Review article

Initial adherence of EPEC, EHEC and VTEC to host cells

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Abstract – Initial adherence to host cells is the first step of the infection of enteropathogenic *Escherichia coli* (EPEC), enterohaemorrhagic *Escherichia coli* (EHEC) and verotoxigenic *Escherichia coli* (VTEC) strains. The importance of this step in the infection resides in the fact that (1) adherence is the first contact between bacteria and intestinal cells without which the other steps cannot occur and (2) adherence is the basis of host specificity for a lot of pathogens. This review describes the initial adhesins of the EPEC, EHEC and VTEC strains. During the last few years, several new adhesins and putative colonisation factors have been described, especially in EHEC strains. Only a few adhesins (BfpA, AF/R1, AF/R2, Ral, F18 adhesins) appear to be host and pathotype specific. The others are found in more than one species and/or pathotype (EPEC, EHEC, VTEC). Initial adherence of EPEC, EHEC and VTEC strains to host cells is probably mediated by multiple mechanisms.

adherence / adhesin / enteropathogenic *E. coli* / enterohaemorrhagic *E. coli* / verotoxigenic *E. coli*

Table of contents

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1. INTRODUCTION

1.1. Enteropathogenic, enterohaemorrhagic and verotoxigenic *Escherichia coli*

The *Escherichia coli* species is a Gram-negative bacterium that belongs to the *Enterobacteriaceae* family. It is the predominant facultative anaerobe of the human colonic flora. However, some strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system. Pathogenic gastrointestinal strains of *E. coli* are classified according to the properties of their virulence: enteropathogenic *E. coli* (EPEC), enterotoxinogenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), verotoxinogenic *E. coli* (VTEC), "diffusely adherent" *E. coli* (DAEC), necrotoxinogenic *E. coli* (NTEC) [77].

The main virulence property of EPEC strains is the production of a specific lesion called "attaching and effacing (A/E) lesion" characterised by loss of microvilli and intimate attachment of the bacteria to the host [71], whereas the main virulence property of VTEC is the production of verotoxins (or shiga-toxins) that are lethal for eukaryotic cells [56]. As for EHEC strains, their main virulence properties are the production of A/E lesions and verotoxins [23].

EPEC strains colonise the small intestine and cause profuse watery diarrhoea in humans. They were responsible for frequent outbreaks of infant diarrhoea in the USA and the United Kingdom in the 1940s and 1950s [92]. In contrast to the limited importance of EPEC in developed countries nowadays, EPEC are still a major cause of child diarrhoea in developing countries, especially in infants younger than 2 years. The death rate in developed countries is low today but it can reach up to 30% in developing countries [77, 96]. Transmission occurs via oral-faecal contact with contaminated hands or foods. According to epidemiological surveys, asymptomatic carriage by humans could also be at the root of contamination [77]. In bovines, infection with EPEC strains is associated with diarrhoea in young calves (1 week to 8 weeks old) [32]. There is no mortality in calves, but diarrhoea can become chronic, causing economic loss as a consequence. In addition to human and bovine infections, EPEC strains are also found in dogs, cats, rabbits, pigs, goats, and sheep [57]. In rabbits, EPEC strains infect neonates and recently weaned animals causing mild to severe diarrhoea, with important economic loss [17]. In dogs, cats and piglets, colonisation by EPEC strains can occasionally lead to diarrhoea. Finally, in goats and sheep, EPEC strains can be found but the association with diarrhoea has not yet been demonstrated [68].

Infections by EHEC strains in humans are characterised by the production of diarrhoea generally accompanied by haemorrhagic colitis (HC) with, in a few percent of cases, renal sequelae (haemolytic uremic syndrome, HUS), which can lead to death. EHEC strains were recognised as a distinct class of pathogenic *E. coli* in 1983 after two outbreaks in the USA [124]. Today, they represent an important problem for public health in developed countries all over the world. The most common EHEC serotype is O157:H7, but other serogroups such as O26, O111, O145 and O103 are also very important in some countries. In the USA, O157:H7 have been estimated to cause 73 000 illnesses annually and non-O157 EHEC serotypes, at least 37 000 illnesses [19]. In Europe and Japan, infection by EHEC also has a real importance as a foodborne disease [23]. In the veterinary field, several serogroups of the EHEC strain (O26, O111, O118 for example) are directly associated with diarrhoea in 2 week to 2 month old calves [46, 73]. The consequences are economic losses due to a delay in growth and weakness of

calves. EHEC strains can also be found in the faecal flora of a wide variety of animals (cattle, sheep, goats, pigs, cats, dogs, chickens, and gulls) [70]. Cattle, other domestic ruminants and wild ruminants are the most important animal species in terms of a reservoir for human infection and are mainly responsible for food contamination [46]. Indeed, in many cases, EHEC strains infect man via vegetal and animal food soiled by ruminant faeces. Other well-recognised sources of contamination are secondary transmissions from infected persons [88, 93].

VTEC strains cause pathology mainly in humans and piglets. But they can also be found in a large spectrum of domestic and wild animals [126]. In humans, VTEC infections are not frequent but, when they occur, they are frequently associated with HUS syndrome. In piglets, VTEC strains are responsible for oedema disease up to two weeks after weaning [74].

1.2. Adherence of EPEC, EHEC and VTEC strains

Pathogenicity of EPEC infection can be tentatively divided into three stages [31]: (1) initial adherence and colonisation of the intestine; (2) translocation of bacterial signals into the eukaryotic cells via a type III secretion system that cause cytoskeleton rearrangements in enterocytes; and (3) intimate adherence of bacteria to eukaryotic cells by specific proteins, the intimins (coding by the *eae* gene). Steps 2 and 3 together cause the formation of A/E lesions. For EHEC infection, a fourth step consists of the production of verotoxins. For VTEC infection, steps 2 and 3 do not exist and verotoxins are produced after intestinal colonisation.

The molecular biology of the production of A/E lesions and the action of verotoxins is today quite well described and understood, even if research remains to be performed to fully understand the in vivo pathogenesis of those strains. On the contrary, the step of intestinal colonisation via specific adhesins initiating the interaction between the bacteria and host tissues and anchoring the bacteria onto the surface of the enterocytes is actually still poorly understood for many strains [120]. The importance of initial adherence in the infection resides in the following: (1) adherence is the first contact between bacteria and intestinal cells without which the other steps cannot occur, (2) adherence is the basis of host specificity for a lot of pathogens, (3) adhesins represent a good target for the development of a specific vaccinal prophylaxis [25].

The aim of this review is to describe the initial adhesins of the EPEC, EHEC and VTEC strains (Tab. I). During the last few years, several new adhesins and putative colonisation factors have been described, especially in EHEC strains [64, 65, 78, 107, 127]. First, this review will describe pathotype (EPEC, EHEC or VTEC) specific adhesins. Then, adhesins present in more than one of the three pathotypes will be described.

2. EPEC SPECIFIC ADHESINS

2.1. Bfp adhesin

2.1.1. Description

In 1991, Gìron et al. [41] showed that EPEC strains express rope-like bundles of filaments, termed bundle-forming pili (BFP), which create a network of fibres that bind the individual bacteria together. An antiserum against BFP reduces the capacity of EPEC to infect cultured epithelial cells. The *bfpA* gene codes for the main subunit. It is part of an operon of 14 genes: *bfpA* (the pre-pilin), *bfpB* (a lipoprotein), *bfpC* (a bitopic cytoplasmic membrane protein), *bfpD* (a hexameric cytoplasmic ATPase), *bfpE* (a polytopic cytoplasmic membrane protein), *bfpF* (a putative cytoplasmic nucleotide-binding protein), *bfpG* (an unknown function protein that interacts with bfpB), *bfpH* (an unknown function protein that is perhaps not expressed), *bfpI* (a pre-pilin-like protein), *bfpJ* (a pre-pilin-like protein), *bfpK* (a pre-pilin-like protein), *bfpL* (a protein localised with both the inner and outer membranes), *bfpP* (a pre-pilin peptidase) and *bfpU* (an unknown function protein that interacts with *bfpB*) [28, 30, 100, 103]. This operon is situated on a 50–70 MDa plasmid called EPEC Adherence Factor (EAF).

Table I. Distribution of the main adhesins present in EPEC, EHEC and VTEC strains.

Adhesin	Present in pathotypes	Host
Bfp	EPEC	Human, dog
AF/R1	EPEC	Rabbit
AF/R2	EPEC	Rabbit
Ral	EPEC	Rabbit
F18	VTEC	Pig
Saa	VTEC	Human, cattle, sheep
EibG	VTEC	Human
Spf	EPEC, EHEC	Human, cattle
Efa1	EPEC, EHEC	Human, cattle
ToxB	EPEC, EHEC, VTEC	Human, cattle
Lpf	EPEC, EHEC, VTEC	Human, cattle
F9	EPEC, EHEC	Human, cattle
Paa	EPEC, EHEC	Human, pig, cattle
Iha	EPEC, EHEC, VTEC	Human, pig, cattle
AIDA	EPEC, VTEC	Human, pig

The presence of the *bfpA* gene has been searched for in human and animal EPEC, EHEC and VTEC strains but also in *Salmonella* sp. serotypes [23, 25, 41, 42, 55, 99, 122]. The gene was only detected in a subclass of human EPEC strains and in a few EPEC strains from dogs, but not in other EPEC, EHEC and VTEC strains. Human EPEC strains harbouring the EAF plasmid are called "typical-EPEC" strains (t-EPEC) and other human EPEC strains that do not carry EAF plasmid are known as "atypical-EPEC" strains (a-EPEC) [119].

2.1.2. Involvement in the adherence

The plasmid containing the Bfp operon is responsible for the localised adherence (LA) phenotype on cell culture [3]. The complementation of a non-adherent *E. coli* strain with the EAF plasmid leads to an increase in adherence to epithelial cells. In 1985, Levine et al. [63] had already observed the importance of the plasmid during infection in vivo. EPEC strain E2348/69 (containing the EAF plasmid) and its mutant cured of EAF plasmid was inoculated into 10 volunteers. Diarrhoea occurred in nine out of the 10 volunteers who ingested the parental strain but in only 2 of the 9 who took

the mutant. The *bfp* cluster is regulated by environmental signals such as temperature, calcium and ammonium [86]. These findings support the pathogenic role of BFP by the initiation of BFP production in the small intestine, but not in the colon and external habitats.

Adherence studies on mouse and human eukaryotic cells have indicated that BFP play an important role in the cell-type-dependent adherence of t-EPEC [111]. Indeed, the adherence of the bacteria is lower on mouse-derived cells than on human-derived cells. BFP are also implicated in biofilm formation. Moreira et al. [72] demonstrated that the genes encoding BFP are expressed during biofilm formation and that mutants that do not express BFP form more diffuse biofilms than does the wild type strain.

The Bfp adhesin contributes to the attachment of the bacteria to eukaryotic cells but does not seem to be necessary to cause disease in humans. Indeed, the a-EPEC strains (that do not carry the *bfpA* gene) are more and more implicated in diarrhoea outbreaks in both developed and developing countries. Various other adhesins (f.i. Paa, LpfAO113, Iha, Ehx, ToxB, LdaG) are present in a-EPEC strains [45] and play probably the same role as Bfp in adherence. Nevertheless, Bfp adhesin seems to be host specific considering that the *bfpA* gene is mostly found in strains isolated from humans. This host specificity could be explained by the fact that the *bfp* cluster is regulated by environmental signals [86] and that human intestines differ from animal intestines [43].

2.2. REPEC adhesins in rabbit EPEC strains

2.2.1. Description

Three different rabbit-specific adhesins exist in rabbit EPEC strains (REPEC): Adhesive factor/Rabbit 1 (AF/R1) [12], Adhesive factor/Rabbit 2 (AF/R2) [38], Ral [1].

The operon of AF/R1 adhesin is composed of seven genes: *afrA* (the structural subunit), *afrB* (an usher protein), *afrC* (a chaperone), *afrD* (an adhesin), *afrE* (an unknown function protein), *afrR* and *afrS* (two transcriptional regulators). *afrA*, *afrB* and *afrC* genes are required

for the expression of the pilus and for the adherence to the host cells and *afrD* and *afrE* genes are only required for the adherence to the host cells [22]. Penteado et al. [83] and Dow et al. [33] studied the prevalence of the AF/R1 adhesin and respectively found the adhesin in 0% and 4.7% of the REPEC (only found in O103:H2 serotype strains).

Fiederling et al. [38] cloned the *afr2* operon and showed that the *afr2G* gene (the major subunit) is homologous to *clpG* from the CS31A adhesin and *faeG* from the K88 (F4) adhesin. Penteado et al. [83] and Dow et al. [33] studied the prevalence of the AF/R2 adhesin and respectively found the adhesin in 83.3% and 23.3% of the REPEC of O103:H2, O132:H2, O153:H7, O126:H-serotypes.

Ral adhesin is coded by an operon situated on the 95 kb-pRAP plasmid (REPEC adherence plasmid) and is homologous to K88, CS31A and AF/R2 adhesins. The operon is composed of seven genes: *ralC*, *ralF* and *ralH* (three putative minor subunits); *ralG* (one putative major subunit); *ralI* (an unknown function protein); *ralD* and *ralE* (two chaperones). Dow et al. [33] studied the prevalence of the Ral adhesin and found that this adhesin is present in 35% of the REPEC of O153:H7, O15, O132:H2, O49:H2, O145 serotypes.

2.2.2. Involvement in the adherence

AF/R1 was first described by Berendson et al. [12]. In their experiments, they infected sections of human, guinea pig, rat and rabbit small intestines with an REPEC positive for AF/R1 and its deleted mutant and they detected the adherence by indirect immunofluorescence technique. The piliated REPEC strain fully adhered only to the rabbit section and only a few colonies of the non-piliated REPEC strain adhered to the small intestine section in comparison with the wild type. Rafiee et al. [87] identified a rabbit ileal microvillus membrane sialoglycoprotein complex with subunits of 130 and 140 kDa as a receptor(s) for AF/R1 fimbriae localised on the rabbit small intestine [94].

AF/R2 was first described in an REPEC O103 strain by Pillien et al. [85]. This adhesin gives the capacity of diffuse adherence (DA)

to rabbit enterocytes and HeLa cells and there is a loss of pathogenicity after inoculation of deleted mutants into the rabbit.

Mutants deleted in the Ral operon show a 10 times lower colonisation of the rabbit intestine and a decrease in the severity of the disease in vivo [1]. Krejany et al. [58] showed that the adherence on the intestinal rabbit loop is lost with a mutant deleted in Ral adhesin.

As for BFP, REPEC adhesins appear to play a role in the attachment of bacteria to eukaryotic cells and seem to be host specific. AF/R1 binds specifically to a sialoglycoprotein complex. This receptor could vary among species as it has already been shown previously [87].

3. VTEC SPECIFIC ADHESINS

3.1. F18 adhesin

3.1.1. Description

In 1995, Rippinger et al. [91] designated two variants (F18ab and F18ac) that correspond to the related fimbrial types F107 [13], 2134P [76] and 8813 [95] of verotoxigenic and enterotoxigenic *E. coli* isolated, respectively, from porcine postweaning diarrhoea and oedema disease. The F18ab variant is mostly associated to the VTEC strains while the F18ac variant is mostly associated to the ETEC strains [26]. This fimbria is a long flexible filament with a maximum 4.6 nm diameter. Mainil et al. [69] showed that the F18 gene is localised on the same plasmid as the adhesin involved in diffuse adherence (AIDA) in *E. coli* isolated from piglets. The operon coding for this fimbria is composed of five genes: *fedA*, *fedB*, *fedC*, *fedE* and *fedF* [47, 48, 98]. The backbone is built from the major subunit, FedA (15.1 kDa); but this subunit is not sufficient for recognising the F18 receptor [47]. FedE (15.9 kDa) and FedF (30.1 kDa) are two minor subunits and are essential for fimbrial adherence [48, 98]. Smeds et al. [98] showed that the FedF protein plays the role of the adhesin of the F18 fimbriae. Indeed, anti-FedF antibodies, unlike anti-FedE serum, are able to inhibit *E. coli* adherence to porcine enterocytes. Moreover, subunit FedF is highly conserved among F18+ *E. coli*

isolates (from different countries and from the two different variants) [109]. FedB (86 kDa) and FedC (23.4 kDa) act respectively in the biosynthesis of the pili and as a chaperone. Several laboratories studied the distribution of the F18 adhesin in a collection of strains. Zweifel et al. [133] searched for the *fedA* gene in 31 VTEC strains isolated from healthy pigs at slaughter and only one strain was positive for this gene. Cheng et al. [26] looked for the presence of F18ab and F18ac variants in VTEC, VTEC/ETEC and ETEC strains isolated from diarrheic piglets. They found F18ab and F18ac adhesins in respectively 62% and 0% of VTEC, in 62.5% and 33% of VTEC/ETEC strains and in 4% and 8% of ETEC strains. Osek et al. [81] found the *fedA* gene in 2.7% of strains isolated from diarrheic piglets and in 2.2% of strains isolated from healthy piglets.

3.1.2. Involvement in the adherence

Several studies have shown that this adhesin allows adherence to microvilli of piglet enterocytes [76, 95, 129, 131]. The F18 receptor (F18R) plays an important role in the VTEC/ETEC infection. Piglets can possess (F18R+) or not the receptor (F18R−) and only those piglets that do possess the receptor are subject to infection with F18+ *E. coli* [40]. The F18R status of pigs is genetically determined [14] and, recently, Coddens et al. [27] have shown that the expression of the F18 receptor is positively correlated with the presence of histo-blood group antigens, that its levels rise with increasing age during the first 3 weeks after birth and that F18 receptor expression is maintained in older pigs (3–23 weeks old). Several laboratories have tried to develop vaccines against F18 fimbria to prevent post-weaning diarrhoea in piglets [15, 35, 110, 123].

3.2. Saa and EibG

3.2.1. Description

In 2001, Paton et al. [82] isolated a gene, named *saa* (STEC autoagglutinating adhesin), from the megaplasmid of an *eae*-negative O113:H21 VTEC strain (98NK2) responsible for an outbreak of HUS in Australia. In the 98NK2 strain, the protein is 516 amino-acids long, including four copies of a 37-aa direct repeat sequence, and is localised in the outer membrane of the cell. Saa produced by other VTEC strains vary in size as a consequence of variation in the number of copies of a 37-aa repeat unit. In 2006, Lucchesi et al. [67] found the existence of 5 variants based on this variation in the number of repeat units present in the 3′ coding region. Saa exhibits 24–27% of identity with two outer membrane proteins, YadA of *Yersinia enterocolitica* (a plasmid-encoded outer membrane protein implicated in epithelial cell adherence and invasion) and Eib of *E. coli* (*E. coli* immunoglobulin-binding protein) [82]. Several laboratories have studied the distribution of the *saa* gene in a collection of strains [18, 24, 50, 80, 112, 132]. The *saa* gene was found in VTEC strains isolated from cattle, humans, sheep and food. There always exists a negative correlation between the presence of the *saa* gene and the gene coding for intimin (*eae* gene).

In 2006, Lu et al. [66] identified a new gene designated *eibG* through the screening of transposon-mutagenised O91 *E. coli.* The gene encodes a 508-amino-acid protein that presents similarity to Eib proteins (*E. coli* immunoglobulin-binding protein). Lu et al. [66] examined the distribution of *eibG* in human O157, O26, O111 and O91 strains. All *eae*-positive and *saa*-positive strains were found to be negative for *eibG*.

3.2.2. Involvement in the adherence

Paton et al. [82] showed that the introduction of the *saa* gene cloned into a plasmid increases 9.7 fold the adherence of *E. coli* JM109 to HEp-2 and a semilocalised adherence pattern. Mutagenesis of this gene in the O113:H21 strain reduces the adherence significantly. The *saa* gene encodes an auto-agglutinating adhesin. In 2008, Toma et al. [114] showed that *saa*-positive VTEC strains exhibit differential binding properties to HEp-2 and Caco-2 cells. On the 32 strains studied for their adherence to epithelial cells in the absence or presence of D-mannose, 13 strains were sensitive to the presence of this sugar. Moreover, a VTEC strain (in which adherence was mannose

resistant) was deleted in the *saa* gene and its adherence to epithelial cells was not significantly decreased compared to the wild type, suggesting that multiple adherence mechanisms are present in *saa*-positive VTEC strains.

EibG is a new immunoglobulin-binding protein and acts as an adhesin in certain strains of VTEC. A mutant deleted in the *eibG* gene was constructed and its adherence phenotype was studied on Hep-2 cells. The mutant did not adhere to epithelial cells and the chain-like adherence pattern (CLA pattern) was restored after the transformation of the mutant with a plasmid carrying only *eibG*. Therefore, Lu et al. [66] suggested that the *eibG* gene is responsible for the CLA pattern.

Saa is present in several species and do not seem to be host specific but is present only in *eae*-negative, *stx*-positive strains and interestingly in *eibG*-negative strains. VTEC strains do not carry the *eae* gene and thus may compensate for this lack by a multiple adherence mechanism.

4. ADHESINS PRESENT IN MORE THAN ONE OF THE THREE PATHOTYPES

4.1. Spf adhesin

4.1.1. Description

The first fimbria to have been described in EHEC strains was part of a cluster called *sfp* (sorbitol-fermenting protein) encoded by a large plasmid in sorbitol-fermenting O157 strains [20]. This fimbria has similarities with the *pap* gene, which codes for the P-fimbriae in uropathogenic *E. coli* strains. The cluster is divided into six genes: *sfpA* (the major pilin), *sfpH*, *sfpC*, *sfpD*, *sfpJ*, and *sfpG* (the adhesin). Several studies showed that *sfpA* is present only in sorbitol-fermenting O157 strains (EHEC and EPEC strains isolated from humans with diarrhoea and HUS) [21, 39, 49]. In 2006, Lee et al. [61] detected *spfA* in sorbitol-fermenting O157 strains isolated from cattle. Recently, Bielaszewska et al. [16] detected the entire cluster in EHEC O165:H25/NM strains isolated from cattle and humans and they suggested that this cluster is acquired indepen-dently by EHEC O165:H25 and sorbitol-fermenting EHEC O157:NM.

4.1.2. Involvement in the adherence

A mutant deleted in the *sfp* gene is not more able to agglutinate erythrocytes. The expression of Sfp and the adherence to Caco-2 and HCT8 cells are increased in anaerobic conditions that imitate intestinal conditions [75]. It has, therefore, been suggested that Sfp production is induced under conditions resembling those of the natural site of infection and that Spf adhesin plays an important role in the adherence of sorbitol-fermenting strains.

4.2. ToxB and Efa1 adhesins

4.2.1. Description

In 2000, Nicholls et al. [78] identified a locus required for the adherence in vitro of an EHEC strain of serogroup O111:H- by transposon mutagenesis. This factor was called Efa1 for "EHEC Factor for Adherence". The *efa1* locus has a size of 9 669 bp and is situated on the pathogenicity island O122 [53]. The *efa1* locus is strongly associated with non-O157 EHEC and EPEC strains and a truncated variant of 1 299 pb is present in O157 EHEC strains [4, 44, 84]. Efa1 has 97.4% homology in amino acid and 99.9% identity in nucleic sequence with Lymphocyte Inhibitory FactorA (LifA), described in EPEC strain E2348/69, which codes for the lymphostatin. This toxin inhibits the proliferation of lymphocytes and the production of interleukin 2 and 4 [54].

In 2001, Tatsuno et al. [107] identified *toxB* on the pO157 plasmid in Sakai O157 EHEC strain. The *toxB* locus is 9.5 kb long and shares homology with the *efa1* locus (28% of nucleic acid sequence and 47% of amino acid sequence) and the gene coding for the toxin B from *Clostridium difficile* (20% of similarities in amino-acid sequence). Tozzoli et al. [118] looked for the presence of the entire *toxB* sequence in EHEC and EPEC strains of several serogroups by PCR and hybridisation. The complete *toxB* sequence is present in all O157 EHEC strains, in about 50% of EHEC O26

strains, in a few EHEC O118 and O123 strains and in a few EPEC O26 and O86 strains but not in O111 and O103 strains. They also suggested the existence of a polymorphism of *toxB* genes among the different *E. coli* serogroups. Finally, Tatarczak et al. [106] detected the *toxB* gene in VTEC strains from humans and cattle.

4.2.2. Involvement in the adherence

The mutant deleted in Efa1 is deficient in adherence on CHO (Chinese Hamster Ovary) cells. Shedding studies on calves aged 4 to 11 days with wild type and mutant strains show that the colonisation of the intestine is influenced by Efa1 for O5 and O111 EHEC strains [101]. In spite of the high homology between *lifA* and *efa1*, mutants deleted in *lifA* produce the A/E lesion and LA.

ToxB contributes to the adherence by the stimulation of the production of EspA, EspB (two translocator proteins) and translocated intimin receptor (Tir). Indeed, a pO157-cured strain of O157 Sakai has its adherence decreased and the expression level of the effectors EspA, EspB and Tir in this strain also decreased. The adherence is restored with the complementation of the pO157-cured strain of O157Sakai with a mini-pO157 plasmid composed of the *toxB* and *ori* regions. Stevens et al. [102] have shown that ToxB influences the expression of the LEE but not intestinal colonisation in sheep and calves in contrast with Tir.

Regarding the prevalence results [4], both genes are present in the majority of the EHEC and EPEC strains and are mostly absent in non pathogenic *E. coli* strains. Therefore, they might play an important role in the adherence of EPEC and EHEC strains.

4.3. Long polar fimbriae

4.3.1. Description

Long polar fimbria (LPF) was first described in *Salmonella enterica* serovar Typhimurium [6]. Then five homologues were described in *E. coli* strains. Two variants were found in O157 EHEC strains: *lpfA1* (localised in pathogenicity island OI141) and *lpfA2* (localised in pathogenicity island OI154). Doughty et al.

[32] identified an *lpf* homologue in O113:H21 VTEC strain (called $lpfA_{O113}$) localised in pathogenicity island OI154. Toma et al. [112] identified another variant in O26:H11 EHEC strain (called $lpfA_{O26}$) localised in pathogenicity island OI141. Finally, Tatsuno et al. [108] identified an *lpf* homologue in EPEC strain E2348/69; however, this Lpf adhesin does not appear to be implicated in the adherence in this strain. Toma et al. [113] studied the distribution of *lpfA1, lpfA2, lpfA_{O113}* and *lpfA_{O26}* among a collection of *E. coli* strains. *lpfA1* and *lpfA2* are found in most O157 EHEC strains and O145 EHEC strains and in a few EPEC and ETEC strains [112, 113]. $lpfA_{O113}$ and $lpfA_{O26}$ are found in strains of different serogroups and pathotypes (VTEC, EPEC, EHEC, EAEC, ETEC) [4].

4.3.2. Involvement in the adherence

Baumler et al. [6] have shown that Lpf facilitates the attachment of the bacteria to murine Peyer patch cells. Torres et al. demonstrated that the adherence to HeLa cells decreases when *lpfA1* and *lpfA2* are mutated [115, 117]. $LpfA_{O113}$ enhances the adherence of O113:H21 VTEC strains on CHO-K1 cells [32]. The LPF is present in a wide range of strains isolated from different sources and belonging to different seropathotypes. Toma et al. [113] suggested that the acquisition of these genes in specific lineage of *E. coli* has probably contributed to the emergence of a pathogen from a typically commensal organism.

4.4. F9 fimbrial adhesin

4.4.1. Description

A novel potential fimbrial operon called F9 has been identified by transposon mutagenesis with O26 and O157 strains [34, 121]. This operon is present in most EHEC strains in all serogroups.

4.4.2. Involvement in the adherence

It has been shown that F9 promotes colonisation in vivo in calves. When complemented in K12, the binding of the bacteria is increased in bovine epithelial cells. However, the complementation of an O157 strain reduces the

adherence. This phenomenon is probably due to a physical competition with the type III secretion system present in O157 strains. A deletion of the operon in O157 strains reduces the shedding of the bacteria but the colonisation of the rectum is still present. Low et al. [64] concluded that F9 is not responsible for the rectal tropism of O157 strains but that it may be involved in the colonisation of other intestinal sites.

4.5. Paa adhesin

4.5.1. Description

Paa (Porcine attaching- and effacing associated) adhesin was first identified in a porcine EPEC strain (PEPEC) by transposon mutagenesis [2, 5]. The *paa* gene of 753 pb encodes a 27.6 kDa protein and is localised on the chromosome. Studies of the distribution in enteric *E. coli* strains have revealed that *paa* is present, on the one hand, in EHEC O157:H7 and O26, and in dog, rabbit, and pig EPEC isolates, and to a lesser extent in human EPEC strains and, on the other hand, in ETEC strains. In 2007, Leclerc et al. [60] studied *paa* in O149 ETEC strains. *paa* in ETEC is carried by high molecular weight plasmids and all *paa*-positive strains possess *estB, elt, astA* and *faeG* genes (coding respectively for the heat-stable enterotoxin B, the heat-labile enterotoxin, the entero-aggregative heat-stable enterotoxin and a part of F4 operon) and more than half also carry the *estA* gene (coding for heat-stable enterotoxin A). Moreover, they suggested that *paa* from ETEC and EPEC/EHEC strains could be derived from a common ancestor because *paa* from ETEC strains and *paa* from EPEC/EHEC strains contain IS signatures.

4.5.2. Involvement in the adherence

The transposon mutagenesis performed by An et al. [2] suggested that Paa plays a role in the A/E mechanism. Indeed, no A/E lesions were induced using a mutant deficient in the *paa* gene and the adherence phenotype was restored after the complementation of the *paa* mutant [5]. In addition, anti-Paa antibodies reduce the proportion of intact villi showing intimate adherence. In vivo, *eae*-positive and *paa*-negative mutants induce less severe or no A/E lesions in piglets that in the end developed no diarrhoea or delayed-onset diarrhoea.

4.6. Iha adhesin

4.6.1. Description

Tarr et al. [105] described an outer membrane protein similar to Iron-Regulated Gene A (IrgA) from *Vibrio cholerae* in cosmid obtained from the O157:H7 strain. This protein was called Iha for "IrgA Homologue Adhesin". This protein is 67 kDa in the O157:H7 strain and 78 kDa in laboratory *E. coli*. The *iha* gene is found in EPEC, EHEC and VTEC strains from humans, cattle and pigs [4, 104, 106, 112]. The *iha* gene is also present in 39% of *E. coli* isolated from patients with urosepsis [51] and it has been shown to be a virulence factor in urinary tract infection-associated strains (UTI) [52].

4.6.2. Involvement in the adherence

The adherence of mutants deleted in *iha* on eukaryotic cells is decreased and the *iha* gene provides an adherence of K12 strains to HeLa and MDBK cells [105]. Rashid et al. [89] and Leveillé et al. [62] showed that the transcription of *iha* is repressed by iron with a direct interaction between Ferric Uptake Regulation protein (Fur) and Iha. Moreover, Iha represents a Fur-regulated catecholate siderophore receptor in UTI strains. Therefore, Iha may be a dual-function virulence factor: the adherence to the host cell and the siderophore receptor activity.

4.7. AIDA adhesin

4.7.1. Description

Benz et al. [7–10] studied an O126:H27 EPEC strain implicated in infantile diarrhoea and presenting a DA phenotype. They showed that this DA phenotype is mediated by a 6 kb DNA fragment present in a 100 kb plasmid. This 6 kb fragment codes for an adhesin, called AIDA-I. The *aida-I* locus is composed of two

genes (1) *aah* an autotransporter adhesin heptosyltransferase encoding AAH protein, which modifies AIDA-I adhesin, and (2) *aidA*, the AIDA, consisting of AIDA-I (orfB) and AIDAc (orfBc). In 2001, Niewerth et al. [79] detected the *aida-I* locus in a porcine VTEC strain involved in oedema disease. The *aida-I* locus is localised on the same plasmid as the gene coding for the F18 adhesin in porcine strains [69]. In 2007, Zhao et al. [130] showed that the *aida-I* locus is occasionally present in porcine strains involved in post weaning diarrhoea and that a transfer between human and porcine strains is possible.

4.7.2. Involvement in the adherence

AIDA-I is involved in the DA phenotype but is also involved in bacteria aggregation and in biofilm formation [90, 97]. There is an intercellular interaction between AIDA-I-AIDA-I but also AIDA-I-Antigen43 (an autotransporter protein), which lead to the aggregation of cells. Several studies have focussed on the receptor of the AIDA-I adhesin. Laarmann et al. [59] found that AIDA-I recognises an integral membrane glycoprotein in HeLa cells as receptor of 119 kDa called gp119. Fang et al. [36] hypothesised the existence of a receptor in porcine intestinal mucus and found two proteins of 65 and 120 kDa (p65 and p120), that bind with high affinity to purified AIDA-I adhesion. In addition, *aida-I* positive *E. coli* binds to these proteins with higher affinity than do *aida-I* negative mutants. Recently, Benz et al. [11] showed that the environmental factors (different growth conditions) and the genetic backgrounds of the strain significantly influence the transcription activity of the genes.

4.8. Other non-specific miscellaneous adhesins

With the sequencing of the O157 strain [44, 84], several fimbriae were identified in silico. Low et al. [65] studied the distribution of 16 fimbrial gene clusters in *E. coli* and the expression of these fimbriae in different conditions. In those 16 identified fimbriae, 4 are specific to O157 strains in comparison with the K12 strain and 4 fimbriae correspond to previously studied adhesins (LpfA1, LpfA2, Curli and Type 1 fimbriae).

Calcium binding Antigen 43 Homologue (Cah) is a protein described in the O157 strain and is homologous to antigen 43 and AIDA-I [116]. This gene is 2 850 pb long and is present in duplicate in O157 strain EDL933. A K12 strain complemented with *cah* produces two proteins (one outer membrane protein and one heat extract protein) and shows the capacity to autoaggregate. In O157, Cah protein participates in biofilm formation and also binds to calcium. This protein is implicated more in autoaggregation properties than in binding to eukaryotic cells.

Recently, several other adherence factors have been described. First, Xicohtencatl-Cortes et al. described the Haemorrhagic Coli Pilus (HCP). HCP is found in EHEC and EPEC strains [4]. The inactivation of the main subunit (*hcpA* gene) in O157:H7 EHEC reduces adherence to cultured human intestinal and bovine renal epithelial cells and to porcine and bovine gut explants [127]. They also found that in addition to promoting bacterial attachment to host cells, HCP also plays a role in the invasion of epithelial cells, in the haemagglutination of rabbit erythrocytes, in biofilm formation, in the specific binding to laminin and fibronectin of the host cells, and in twitching motility [128]. Then, in 2008, Wells et al. [125] described a novel autotransporter protein, called EhaA (EHEC autotransporter) implicated in adherence and biofilm formation in the O157:H7 strain. Finally, Ferreira et al. [37] described the implication of the *pst* operon (the phosphate-specific transport system) on the adherence to host cells in an EPEC strain. Indeed, in the absence of *pst* there is a decrease in the expression of the main EPEC adhesins and a reduction in bacterial adherence to epithelial cells in vitro.

5. CONCLUDING REMARKS

Considering that adherence is the basis of host specificity for a lot of pathogens and that ruminants (especially cattle) are considered to represent the main reservoir of EHEC and

VTEC strains for humans, we could wonder about the distribution of the EPEC, EHEC and VTEC adhesins among humans and animals. Based on different prevalence studies, we can affirm that, except for a few adhesins (Bfp, REPEC and F18 adhesins), EPEC, EHEC and VTEC adhesins do not seem to be host specific. There are several possible explanations. First, the host specificity is based on other factors. This host specificity may be based upon another adhesin not yet discovered or another property such as the following: (i) differences in the sequences of genes coding for some adhesins present in human and bovine strains, resulting in host and tissue tropism, as already described in other families of fimbrial (P family) or afimbrial (AFA family) adhesins [12, 35]; (ii) variation in the expression of some adhesin-encoding genes according to the growth environment (bovine or human intestines; intestinal segments; age of the host; etc.), as observed for other genes [29]; and/or (iii) properties other than adherence such as an intermediate metabolism, which allows the bacteria to be better adapted to a bovine intestinal environment, such as the young calf intestine [34, 121]. Second, there is no actual host specificity and the strains carrying specific adhesins (Bfp, REPEC and F18 adhesins) could represent a subgroup of strains specifically adapted to one host and/or one environment. Therefore, the other EPEC, EHEC and VTEC strains could be considered as only one group that carry several adhesins without any specificity and acting together with the aim to attach the bacteria to the host cells. Thus, it is not surprising that the bacteria could have different adhesin profiles that are not linked to one specific host or to one specific pathotype.

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