAIRBROTHER (1994) isolated an *E. coli* strain of O1 serogroup from a septicaemic turkey that expressed P fimbriae related to F11 fimbriae.

In this study, sixty-nine (76.6%) of avian cellulitis *E. coli* isolates from different serogroups showed MSHA pattern and expressed F1 fimbriae

after growth in TSB. The high prevalence of F1 expressing isolates from avian cellulitis suggests a role of this fimbriae in the pathogenesis of cellulitis. *E. coli* strains that cause septicaemia in poultry often possess F1 fimbriae (DOZOIS et al., 1994). In Japan, 72.2% of *E. coli* strains were isolated from chickens with colisepticemia expressing type 1 fimbriae, while O2 and O78 were predominant serogroups (IKE et al., 1990). ERGANIS et al. (1992) proved 76.1% of Turkish *E. coli* strains (13 different serogroups) isolated from hens with colibacillosis, showing positive reaction with anti-F1 fimbrial serum in slide agglutination test. In the present study, among 90 *E. coli* isolates, O78 serogroup was the most frequent, while thirty-nine isolates from this serogroup possessed F1 fimbriae. DERAKHSHANFAR and GHANBARPOUR (2002) and CAYA et al. (1999) reported that O78 and O2 are the most frequent O groups in avian isolates. O78 serogroup is known to include virulent strains associated with severe *E. coli* infection in poultry. It is also recognized as one of the serogroups associated with enterotoxigenic *E. coli* strains that can affect humans. This could have public health implications (MESSIER et al., 1993).

PEIGHAMBARI et al. (1995b) concluded that *E. coli* from avian cellulitis were predominantly of the same O groups as those associated with respiratory and septicaemic diseases in poultry. GOMIS et al. (2001) reported that *E. coli* derived from cellulitis lesions produced virulence factors similar to those found in *E. coli* isolated from other colibacillosis lesions in poultry.

Assays for the presence of fim and pap DNA sequences produced variable results, but suggested that cellulitis isolates may express F1A and/or MRHA fimbriae (NGELEKA et al., 1996). Results of our study and comparison with other reports suggest that avian cellulitis *E. coli* isolates have virulence factors (such as fimbriae) similar to those of colisepticemic *E. coli* isolates. Studies on fim and pap fimbrial genes would assist in elucidating similarity and/or difference(s) between fimbriae of cellulitis and colisepticemic *E. coli* isolates.

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SAŽETAK

Avijarni celulitis uzrokuju virulentni sojevi *E. coli*. Svrha istraživanja bila je ustanoviti P (F11) i F1 fimbrije u 90 izolata *E. coli* uzročnika avijarnog celulitisa. Izolati su bili šest puta uzastopno pasirani na čvrstoj podlozi i bujonu za rast fimbrija. Pet (5,5%) izolata serološke skupine O bili su manoza-rezistentne hemaglutinacijske aktivnosti (MRHA) uzgojeni na čvrstoj hranjivoj podlozi.

Postupkom poliakrilamid gel elektroforeze (SDS-PAGE) sirovi fimbrijski ekstrakti MRHA soja sadržavali su veću fimbrijsku podjedinicu od 18 kDa. Ta je podjedinica također reagirala s protuserumom za F11 u testu imunobloting. Šezdesetdevet (76,6%) izolata *E. coli* iz različitih seroloških skupina uzgojenih na statičnom hranjivom bujonu sadržavalo je manoza osjetljive hemaglutinacijske uzorke. Imunobloting testom dokazano je da sirovi fimbrijski ekstrakti manoza osjetljivih izolata sadrže jednu podjenicu molekulske mase od 17 do 17,5 kDa što je otkriveno apsorpcijom anti F1A seruma. Čini se da izolati *E. coli* koji uzrokuju avijarni celulitis imaju F1 i P fimbrije slične onim izolatima *E. coli* koji uzrokuju koliseptikemiju.

Ključne riječi: celulitis, tovni pilići, *E. coli*, fimbrije F1, fimbrije P