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Review article

Whatever makes them stick – Adhesins of avian pathogenic *Escherichia coli*

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) is associated with extraintestinal infections and the development of colibacillosis, causing high mortality in farm birds and extensive losses in the poultry industry worldwide. The virulence of APEC is a complex phenomenon associated with numerous mechanisms involving a variety of extracellular and intracellular structures to overcome host barriers. Initial bacterial attachment or adhesion to host cells is vital to bacterial pathogenesis and is determined by various adhesins. These proteins protect pathogens against possible host defense mechanisms, enabling the effective use of other virulence attributes. Considering this property, the current review provides a systematic and in-depth analysis of the latest information on adhesins analyzed in APEC strains. This review discusses in detail each of the adhesin types, such as fimbrial chaperone-usher, fimbrial curli, nonfimbrial and atypical adhesins, and their components, presenting an opportunity to gain a better understanding of different adhesins and mechanisms employed in pathogenesis. Additionally, the article scrutinizes and notes missing information and potential studies that need to be undertaken to develop a complete understanding of APEC adhesion.

1. Introduction

Escherichia coli primarily colonizes the digestive tract of both humans and animals, where as a commensal, it is part of the natural microbiome and contributes to the maintenance of the organism's homeostasis. It is an opportunistic bacterial species and becomes pathogenic under the compromised immune system of the host (Stordeur et al., 2002). Additionally, there are groups of highly adapted strains that have acquired attributes of virulence and effectively infect animals. Given that the set of virulence factors and pathogenicity to a particular host are identical, these groups are referred to as pathotypes. However, taking into account the location of infection, *E. coli* strains are classified into two categories: intestinal pathogenic *E. coli* (InPEC) and extraintestinal pathogenic *E. coli* (ExPEC). InPECs are further categorized as the following pathotypes: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), enteroinvasive (EIEC), diffusely adherent (DAEC) and Shiga toxin-producing (STEC). Classification of extraintestinal strains involves pathotypes that are particularly characteristic of humans, uropathogenic (UPEC), sepsis-associated (SEPEC), and neonatal meningitis (NMEC), and those responsible for infections in animals, such as avian pathogenic *E. coli* (APEC), the etiological factor of colibacillosis (Ewers et al., 2007; Johnson et al., 2007). A high mortality rate is observed in farm birds due to APEC, causing significant losses in the poultry industry worldwide. Apart from the economic impact, it is considered a threat to human health because of its zoonotic potential (Ewers et al., 2007; Antão et al., 2009). For more detailed information about APEC epidemiology, pathogenesis, clinical manifestations and diagnosis of colibacillosis, see the review by Nolan et al. (2017).

The virulence of microorganisms, including APEC, is a complex phenomenon associated with numerous mechanisms involving various extracellular and intracellular structures. There are several well-understood bacterial mechanisms and strategies that pathogens use to establish close contact with the host, to overcome its barriers and then to provide appropriate conditions for survival and replication. Colonization and infection begin with the pathogen's proximity to the host's tissues, while the moment of initial contact determines its success in

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overcoming the barrier responsible for mechanical removal of pathogens (Antão et al., 2009). In the case of animals, this natural protection mechanism, constituting the first line of defense, involves the production of secretions and excretions - saliva, tears, urine, mucus, as well as some physiological activities - intestinal peristalsis, sneezing and coughing. Adhesion, *i.e.*, attachment to host cells, protects pathogens against possible defense mechanisms and removal from the host surface. Additionally, it enables the effective use of other virulence attributes, including several secretion systems, which allow bacterial effector proteins to be introduced into host cells. A special role at this stage is played by the extracellular bacterial appendages, including fimbriae and a number of other adhesins responsible for highly specific binding to receptors on the host cell surface (Rendón et al., 2007).

The characterization and role of individual adhesins in APEC pathogenesis have been allotted little attention in previously published reviews (Dho-moulin et al., 1999; Dziva and Stevens, 2008). Due to the wider scope of these articles, which include other virulence determinants, only the most common adhesins were discussed. Considering this, the aim of this review is to collect and systematize the available scientific reports, focusing on adhesins identified in APEC strains, to achieve a better understanding of different mechanisms involved in their contribution to pathogenesis.

The current system of classification distinguishes between several types of bacterial adhesins participating in the initial stage of infection. The basic systematic classification is the result of differences observed after stabilization with appropriate antibodies and takes into account fimbrial and nonfimbrial adhesins (Shu Kin So et al., 2007). Another way to characterize and classify adhesins is based on their ability to bind and agglutinate red blood cells. Hemagglutination in the presence and absence of mannose provides information about the receptor type involved in the interaction of bacteria with host cells (Labigne-Roussel et al., 1984; Nowicki et al., 1990). Adhesins cause mannose-sensitive agglutination, as the receptor contains a mannose-containing glycan structure, and in the case of mannose-resistant agglutination, adhesion to erythrocytes is mediated by a nonmannose-containing receptor. In addition, due to numerous biochemical and genetic comparisons, a nomenclature was developed that classifies fimbriae depending on differences in their synthesis processes. On this basis, the following fimbriae are distinguished: chaperone-usher fimbriae biosynthesized with the participation of chaperone proteins (Snyder et al., 2005; Korea et al., 2010), type IV pili assembled by a type II secretion system, and curli formed as a result of nucleation and precipitation processes (Barnhart and Chapman, 2006; Shu Kin So et al., 2007) (Fig. 1). It should be emphasized that the adhesion process also involves structures and mechanisms that indirectly contribute to the adhesion and colonization of host tissues. These structures, whose primary function is not adhesion-related, are known as atypical structures. In APEC strains, this role is fulfilled by flagella, lipopolysaccharide (LPS) and the type VI secretion system (Table 1).

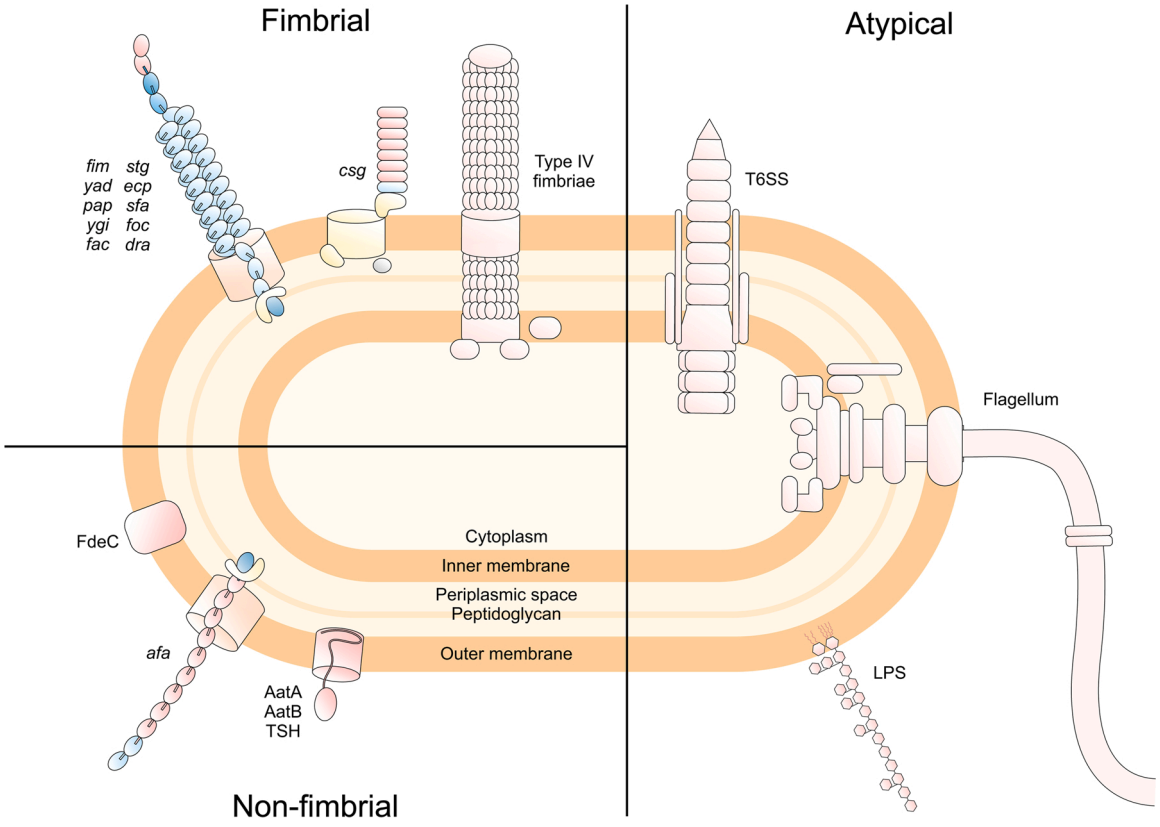

Fig. 1. Schematic representation of the adhesins identified in APEC strains.
Adhesins found on the surface of APEC cells may be classified as fimbrial, nonfimbrial, and atypical. Fimbrial adhesins (top-left) include different types of structures and are classified depending on their biosynthesis process. Synthesis of the first type is facilitated by chaperone proteins and therefore they are called chaperone-usher fimbriae (*fim*, *yad*, *pap*, *ygi*, *fac*, *stg*, *ecp*, *sfa*, *foc*, and *dra*), whereas the second type, *i.e.*, curli fimbriae (*csg*), is assembled in a process of nucleation and precipitation, and the third type, represented by type IV fimbriae, is biosynthesized by a type II secretion system. Nonfimbrial adhesins (bottom-left) comprise afimbrial adhesins (*afa*) and autotransporters. Autotransporters are further divided into classical autotransporters (AatA, AatB, and temperature-sensitive hemagglutinin, TSH) and inverse autotransporters (intimin-like FdeC). Atypical adhesins (right) are a group of structures that contribute to adhesion while possessing other primary functions. These adhesins include the type VI secretion system (T6SS), flagella, and lipopolysaccharide (LPS). Depicted structures are not to scale.

Table 1
APEC adhesin characteristics.

Type	Adhesin name	Cluster/Gene name	Receptor	Adhesion in chicken *in vitro/in vivo* model
FIMBRIAL chaperone-usher	Type 1 fimbriae	*fim*	Mannose oligosaccharides	Trachea and intestines, ileal and colonic enterocytes
	P fimbriae	*pap*	Specific glycosphingolipids (α-D-Galp-(1−4)-β-D-Galp)	Ileal and colonic enterocytes
	Stg fimbriae	*stg*	Not identified	Respiratory epithelium (lungs, air sacs)
	S fimbriae	*sfa*	Neuraminic (sialic) acid; β-GalNac-1,4-Gal	Not determined
	AC/I fimbriae	*fac*	Neuraminic (sialic) acid	Trachea
	F1C fimbriae	*foc*	Glicolipid-containing lactosyl ceramide; β-GalNac-1,4-Gal	Not determined
	Dr fimbriae	*dra*	Dr antigen; Decay-accelerating factor (DAF)	Not determined
	Escherichia common pilli (ECP)	*ecp*	Not identified	Spleen and liver epithelium, erytrocytes
	Yad fimbriae	*yad*	Not identified	Spleen and lung epithelium
	Yqi fimbriae	*yqi*	Not identified	Fibroblasts and lung epithelium
FIMBRIAL curli	Curli fimbriae	*csg*	Matrix and serum proteins – plasminogen, fibronectin, laminin	Trachea and intestines
FIMBRIAL type II secretion system	Type IV fimbriae	*pil; rci*	Not identified	Not determined
	Afimbrial adhesins (AFA)	*afa*	Decay-accelerating factor (DAF)	Not determiend
NONFIMBRIAL	Temperature-sensitive haemagglutinin	*tsh*	Hemoglobin, fibronectin, type IV collagen	Erythrocytes, air sacs
	Autotransporter adhesins A/B	*aatA/aatB*	Not identified	Fibroblasts, lung epithelium
	FdeC	*fdeC*	Not identified	Intestinal epithelium
	Flagella	*fliC*	Not identified	Intestinal epithelium
ATYPICAL	LPS	*waa/rfa*	CD14/TLR4/MD2	Fibroblasts, Intestinal epithelium
	Type VI secretion system	*vgrG/clpV*	Not identified	Epithelial cells

2. Fimbrial adhesins

2.1. Chaperone-usher

2.1.1. Type 1 fimbriae

Type 1 fimbriae are the most common and best-characterized adhesins in the *Enterobacteriaceae* family. This type of fimbriae belongs to the group of mannose-dependent fimbriae, as their binding ability to receptors is inhibited by mannose or its analogs (Table 1). Analyses carried out in 2004 revealed that they are present in almost all APEC strains (Ewers et al., 2004). Type 1 fimbriae are encoded by chromosomal operon of nine *fim* genes, as shown in Fig. 2. Four of these genes, *fimA, fimF, fimG* and *fimH,* are responsible for the biosynthesis of the fimbrial shaft; *fimC* and *fimD* encode chaperone and usher, respectively; and *fimI* expresses a fimbriae-like protein, the role of which is not yet fully understood (De Pace et al., 2010). At the distal end of each appendage, the FimH protein is present and is responsible for binding to glycoprotein receptors on host cells. Expression of type 1 fimbriae is phase-variable and is controlled by *fimB* and *fimE* genes. Strains possessing this adhesin can alter its fimbriation back and forth, *i.e.*, from fimbrial to an afimbrial phase and *vice versa*. Importantly, *in vivo* studies using APEC strains showed the expression of genes encoding type 1 fimbriae in the trachea and, to a lesser extent, in air sacs and lungs. Its presence has not been reported in other organs or blood, indicating a key role in colonization of the lower respiratory tract (La Ragione et al., 2000a, 2000b). In 1998 and 2000, studies undertaken to compare the degree of adhesion of wild-type APEC strains and *Δfim* and *ΔfimH* mutants showed that the introduced mutations did not affect bacterial ability to bind to chicken cells of trachea and air sacs (Marc et al., 1998). Research conducted in 2000 by Arne et al. led to similar conclusions, where the authors examined the binding ability of a *ΔfimH* mutant to chicken tracheal cells and demonstrated superior adhesive properties of

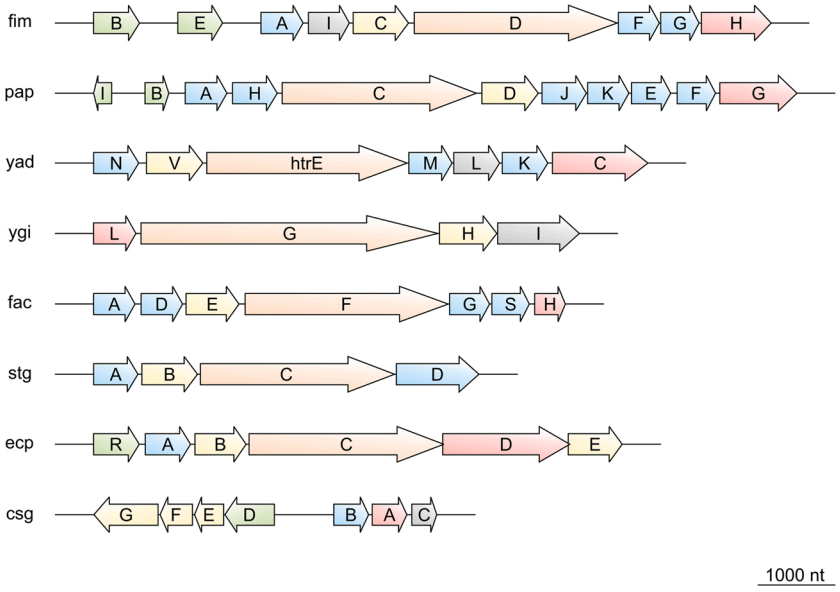

Fig. 2. Genetic organization of operons encoding fimbrial adhesins.
Type 1 fimbriae (*fim*) are the most common and best-characterized adhesins encoded by the chromosomal operon of nine genes. P fimbriae (*pap*) are encoded by a cluster of eleven genes and are morphologically identical to type 1 fimbriae. The Yad fimbriae (*yad*) operon of seven genes was identified as a homolog of the *fim* cluster. Ygi fimbriae (*ygi*) are encoded by a cluster of four genes found exclusively in extra-intestinal *E. coli* pathotypes. Avian *E. coli* I fimbriae (*fac*) is composed of seven genes showing high similarity to the *sfa* cluster. The Stg fimbriae (*stg*) operon consists of four genes and was identified due to sequence homology to the *stg* cluster from *Salmonella* Typhi. *Escherichia* common pili (*ecp*) are encoded by an operon of six genes that are widespread among both pathogenic and nonpathogenic *E. coli* strains. All adhesins mentioned above belong to the chaperone-usher group of fimbriae. The curli fimbriae (*csg*) encoded by a cluster of seven genes belong to a separate group of fimbrial adhesins. Colors correspond to the functions performed by gene products: green – regulatory, blue – structural, yellow – chaperone, orange – usher, red – adhesion, gray – unknown.

this mutant compared to the wild-type strain (Arne et al., 2000). These results may be a consequence of the compensatory effect, in which suppressed expression of one fimbrial cluster may result in overexpression of other adhesins and consequently increase adhesion capacity of the deletion mutant to a cell line or tissue. Similar observations were made in APEC χ7122 *Δstg* and UPEC CFT073 *Δfim* mutants, which showed a higher level of adhesion than wild-type strains (Snyder et al., 2005; Lymberopoulos et al., 2006).

2.1.2. P fimbriae

Morphologically, type 1 and P fimbriae are identical, but P fimbriae are distinguished by specific binding to glycolipid receptors containing Gal-a-(1,4)Gal galactoside residues (Table 1) (Ewers et al., 2004). P fimbriae are found in 25 % of APEC isolates (Ewers et al., 2007), and their presence is determined by the expression of the chromosomal *pap* operon, composed of 11 genes, located on the pathogenicity island. P fimbriae are composed of a proteinaceous fragment (PapG) responsible for the binding of the adhesin to the host receptor and a structural subunit (PapA). They belong to the group of mannose-resistant adhesins and exhibit binding ability to receptors even in the presence of mannose. With regard to other adhesins, P fimbriae have not been analyzed thoroughly, and their role in the pathogenesis of APEC remains unclear (Stordeur et al., 2004). According to the results of *in vivo* experiments, P fimbriae are involved in colonization of the lower respiratory tract by APEC, as indicated by their increased expression in air sacs and lungs (Kariyawasam and Nolan, 2009). Additionally, *pap*-positive strains are more often isolated from chickens suffering from diseases of these parts of the respiratory system compared to healthy birds (Dozois et al., 1995). In contrast, *in vitro* studies excluded participation of these adhesins in attachment to the upper respiratory tract, trachea and pharynx, which may be explained by the lack of appropriate galactoside receptors in these organs (Kariyawasam and Nolan, 2009).

2.1.3. Yad fimbriae

In recent years, thanks to rapidly developing bioinformatics and increasing popularity of sequencing methods, the *yad* operon was identified, which is a homolog of the *fim* cluster (Fig. 2) (Korea et al., 2010). Deletion of the *yadL* gene in the APEC O78 strain slightly decreased the adhesive capacity of this strain, which confirmed Yad as a virulence factor and adhesin of APEC (Dziva et al., 2013) (Table 1). Moreover, a 2015 study by Verma et al. carried out on an *in vivo* model, suggesting a role for Yad in the initial stages of infection. This is indicated by increased expression of the *yadC* gene in the lungs compared to the expression levels observed in the spleen (Verma et al., 2015).

2.1.4. Ygi fimbriae

The putative *yqi* gene cluster was identified in 2009 in the genome of the APEC IMT5155 strain (Table 1). It was observed that the cluster included *yqiG* and *yqiH* genes coding for auxiliary proteins, *yqiI* with unknown function and *ygi* (or *ygiL*), which displays adhesive properties (Fig. 2) (Antão et al., 2009). Comparative analyses have shown that this cluster occurred only in extraintestinal *E. coli* pathotypes, UPEC (65.9 %), NMEC (60.0 %), APEC (54.4 %), and SEPEC (52.6 %), and was not present in a group of intestinal pathogens and nonpathogenic strains (Antão et al., 2009). The hypothesis is therefore that Yqi is a unique virulence factor utilized in extraintestinal infections, including respiratory tract infections of APEC etiology. According to Antão et al., deletion of the *yqi* gene in the APEC IMT5155 strain results in a significant reduction in adhesion to fibroblasts and epithelial cells. *In vivo* experiments confirmed that Ygi fimbriae increased the capacity for colonization of chicken lungs. Importantly, no analogous observations have been made in the case of infections of other organs, such as the brain, lungs, kidneys, spleen, or heart, which may indicate that Yqi binds to a previously unidentified receptor present only on pulmonary epithelial cells (Antão et al., 2009).

2.1.5. AC/I fimbriae

In 2000, a new fimbrial cluster named AC/I (avian *E. coli* I) or *fac* was identified in the genome of the APEC O78 strain (Fig. 2). These fimbriae do not have the ability to hemagglutinate erythrocytes but are involved in adhesion to avian tracheal cells (Babai et al., 2000). They are present in only 10 % of APEC isolates, mainly in strains belonging to the O78 serotype, possibly increasing bacterial virulence in avian colisepticemia (Table 1) (Yerushalmi et al., 1990).

2.1.6. Stg fimbriae

In 2006, a new gene cluster was identified in the APEC O78:K80 genome due to observed homology and was named the ortholog of the *stg Salmonella* Typhi cluster (Lymberopoulos et al., 2006). Lymberopolous et al. showed that *stgC* sequences were present in 133 of 298 (44.6 %) APEC isolates. The *stg* operon consists of four genes, *i.e.*, *stgABCD* (Fig. 2), whose protein products have the following functions: StgA - main constituent of the fimbrial shaft; StgB - periplasmic chaperone; StgC - transport of StgA and StgD across outer cell membrane; and StgD - functional adhesin responsible for interaction with receptors. As indicated by the study with the APEC O78 strain, Stg fimbriae play an important role in the colonization of the avian respiratory tract. An experiment comparing adhesion levels of the wild-type strain and *Δstg* mutant showed that deletion of the gene cluster reduced the adhesion capacity to the air sacs. However, the mutation did not affect the degree of lung colonization. Moreover, induced expression of *stg* in a nonpathogenic strain of *E. coli* K-12 contributed to an increase in adhesion to avian and human lung cells *in vitro* (Lymberopoulos et al., 2006).

2.1.7. Escherichia common pili (ECP)

The term ‘common pili’ reflects the widespread distribution of this adhesin among both pathogenic and nonpathogenic strains of *E. coli* (Rendón et al., 2007). Their function is conditioned by the expression of an operon composed of six genes: *ecpRABCDE*, of which EcpA is the main subunit of fimbriae, EcpD is responsible for binding to the receptor, EcpR is a transcription regulator, and EcpB, EcpC and EcpE are chaperones (Fig. 2) (Rendón et al., 2007). The presence of the *ecpA* gene was confirmed in 76 % of APEC isolates, which suggests that this adhesin is an important virulence factor involved in the initial stage of infection (Stacy et al., 2014). This is supported by the results of studies in an *in vivo* chicken model, which showed that the deletion of the *ecpA* and *ecpD* genes reduced the virulence of APEC to one-day-old chickens. Additionally, it has been proven that the *ΔecpD* mutant’s ability to cause systemic infection was significantly reduced along with the degree of liver and spleen colonization (Stacy et al., 2014). The role of ECP in the pathogenesis of APEC and EHEC was also verified in an *in vitro* model. These fimbriae have been shown to be involved in adhesion to HeLa and HEp-2 cell lines (Rendón et al., 2007; Stacy et al., 2014). In 2020, a mutant with insertional inactivation of the *ecpR* gene - a positive regulator of the expression of the *ecp* operon - was subjected to a similar analysis. This strain has been shown to adhere more strongly to the CHIC-8E11 cell line than to the wild-type strain (Table 1) (Ali et al., 2020). Therefore, it is suggested that these observations could be explained by the phenomenon of compensation, in which lack of ECP fimbriae expression leads to overexpression of other adhesin-encoding genes (Ali et al., 2020). However, this hypothesis requires further in-depth analysis.

2.2. Curli

The term ‘curli’ was proposed in 1989, referring to the third class of *E. coli* surface organelles, in addition to flagella and other types of fimbriae described thus far. These organelles are composed of subunits of one type of protein called curlin, encoded by the *csgA* gene (Barnhart and Chapman, 2006). Curli fimbriae are particularly characteristic of *E. coli* strains isolated from human infections, but their role among APEC

isolates was also analyzed, demonstrating that hemagglutination capacity, fibronectin binding and curli biosynthesis occur synergistically (Table 1). It was also observed that 99 % of *E. coli* strains isolated from birds have the *csgA* gene, which is responsible for biosynthesis of this adhesin. Curli fimbriae are encoded by the chromosomal *csg* gene cluster, consisting of two differentially transcribed operons - *csgABC* and *csgDEFG*. As shown by studies in an *in vivo* chicken model, the presence of curli is important for the adhesion of the *E. coli* O78 strain and its colonization, especially in the cecum. Additionally, it is crucial for the binding of bacteria with extracellular matrix proteins and other plasma proteins, such as fibronectin, plasminogen and laminin (La Ragione et al., 2000b; Gophna et al., 2002). *In vitro* studies using *E. coli* O78 and intestinal explants also indicate the participation of curli fimbriae in adhesion. It was demonstrated that the mutant with insertional inactivation of the *csgA* gene showed an 80 % reduction in adhesion compared to the wild-type strain (La Ragione et al., 2000a). In addition, based on *in vitro* studies using the same strain, it was found that curli fimbriae mediated bacterial internalization by HeLa eukaryotic cells (Gophna et al., 2001).

3. Nonfimbrial adhesins

3.1. Afimbrial adhesins (AFAs)

As early as 1984, Labigne-Roussel and coworkers showed that among ExPEC strains, there are isolates capable of agglutinating erythrocytes, and the process was not inhibited by the presence of D-mannose. This phenomenon has been referred to as mannose-resistant hemagglutination (MRHA) and is dependent on the presence of AFA-I protein, which is characteristic of uropathogenic strains (Labigne-Roussel et al., 1984). Importantly, bacteria lacking adhesins were unable to effectively colonize epithelial cells, indicating the role of nonfimbrial adhesins in the first stage of pathogenesis. As shown by phylogenetic analysis, this protein is much less common in APEC strains and occurs in less than 10 % of isolates (Alizade et al., 2017). It is encoded by a cluster of five genes, *i.e.*, *afaC, afaB, afaD, afaE*, and *afaA*, with a total length of 6.7 kb (Fig. 3) (Labigne-Roussel and Falkow, 1988). Of these, only *afaB*, *afaC*, and *afaE* are responsible for adhesin expression and adherence to epithelial cells (Labigne-Roussel et al., 1984). AfaB is the major structural protein, while AfaC is the precursor to MRHA. Subsequent studies using hybridization and Western blotting proved that the gene clusters structurally related to the *afa* operon described earlier were present in the genomes of ExPEC but encoded antigenically different adhesins. All clusters share the presence of a highly conserved 4.1 kb region consisting of *afaB, afaC* and *afaD* genes and *afaE* sequences of high heterogeneity. Thus, the presence of four different operons, *afa-1, afa-2, afa-3* and *afa-4*, encoding Afa-I, Afa-II, Afa-III and Afa-IV adhesins, respectively, was demonstrated (Labigne-Roussel and Falkow, 1988). Afa-III and Afa-I belong to the group of hemagglutinins, which also includes Dr fimbriae. Together, they form a heterogeneous family referred to as the family of Dr adhesins (Nowicki et al., 1990). A few years later, changes in the nomenclature were made due to the diversity of *afaE* genes, and from then on, AfaE-I - AfaE-IV adhesins were distinguished. In 1995, AfaE-V was described as characteristic of strains isolated from humans, followed by AfaE-VII and AfaE-VIII identified in oxen (Lalioui et al., 1999). In 2002, Stordeur et al. detected a cluster of *afa-8* genes in *E. coli* strains infecting birds, which, as they found, contributed to the increase in bacterial virulence in chickens in a manner similar to *pap*--positive strains (Table 1) (Stordeur et al., 2002).

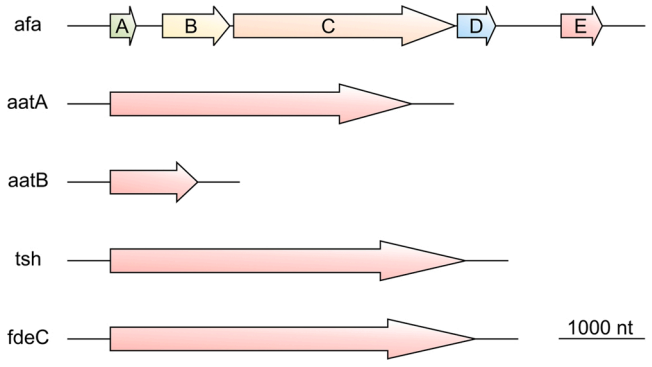

Fig. 3. Genetic organization of operons encoding nonfimbrial adhesins.
The group of nonfimbrial adhesins comprises afimbrial adhesins (*afa*) and autotransporters (AatA, AatB, TSH, FdeC). Seven types of clusters encoding Afa adhesins have been identified until now, but all of them share the presence of *afaB*, *afaC*, *afaD*, and *afaE* genes. Due to the genetic diversity of *afaE*, only a cluster encoding Afa-I adhesins is depicted. Colors correspond to the functions performed by gene products: green – regulatory, blue – structural, yellow – chaperone, orange – usher, red – adhesion.

3.2. Autotransporters

3.2.1. AatA and AatB

Afimbrial adhesins identified in APEC strains also include autotransporters, classified into the type V secretion system (Table 1). They are a product of the expression of the *aatA* gene, present in over 40 % of the genomes of APEC, and *aatB*, whose prevalence in this pathotype is 26.4 % (Li et al., 2010; ZhuGe et al., 2013). Structurally, AatA and AatB are classic autotransporters composed of three domains: signal peptides, passenger domains and translocation domains (ZhuGe et al., 2013). The AatA amino acid sequence shares a high similarity with that of the AidA-I adhesin, which is responsible for the diffusively adhering phenotype of the *E. coli* O157:H7 strain (Konieczny et al., 2001). This suggests a possible role for AatA as an adhesin. As demonstrated in 2010, overexpression of the *aatA* gene increased the adhesive capacity of the APEC strain to a chicken fibroblast cell line (Li et al., 2010). Three years later, the *aatB* gene was identified, the deletion of which also resulted in a change in the adhesive phenotype. An *ΔaatB* mutant adhered less to the chicken DF-1 fibroblast cell line than the wild-type strain, while in an *in vivo* duck model, its reduced lung colonization capacity during systemic infection was demonstrated (ZhuGe et al., 2013).

3.2.2. Temperature-sensitive hemagglutinin (TSH)

TSH is an APEC-expressed protein that has the ability to hemagglutinate chicken erythrocytes at 26 °C in a low-osmolarity environment (Dozois et al., 2000). Its amino acid sequence is highly similar to *Neisseria gonorrhoeae* and *Hemophilus influenzae* IgA-type serine proteases, and since TSH is secreted in a similar manner to these proteases, it has been classified as a subfamily of autotransporter proteins called *Enterobacteriaceae* serine protease autotransporters (Kobayashi et al., 2010). TSH is synthesized from a 140 kDa precursor, which is then cleaved in the bacterial periplasm into two subunits. The smaller subunit serves as the passenger domain, and the larger subunit is secreted into the external environment. Prior to cleavage, it remains temporarily bound to the outer membrane, where it participates in the adhesion process. After being released into the environment, it shows proteolytic activity, which indicates that TSH is a bifunctional protein with adhesive and hydrolytic abilities (Kostakioti and Stathopoulos, 2004). As indicated by scientific reports, apart from adherence to erythrocytes, hemoglobin and extracellular matrix proteins including fibronectin and collagen IV, hemagglutinin also has a proteolytic effect on casein (Table 1) (Kobayashi et al., 2010). Importantly, the gene encoding TSH, *tsh*, was found only in APEC genomes. It has not been identified in *E. coli* isolates from healthy chickens, suggesting involvement in pathogenesis. Additionally, the results of the study completed in 2000 suggest that TSH may contribute to the colonization of bird air sacs but is not involved in further stages of infection (Dozois et al., 2000).

3.2.3. FdeC

The FdeC adhesin is referred to as an intimin-like adhesin due to its high functional and structural similarity to intimin (Nesta et al., 2012). Its functionality is conditioned by the expression of the *fdeC* gene and is characterized by more than 91 % similarity of its amino acid primary structure among pathotypes characteristic of both humans and animals. Based on an analysis using 128 isolates, it was shown that its occurrence ranged from nearly 29 % among nonpathogenic strains up to 100 % among EPEC, EAEC and STEC (Nesta et al., 2012). The results obtained from adhesion assays with the CHIC-8E11 chicken enterocyte cell line and APEC IMT5155 strain indicated the influence of FdeC on adhesion to these cells (Table 1). Ali et al. observed that in the case of the tested cell line, the *fdeC* transposon mutant was characterized by a greater degree of adhesion than the wild-type strain (Ali et al., 2020).

4. Atypical adhesins

4.1. Flagella

Flagella are long protein structures (approx. 10 μm) composed of three characteristic parts. In bacterial cell envelopes (cytoplasmic and outer membranes), they are anchored by the basal body, connected to the fiber *via* a flexible connector or a hook constructed from the FlgE protein. The basal body consists of four rings and is responsible for motor movement, while the fiber is composed of spiral rows of axially arranged flagellin monomers called FliC. The FliD protein located at the distal end of the fiber acts as a cap (Macnab, 2003). Flagella are primarily characterized as virulence factors that allow the movement of microorganisms and enable access to hard-to-reach niches. However, they have also been shown to be involved in the formation of bacterial biofilms and possess an immunomodulatory function by inducing interleukin-8 synthesis in intestinal epithelial cells (Zhou et al., 2003). Other scientific reports indicate the participation of flagella in adhesion and invasion by avian pathogenic *E. coli* O78:K80. An analysis carried out using mutants with insertional inactivation of the *fliC* gene showed a significant reduction in adhesion capacity of the mutant to the HT2916E mucin-secreting cell line compared to the wild-type strain. However, a similar effect was not observed in the absence of mucin, which proves flagella is a factor determining the penetration of the mucin layer in the process of APEC adhesion to epithelial cells (La Ragione et al., 2000a). In addition, experiments with one-day-old chicks have shown that flagella favor the persistence of APEC in the chicken gut (La Ragione et al., 2000a). However, further research is required to clearly define the role of this virulence factor in adhesion. This is indicated by experiments carried out with the APEC ONT:H31 strain and mutants with the *flgE* gene deletion. This mutation did not affect adhesive capacity in an *in vitro* assay with chicken CEF fibroblasts (de Paiva et al., 2016). On the other hand, screening for the adhesion of APEC IMT5155 strain transposon mutants to CHIC-8E11 chicken intestinal epithelial cells showed that strains with insertional inactivation of the *flgK* and *fliP* genes exhibited reduced adhesion to these cells (Table 1) (Ali et al., 2020).

4.2. Lipopolysaccharide

The main component of the outer bacterial membrane, known as LPS, is also referred to as an 'atypical adhesin' (Fig. 1). Lipopolysaccharides play a vital role in the pathogenesis of gram-negative bacteria, including *E. coli*. LPS consists of 3 regions: lipid A, the oligosaccharide core and an O-antigen. Lipid A is responsible for the toxic effects of LPS, which is why it is referred to as an endotoxin. In turn, the task of the O-antigen is to enable efficient bacterial colonization of host tissues and to ensure resistance to the complement system (Bravo et al., 2008). Despite the high diversity of the O-antigen, only three biosynthesis pathways have been identified: Wzy-dependent, ABC transporter-dependent and synthetase-dependent. As indicated by the latest research, deletion mutations of the *wza* and *waaL* genes affect the adhesion phenotype of APEC strains, which indicates that the O-antigen is an adhesive factor. The *Δwse* mutant adhered to a chicken fibroblast cell line much less strongly than the wild-type strain along with reduced mobility and biofilm formation capacity (Zuo et al., 2019). A similar outcome was obtained in studies using the *ΔwaaL* mutant, which showed lower adhesion to fibroblasts than the wild-type strain (Han et al., 2014). Ali et al. (2020) indicated that transposon mutants of the *wzx, fdtA,* and *rfbD* genes and of the gene encoding glycosyltransferase group 1 (AJB35136) lost their ability to adhere to CHIC-8E11 cells. This is the only study performed on chicken intestinal epithelial cells involving the CHIC-8E11 cell line and the APEC IMT5155 strain and demonstrates the effect of LPS on adhesion (Ali et al., 2020).

4.3. Type VI secretion system

The type VI secretion system (T6SS) is a protein secretion system that can impact prokaryotic and eukaryotic cells by delivering a broad range of toxins. Its role in bacterial pathogenicity can be either direct or indirect. It was demonstrated that in pathogenic *E. coli* strains, T6SS is involved in cell adhesion, survival in macrophages or systemic proliferation. Although the functions of most T6SS proteins remain unknown, important roles of three of them have been identified, *i.e.*, hemolysin-regulated protein (Hcp), valine-glycine-motif repeating protein (VgrG) and protein providing energy for transport (ClpV). The Hcp protein is suggested to form hexamers with both secretory and structural functions. VgrG, with various extensions of the C-terminal domain, is involved in diverse processes, such as host actin cross-linking, peptidoglycan degradation, and ADP-ribosylation of host proteins. The action of ClpV is based on the creation of a hexameric channel that allows ATP hydrolysis-dependent transport of proteins (Ma et al., 2014). De Pace et al. showed that mutations in the core genes *clpV* and *hcp* in the APEC SEPT362 strain reduced adhesive capacity and actin rearrangement in epithelial cells (Table 1). Moreover, deletions of T6SS genes reduced the expression of type 1 fimbriae and flagella, structures necessary for the successful initial phase of pathogenesis and biofilm formation (De Pace et al., 2010).

5. Conclusions

We have presented various studies describing advancements in research dedicated to APEC adhesins and their role in colonization and pathogenesis in the host. Although much has been revealed about this topic, there are still significant scientific questions that remain unanswered.

Dozens of adhesion factors have been identified in *E. coli*, but the prevalence of the majority of adhesion factors in APEC has not been evaluated thus far. There is no doubt that a large-scale bioinformatic analysis should also be performed to identify and study the adhesiome of APEC. Moreover, such analyses should focus not only on the frequency of previously reported adhesion factors but also on searching for new adhesins that might be specific to APEC with the use of prediction tools. Knowledge of the complete adhesiome in APEC will be helpful in the selection of the most prevalent adhesins and the design of effective combination vaccines to prevent or lower APEC infections in poultry with a reduced probability of vaccine antigenic escape by pathogens and therefore curtail the use of antimicrobials.

New adhesins will require determination of their biological role in the pathogenesis of avian colibacillosis. There are already known adhesins, *e.g.*, S fimbriae, F1C fimbriae, Dr fimbriae, and type IV fimbriae, that have been detected in APEC genomes, but their roles in APEC virulence have not been studied so far, while others, such as FdeC or Ygi fimbriae, have been studied infrequently. Another important issue that should be taken into account in such studies is strains. In previous works, which are included in this review, 17 different strains were used to investigate the adhesion of APEC. Different genomic backgrounds, such as adhesiome and/or single nucleotide polymorphisms (SNPs) in

coding sequences of adhesins, might have a substantial impact on the results. We anticipate that the results from bioinformatics analysis will provide information that will aid in the selection of representative strains with known adhesin profiles and adhesin variants. The influence of SNPs in adhesins has been studied in many *E. coli* pathotypes, but reports concerning APEC are missing. Therefore, such analyses would provide new information regarding the evolution of virulence and host adaptation of APEC *via* point mutations in adhesins. Our review also highlights that current adhesin research lacks information about receptors for numerous adhesins (Table 1). We think that a deeper understanding of the adhesion process by receptor determination can be a starting point leading to new and innovative methods of prevention and treatment of bacterial diseases based on blocking adhesin-receptor interactions and inhibiting the biosynthesis of bacterial adhesins or host receptors. Such studies require proteomics analyses, extensive screening of receptor/chemical compound libraries and adhesin or receptor crystal structure determination. The results of these studies would further contribute to understanding the mystery of APEC adhesins and provide new tools to meet the challenges of prevention or treatment of antibiotic-resistant bacterial infections.

Declaration of Competing Interest

The authors report no declarations of interest.

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