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Transport proteins promoting *Escherichia coli* pathogenesis

Fengyi Tang¹ and Milton H. Saier Jr.*

Department of Molecular Biology, Division of Biological Sciences, University of California at San Diego, La Jolla, CA 92093-0116, USA

Abstract

Escherichia coli is a genetically diverse species infecting hundreds of millions of people worldwide annually. We examined seven well-characterized *E. coli* pathogens causing urinary tract infections, gastroenteritis, pyelonephritis and haemorrhagic colitis. Their transport proteins were identified and compared with each other and a non-pathogenic *E. coli* K12 strain to identify transport proteins related to pathogenesis. Each pathogen possesses a unique set of protein secretion systems for export to the cell surface or for injecting effector proteins into host cells. Pathogens have increased numbers of iron siderophore receptors and ABC iron uptake transporters, but the numbers and types of low-affinity secondary iron carriers were uniform in all strains. The presence of outer membrane iron complex receptors and high-affinity ABC iron uptake systems correlated, suggesting co-evolution. Each pathovar encodes a different set of pore-forming toxins and virulence-related outer membrane proteins lacking in K12. Intracellular pathogens proved to have a characteristically distinctive set of nutrient uptake porters, different from those of extracellular pathogens. The results presented in this report provide information about transport systems relevant to various types of *E. coli* pathogenesis that can be exploited in future basic and applied studies.

Keywords

Escherichia coli; Pathogenesis; Transporters; Toxins; Iron acquisition; Intra vs. extracellular pathogens

1. Introduction

Escherichia coli is a major source of infant mortality, adult diarrhea and urinary tract infections worldwide [1,2]. Uropathogenic strains are responsible for up to 80% of urinary tract infections (UTIs) and a majority of pyelonephritis cases [3]. Enteroaggregative and enterotoxigenic pathovars have been the leading causes of travellers' diarrhea, which has significantly contributed to infant mortality [4]. Enterohaemorrhagic serotypes are currently the leading cause of haemorrhagic colitis, which can lead to fatalities [5].

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*Corresponding author. Tel.: +1 858 534 4084; fax: +1 858 534 7108. ftang@ucsd.edu, aftang@gmail.com (F. Tang), msaier@ucsd.edu (M.H. Saier).

¹Tel.: +1 408 507 2686.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.micpath.2014.03.008>.

Some probiotic strains of *E. coli* including the *E. coli* Nissle and O83 strains contribute beneficially to human physiology [7,8]. Current research on probiotic *E. coli* in humans has proven to be clinically useful in the treatment of colitis and inflammatory bowel disease (IBD) syndromes and in combating pathogenic *E. coli* strains [9,10]. *E. coli* can have tremendously varied effects on humans and other animals, a consequence of its genetic diversity.

Prominent types of *E. coli* pathogens have been identified based on varying mechanisms of infection. There are five types of diarrheagenic strains that pertain specifically to the alimentary system: enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), and enteroaggregative (EAEC). Diffusely adherent (DAEC) strains can infect both the urinary tract and the gastrointestinal tract due in part to their diverse mechanisms of host adhesion [6]. In our study, seven representative *E. coli* pathogens as well as *E. coli* K12 were examined. We use the first three letters/numbers of these strain designations as abbreviations to refer to these strains. Table 1 presents these abbreviations as well as basic information about the eight strains of *E. coli* examined.

The general paradigm of the pathogenic mechanism associated with *E. coli* virulence involves (1) adhesion, (2) protein injection into host cells, (3) subversion of signaling mechanisms and (4) colonization leading to impaired immune responses, membrane potential disruption and cytoskeletal manipulation [11]. During the injection stage, *E. coli* typically secretes effectors into host cells using one or more protein secretion systems, either to escape the immune response or to alter signaling pathways. Host membrane potential disruption and apoptosis induction are employed by several pathovars.

Virulence genes are often clustered in “pathogenicity islands” that reflect horizontal transfer [12]. Horizontal gene transfer has allowed *E. coli* to survive under a variety of *in vivo* conditions, increasing the virulence of existing pathogens and creating new ones [13,14]. Because the pan genome of *E. coli* far exceeds the core genome, perhaps by as much as 20-fold [15], this increase in genetic plasticity allows for fluctuations in gene content, further contributing to divergence of *E. coli* pathogenic mechanisms [16].

2. Methods

2.1. G-BLAST search of *E. coli* proteomes

The proteomes of eight *E. coli* strains were screened for homologs of all proteins contained in the Transporter Classification Database (TCDB; www.tcdb.org) as of September, 2012 using a program designed for this purpose, G-BLAST [17]. The program retrieves information for both the genome query and the TC top hit sequences, TC#, protein size in number of amino acid residues, number of predicted TMSs using the HMMTop 2.0 Program, e-value for the query and hit proteins, regions of sequence similarity and regions of TMS overlap. FASTA-formatted protein sequences from the completed genome were used. Each putative open-reading frame was used as a query in the BLASTP software to search for homologous proteins in TCDB. The low complexity filter was not used as it is normally of value only for larger datasets including proteins with multiple repeat elements. In addition, each open reading frame [18] was scanned with the HMMTOP 2.0 program [19]

to predict the number of putative transmembrane segments (TMSs). The Web-based Hydropathy, Amphipathicity and Topology (WHAT) program [20] was used with a window size of 19 residues and an angle of 100° to display the hydropathy plot for individual proteins in order to resolve the differences in the numbers of TMSs between the proteins retrieved and their TCDB homologs. The plot generated by WHAT allows the user to judge if a program such as HMMTOP has missed a TMS or has predicted a TMS inappropriately. A cut-off value of 0.0001 was used with the G-BLAST program so proteins retrieved with larger values (greater sequence divergence) were not recorded. Proteins with no predicted TMSs were eliminated so that only integral membrane proteins, primarily multi-spanning membrane proteins, were retrieved. Proteins with only an N-terminal signal sequence are numerous because these proteins include almost all periplasmic, outer membrane and secreted proteins that are exported via the general secretory pathway (Sec) or twin arginine translocase (TAT). The topological prediction programs often miss these TMSs, recording them to have zero TMSs. Consequently, the numbers retrieved were not reliable and were therefore not always recorded. For example, single TMS proteins such as extracytoplasmic solute binding receptors of ABC transport systems were often predicted to lack a TMS, and therefore these proteins were not included in our study of the integral membrane transport proteins.

2.2. Identification of distant transport protein homologs

Proteins retrieved between the values of 0.0001 and 0.1 were examined manually to determine the likelihood that these proteins were members of recognized transport protein families, or if they might comprise representatives of novel families of putative transport proteins. A total of 82 non-orthologous homologous proteins were retrieved using the 0.0001–0.1 cutoff, but only 10 proved to be recognizable transport proteins. These were incorporated into TCDB. The 10 proteins were manually examined by conducting searches as follows. (1) TC-BLAST searches provided preliminary evidence for family assignment. (2) NCBI BLAST searches provided confirmation or refutation of family assignment based on the conserved domain database (CDD) and hits obtained with values to the query sequence of less than 1×10^{-7} . (3) Topological analyses revealed similarities and differences between the query sequence and members of the assigned family. (4) Proteins proving to represent new potential families were included in TC subclass 9.B.

Candidate proteins were subsequently examined in greater detail to estimate their substrate specificities. On the basis of the numbers and locations of TMSs as well as degrees of sequence similarity with entries of known function in TCDB, transport proteins were classified into families and subfamilies of homologous transporters according to the classification system presented in TCDB. Regions of sequence similarity were examined using the WHAT program which shows hydropathy plots to ensure that homology was in a transmembrane region of 3 or more TMSs and not only in hydrophilic domains. Proteins encoded within single multicistronic operons were often identified in order to gain evidence for multicomponent systems and to help deduce functions. Operon analyses (genome context, a.k.a., synteny analyses) were performed for candidate proteins with assigned or unassigned transport functions as described in Castillo and Saier (2010) and Reddy et al. (2012) [21,22].

2.3. Overview of programs used

Transport proteins thus obtained were systematically analyzed for unusual properties using published [17] and unpublished in-house software. Among the programs described by Reddy and Saier [17], used in this report, were the GSAT, Protocol1, Protocol2, TSSearch, SSearch and GBlast programs. Unpublished software was used to tabulate information according to TC# or other criteria such as substrate type. Unusual characteristics of the query *E. coli* sequences were identified based in part on topologies that differed from corresponding family members in TCDB as well as e-values obtained with G-BLAST. Unusual properties can result from events such as genetic deletion and fusion, sometimes resulting in the gain or loss of extra domains or the generation of multifunctional proteins. Such results can be reflective of the actual protein sequence, but they can also be artifactual, due to sequencing errors or incorrect initiation codon assignment. In the latter cases, but not the former, the protein sequences were either corrected when possible or eliminated from our study.

Orthologous relationships among homologs were estimated by measuring the percent identities. We initially used an arbitrary cutoff of 95% identity, a value appropriate in view of the similarities of the strains considered. Homologs with lower percent identities were examined manually, revealing occasional sets of orthologs that exhibited less than 95% identity (See Table S1 for orthologous assignments).

3. Results

3.1. Overview of transporter types

According to the transporter classification system, there are five defined classes of transport systems (classes 1–5) as well as a class of transporter auxiliary proteins (class 8) and a class of incompletely defined transporters (class 9). These will be considered in sequence.

3.1.1. Pore-forming proteins—Eight *E. coli* genomes were analyzed for the occurrence of transport proteins using the Transporter Classification Database (TCDB; www.tcdb.org [23]) and the G-Blast program [17]. The results are summarized according to TC subclass in Table 2, Fig. 1, and Table S1. Examining the total number of transport proteins present in these 8 genomes, we see that *E. coli* K12 has the fewest at 1128. The most found in any genome is 1287, 159 more than in K12. However, the seven pathogenic species combined contained 838 unique transport proteins lacking in K12.

TC subclass 1.A in TCDB includes α -type channels. The eight strains have 37–42 such channel proteins. Surprisingly, however, the pathogenic organisms possess 24 non-orthologous channel proteins lacking in K12 (Table 2). It appears unlikely that α -type channels are major contributors to *E. coli* pathogenesis (Fig. 1).

TC subclass 1.B includes outer membrane β -type porins. In this subclass, we find striking differences between the organisms, where K12 has far fewer than any of the other strains. The largest number of these porins is 108 observed in ABU, and the seven pathogens possess 151 porins proteins non-orthologous to those in K12 (Table 2). This observation suggests that porins contribute to pathogenesis as confirmed and explained below.

TC subclass 1.C includes pore-forming toxins. K12 has only nine such proteins, and E24 has just two more, but all the others have between 15 and 20 such toxins, and 51 toxins, non-orthologous to those in K12, were identified (Table 2). As expected, an array of toxins characterizes each of the pathogens. It should be noted that toxins that do not form transmembrane pores were not included in our study.

Small α -type channel-forming holins (TC subclass 1.E.) function in release of auto-lysins which allow phage particle release when phage-encoded, or self-destruction by a suicide-type process when chromosomally encoded [17]. The *E. coli* strains have 3–7 holins each. However, 19 of these proteins are non-orthologous to those found in K12 (Table 2). It is possible therefore that chromosomally encoded holins play important roles in pathogenicity, possibly in programmed cell death as an aid to biofilm formation and communal life [24-26].

3.1.2. Secondary carriers—The largest number of transport systems, but not the largest number of transport proteins, includes secondary carriers belonging to TC subclass 2.A. The eight strains have anywhere from 309 to 325 such systems. K12 has 311, and 96 proteins non-orthologous to those in K12 were identified, many of which are likely to contribute to specific types of pathogenicity.

3.1.3. Primary active transporters—TC subclass 3.A consists of pyrophosphate-hydrolysis driven primary active transporters, usually multi-component systems. K12 has the fewest such systems (340), while UMN has the most (390), and 155 proteins found in pathogens appear to have no orthologs in K12. Many of these systems play essential roles in virulence. The strains examined have 3–5 decarboxylation driven sodium pumps, which probably play a housekeeping function in the maintenance of ionic homeostasis. Subclass 3.D, ion (H^+ or Na^+)-pumping electron carriers, also exhibit little variation with only seven proving to be non-orthologous to those of K12.

3.1.4. Group translocators—TC class 4 includes group translocators that modify their substrates during transport. Subclass 4.A includes the proteins of the multi-component sugar transporting phosphotransferase system (PTS) [27]. While K12 has the fewest such systems (49), both CFT and ABU have 13 more (62). In fact, 43 PTS proteins proved to be non-orthologous to those found in K12. These systems, likely to be important virulence factors, will be analyzed below.

Each of the eight different strains has between 7 and 12 TC subclass 4.D group translocating glycosyl transferases (4.D [28,29]). Interestingly, the pathogens possess eight sets of orthologs not found in K12, suggesting that unique glycosyl transferases may serve different functions in the different pathogens. Some of them could function in the production of capsules, known to be virulence factors in many pathogenic bacteria [30].

3.1.5. Transmembrane electron carriers—Electron-carriers (TC class 5) that transfer electrons from one side of the membrane to the other, thereby influencing cellular energetics [31], do not vary in numbers appreciably between the eight strains.

3.1.6. Poorly characterized transporters—Tremendous variation is observed between the numbers of poorly characterized transporters found in subclass 9.A. K12 has the fewest, with only 42 such proteins while 559 has over twice this number with 87. Most of the pathogens have between 60 and 70 such proteins, and 94 of these proteins are lacking in K12. This shows that the pathogens possess large variable numbers of poorly characterized transporters that are likely to serve highly specific functions within the cell. It is probable that they remain poorly characterized because they are not found in *E. coli* K12 and other commonly studied model bacteria. Elucidation of their functions and mechanisms of action is likely to prove particularly interesting and important for specific aspects of *E. coli* pathogenesis. In contrast to the situation observed for subclass 9.A, the strains examined have relatively little variation in the numbers of subclass 9.B members. The smallest number is found in O15 (81), and the largest number is found in CFT (91) with K12 having 90. Nevertheless, the pathogens collectively have 26 such proteins lacking in K12.

3.2. Pathogenicity-related transport protein analyses

Transport proteins likely to be important in pathogenicity include (1) toxins, (2) trans-envelope protein secretion systems, (3) outer membrane protein secretion systems, and (4) outer membrane iron-siderophore receptors that function with cytoplasmic membrane ABC-type iron uptake porters. The transport proteins that fall into these four classes are summarized in Tables 3-6, respectively.

3.2.1. Toxins identified in *E. coli* strains—Table 3 summarizes the toxins identified in the eight *E. coli* strains included in this study. In addition to the pore-forming subunits that function in conjunction with type III secretion systems (IIITCP), seven families of toxins are represented. These will be discussed sequentially according to their TC numbers.

HlyE is a well-characterized toxin found in *E. coli*, *Salmonella* and *Shigella* species. Its high resolution X-ray structure is available, and models of its membrane-inserted homooligomeric pore complex have been proposed [32,33]. Five of the eight *E. coli* strains examined possess this toxin.

Another hemolysin is the *E. coli* Hemolysin A (HlyA), part of the RTX superfamily of pore-forming proteins [34]. The general mechanism proposed for HlyA is as follows: the toxin (1) recognizes and binds to β 2-integrin receptors on host cell membranes, (2) inserts into the host membrane forming nonselective pores, and (3) transports a toxin that inhibits host protein kinase B, leading to apoptosis of the host cell [35]. This toxin was found only in two intracellular strains, both of which have HlyE as well.

Type 3 Secretion System (T3SS) pore-forming proteins (IIITCP) are typically involved in attaching/effacing mechanisms in enteropathogenic strains [15]. The EspB/D complex and other homologous pore-forming protein pairs are secreted into the host cell and oligomerize to form translocation pores at the site of contact. EspB with EspD, for example, provide translocation functions for *E. coli* effector proteins and inhibit host phagocytosis by altering host cell cytoskeletal functions [36]. IIITCP family proteins were found in two pathogens.

The prophage-encoded shiga toxin (Stx) is a heteromeric protein with a glycolipid-recognizing, pore-forming, pentameric B subunit and a single A chain with N-glycosidase activity that results in cleavage of a single adenine in the 28S rRNA, resulting in blockage of protein synthesis [37]. Two StxB pore-forming toxins appear to be encoded in the O15 genome, although no such toxin was identified in the other strains.

Clostridial cytotoxins (CCT) are tripartite proteins with N-terminal catalytic domains, C-terminal receptor regions and central channel-forming domains [38]. Upon endocytosis into the host cell, the channel-forming domain is activated by acidic pH, which induces conformational changes promoting insertion into the endosomal membrane, concomitant with channel formation. The catalytic domain of CCT is translocated across the membrane, resulting in inactivation of Rho family GTPases and reorganization of the host actin cytoskeleton [39]. Two homologs of the clostridial cytotoxin were identified (see Table 3).

Serratia pore-forming toxins (S-PFT) (1.C.75) are exported through a two partner secretion system (TPS; TC# 1.B.20) [40]. Three organisms possess this type of pore-forming hemolysin, ABU, CFT and O15. The former two have huge toxins of 3216 amino acids that are nearly identical. By contrast, the O15 homolog is 1270 amino acids in length and corresponds in sequence to the N-terminal portion of the larger toxins.

Toxins of the Cytolethal Distending Toxin (CDT) family are tripartite protein complexes, CdtABC, that induce DNA cleavage, cell cycle arrest, and apoptosis in host epithelial and immune cells [41]. The catalytic subunit of the complex possesses phosphodiesterase activity that cleaves the host DNA, causing G2/M cell cycle arrest [42]. CdtA and C are dimeric subunits that deliver CdtB to the host cell cytoplasm [43]. The complete CdtA/B/C trimeric toxin system (1.C.98.1.1) was found in only one of the eight *E. coli* strains, APE.

3.2.2. Trans-envelope protein secretion systems in pathogenicity—Table 4 summarizes the various protein secretion systems present in the *E. coli* strains examined. These include (1) type I secretion systems (T1SS; ABC; TC#3.A.1), (2) type II secretion systems (T2SS; SEC-SRP complexes; TC#3.A.5) for secretion across the inner membrane, acting together with the Main Terminal Branch (MTB; TC#3.A.15) for secretion across the outer membrane, (3) type III secretion systems (T3SS; Fla; Path; TC#3.A.6), (4) type IV secretion systems (T4SS; Conj; Vir; TC#3.A.7), and (5) type VI secretion systems (T6SS; TC#3.A.23) [44].

Type I secretion systems (T1SSs) identified are members of the ABC Protein Exporter-1 (TC#3.A.1.109) and Protein Exporter-2 (TC#3.A.1.110) families within the ABC functional superfamily [45]. ABC exporters consist of two transmembrane domains and two cytosolic ATP-hydrolyzing domains. T1SS efflux involves ATP hydrolysis-coupled translocation of the substrate across the two bacterial membranes in a single energy-coupled step, utilizing an outer membrane factor (OMF; TC#1.B.17) and a membrane fusion protein (MFP; TC#8.A.1) to couple cytoplasmic membrane transport with that across the periplasm and outer membrane ([46,47]). Protein exporter families of this type were found in abundance in the pathovars.

Type III secretion systems are found in numerous Gram-negative bacterial strains and are used for secretion of flagellar and pathogenicity effector proteins. Effector proteins can be exported across both bacterial membranes as well as the host cell membrane in a single step. The type III protein secretion system includes a needle complex, the injectisome, which attaches to the host cell membrane [48-51]. Several of these systems were present in *E. coli* pathogens (Table 4).

As expected, the complete general secretory pathway (TC#2.A.5.1.1) and the complete flagellar protein export complex (TC#3.A.6.2.1) were found in all strains. The UMN pathovar appears to possess six additional key components of the flagellar system (Table 4).

T4SSs vary tremendously with respect to sequence divergence and numbers of constituents required for function. An examination of potential T4SS components in the eight *E. coli* strains revealed the presence of potential subunits, suggesting that some of these organisms utilize this mechanism to promote pathogenesis.

Type VI secretion systems are found in multiple Gram-negative bacteria. Key constituents include an ATPase protein, ClpV, a phage tail-like protein that spans the outer membrane, and a “tail-spike” protein, VgrG, which penetrates the host membrane and dissociates from the complex to allow contact-dependent translocation of proteins into the host cell cytoplasm [52]. In the *E. coli* strains studied here, two sets of complete T6SS complex homologs were identified (VasA-L of 3.A.23.1.1 and EvpA-P of 3.A.23.2.1) in several strains.

3.2.3. Outer membrane protein secretion systems—Table 5 summarizes the outer membrane protein secretion systems present in the eight *E. coli* strains studied. These include members of the following families: Autotransporter-1 (TC#1.B.12), Autotransporter-2 (TC#1.B.40), outer membrane factors (OMF; TC#1.B.17), fimbrial usher proteins (FUP; TC#1.B.11), two partner secretion systems (TPS; TC#1.B.20), secretins (TC#1.B.22), outer membrane protein insertion porins (OmpIP; TC#1.B.33), curli fiber subunits (CsgA; TC#1.B.48), and putative Autotransporter-3 (Invasins; TC#1.B.54).

Autotransporters are virulence factors that insert into the outer bacterial membrane to form transmembrane β -barrels that export their extracellular protein domains. An Autotransporter-1 protein consists of an N-terminal cleavable secretory signal, an exported passenger domain of variable lengths, and a C-terminal 250–300 amino acyl residue domain that inserts into the outer membrane, giving rise to a 12 TMS β -barrel structure [53].

Autotransporter adhesins (e.g., AidA; TC#1.B.12.1.1) were present in all eight *E. coli* strains examined, but virulence factor-associated autotransporters were present in only certain pathogens. For example, fibronectin binding proteins and a tracheal colonization factor autotransporter were identified only in pathovars (Table 5). Overall, the most Autotransporter-1 proteins were found in ABU with sixteen homologs; fourteen were found in 559, twelve in CFT and UMN, ten in APE and E24, eight in O15, and only five in K12.

Autotransporter-2 family proteins have trimeric structures organized into three domains: an N-terminal head that adheres to the host cell membrane, a stalk, and a C-terminal anchor,

rich in glycine, which forms a β -strand domain that oligomerizes to form a pore for auto-transport [54]. Haemagglutinins, a dissimilar adhesin, YadB [55], and other Autotransporter-2 family members were identified in specific pathovars (Table 5). Interestingly, AT-2 family proteins are far less prevalent than AT-1 family proteins in all strains and are lacking in K12.

Invasins or intimins are also called Autotransporters-3, but a function in autotransport is not well established [56]. The N-terminal domain serves as an anchor and inserts a pore-like β -barrel in the outer membrane. The C-terminus contains folds that bind to Tir (translocated intimin receptor) and β -1 integrins on host cells, leading to pathogenesis in enterohaemorrhagic and enteropathogenic strains [57,58]. In our study, intimin/invasin proteins were identified across all serotypes and were found in abundance in six of the seven pathogens. The UPEC strains have more intimins/invasins than the enteropathogenic strains.

Outer membrane factors (OMFs; 1.B.17) and membrane fusion proteins (MFPs; TC#8.A.1) function with Major Facilitator Superfamily (MFS) type porters, Resistance-Nodulation Cell-Division [59] family proteins (2.A.6), ATP-Binding Cassette (ABC) porters and Aromatic Acid Exporters (AAE, TC#2.A.85) to form complexes that export various drugs, oligosaccharides, proteins, aromatic acids and siderophores across both the inner and outer membranes in a single energy-coupled step ([60-64]). OMF proteins form homotrimeric, 12-stranded β -barrel complexes that form holes in the outer membrane for translocation. The OMF component, TolC (TC#1.B.17.1.1), was identified in all eight strains. TolC-dependent exporters mediate translocation of hemolysins, drugs and various siderophores [65,66]. Additional TolC-like proteins were found in the pathogens but not in K12.

The Fimbrial Usher Proteins of the FUP family function in conjunction with periplasmic chaperone proteins in fimbrial assembly [67]. The usher proteins contain central domains that span the outer membrane 24 times to form β -barrel pores. The C-terminal domains act as “plugs” that lie within the lumen of the β -barrels [68]. These plug domains, along with helical folds, regulate channel opening and export of proteins that contribute to fimbrial assembly [69]. FUPs were identified in all strains of *E. coli* but were present in greater numbers in the pathovars (Table 5).

Homologs of a single two-partner secretion (TPS) system (ShlB; TC#1.B.20.1.1) were identified. The oligomeric β -barrel channel of ShlB serves in secretion of the effector protein across the outer membrane [71]. These TPS systems were found exclusively in O15, where two paralogues were present, and in ABU, where a single protein was identified.

A secretin (TC#1.B.22) consists of an N-terminal periplasmic domain and a C-terminal domain responsible for forming large channels via homomultimeric ring structures [72]. Secretins participate in various transport processes such as fimbrial protein export, T2SS, T3SS, and phage export [73,74], and as expected, a number of these were identified in the pathogens (Table 5). Also as expected, outer membrane protein (OMP) insertion porins (OmpIP or BAM, TC#1.B.33), required for OMP insertion, were ubiquitously present, and all six required subunits were identified in all eight strains.

3.2.4. Iron scavenging outer membrane receptors—Table 6 lists outer membrane receptors of TC family 1.B.14, most of which are involved in the uptake of iron-siderophore complexes. This table also includes a cobalamin receptor and a copper receptor and indicates the occurrence of the TonB protein, which together with other proteins, is involved in OMR energization. TolA homologs involved in outer membrane stabilization and colicin uptake are also tabulated. For some of the OMRs, a single ortholog was found in all eight organisms (e.g., receptors for ferric copragen, ferrichrome, catecholates, and cobalamin). Some organisms possess more than one gene encoding a specific type of iron complex uptake receptor. These include: 1.B.14.1.4 (catecholates) where four of the organisms have a single gene encoding this system, but three organisms have two, and one (CFT) has three (Table 6). A similar situation was observed for 1.B.14.7.2, an iron pesticin uptake receptor. Six additional types of outer membrane receptors specific for iron complexes were found in some of these *E. coli* strains but not others. Similarly, only four of the pathovars possess the copper receptor (TC#1.B.14.4.1). All in all, each of these organisms possesses nine to fifteen iron uptake receptors except for K12 and E24, which have only six. Interestingly, the uropathogenic strains all possess more (ten to fifteen) iron complex receptors than the other serovars.

Table 6 also includes the energizing modules of family 2.C.1, TonB, which energizes OMR-mediated uptake, and TolA, which provides outer membrane stability and mediates colicin uptake [Table S1[75]]. It can be seen that while *E. coli* K12 has just one of each of these two proteins, four of the pathogens have two TonB's, five of them have two TolA's, and one (559) has three TolA's. TonB, but not TolA, associates with ExbB–ExbD or TolQ–TolR to energize uptake of iron-siderophore complexes and other large molecules across the outer membranes of Gram-negative bacteria via outer membrane receptors (OMR; TC#1.B.14). The dissimilar Tol complex includes six proteins, TolA, TolB, YbgF, Pal and the pmf-dependent energizer, TolQR. This complex is involved in the maintenance of outer membrane stability and facilitates late stages of cell division.

3.2.5. Inner membrane iron uptake systems—Table 7 summarizes inner membrane iron uptake systems identified in the eight *E. coli* strains. These systems function to transport iron from the periplasm into the cytoplasm of the bacterium, cooperating with the outer membrane iron-scavenging OMR complexes, which import iron from the surrounding extracellular environment into the periplasm. Various mechanisms are employed for inner membrane transport of iron/iron-chelate complexes. These include the CorA heavy metal ion uptake channels (1.A.35), the zinc–iron permeases (ZIP (2.A.5)), metal ion transporters (Nramp (2.A.55)), GTPase-coupled ferrous iron uptake systems (FeoB (9.A.8)), and ATP-binding Cassette (ABC (3.A.1)) transporters.

Zinc–Iron Porters (ZIP) have about eight TMSs with histidine-rich repeats near the N- and C-termini [76]; a cation symport mechanism may be operative [77]. A single such transport protein was found in each of the eight strains examined (Table 7). Metal ion transporter (Nramp) homologs generally function by a symport mechanism in a 1:1 ratio of ferrous iron and H⁺ although stoichiometrics have been reported to be variable [78]. A single Nramp protein was found in each of the eight strains of *E. coli* examined (Table 7). Another iron influx system, FeoB (TC#9.A.8), has N-terminal catalytic domains with sequences

homologous to GTP-binding domains [79]. Their C-termini catalyze transport, possibly coupled to K^+ -activated GTPase activity. GTP hydrolysis has been reported to be required for transport, and if there is direct energy coupling, the mechanism may resemble that of ABC transporters ([80-83]). Two FeoB homologs were identified in each of the eight strains of *E. coli*.

Four types of inner membrane ABC iron-uptake systems were identified: ferric porters (TC#3.A.1.10), iron chelate porters (TC#3.A.1.14), manganese/zinc/iron chelate porters (TC#3.A.1.15), and homologs of the *Brachyspira* iron transporters (TC#3.A.1.20). A complete four-subunit ferric-citrate uptake system (TC#3.A.1.14.1) was identified in five of the eight strains. Complete ferric-enterobactin and ferric-hydroxamate uptake systems were identified in all eight strains of *E. coli* examined. However, a second complete ferric-enterobactin system (TC#3.A.1.14.2) was identified in two strains.

Homologs of the *Shigella* heme uptake system (ShuTUV (3.A.1.14.18)) were present in five pathogenic strains as complete systems. Complete systems corresponding to the *Salmonella* Mn^{2+} and Fe^{2+} uptake system (3.A.1.15.7) were identified in four pathovars (see Table 7).

Fig. 2 reveals the correlation between numbers of OMRs and the number of iron ABC uptake systems. The juxtaposition of the two datasets demonstrated that the UPEC strains, ABU and CFT, contain the greatest numbers of both OMRs and inner membrane iron-siderophore transporters. E24 and K12 contain the smallest numbers of both types of systems. A clear correlation between the numbers of iron-siderophore OMRs and inner membrane transporters is evident for the eight strains of *E. coli* (RSQ value = 0.90). The slope of the trendline (SLOPE = 1.85) confirms the positive correlation between the two sets of values (see Fig. 3).

3.2.6. Nutrient transporters: intracellular vs. extracellular pathogens—We identified numerous nutrient uptake porters that are uniquely found only in the three intracellular pathogens or only in the extracellular pathogens (Table 8). Transporters found exclusively in the intracellular pathovars include certain α -type channels (TC#1.A), β -barrel porins (1.B), holins (1.E), MFS porters (2.A.1), multidrug resistance (MDR), RND-type efflux pumps (2.A.6), ABC porters (3.A.1), PTS sugar uptake transporters (4.A), and various TC class 9.B proteins. Thus, the APE, CFT and ABU strains, all primarily intracellular pathogens, possess many transport systems that take up metabolic products produced via host intracellular metabolic processes. Examples include a phosphoglycerate:Pi antiporter (2.A.1.4.2) that takes up glycolytic intermediates, 2- and 3-phosphoglycerates as well as phosphoenolpyruvate, a pmf-dependent acetate uptake system (TC#1.A.14.2.2), an ATP-dependent ribose porter (3.A.1.2.1) and a phosphoenolpyruvate (PEP)-dependent D -tagatose/ D -psicose/ D -sorbitol PTS uptake system (4.A.2.1.9). While ribose is a substrate of RNA biosynthesis and a product of RNA degradation, the latter 2-keto hexoses are intermediates of several intracellular metabolic pathways and of nutritional value ([84,85]). These intracellular pathogens also uniquely possess a citrate: H^+ symporter (2.A.1.6.1), a fumarate-malate transporter (2.A.13.1.2), a citrate:succinate antiporter (2.A.47.3.2), a tripartite tricarboxylate (citrate, isocitrate and cisaconitate) transporter (2.A.80.1.1), and a putative C4-dicarboxylate transporter (9.B.50.1.2), all of which are specific

for intermediates in the Krebs (TCA) cycle, and none of which are found in the extracellular pathogens. In addition to Krebs cycle metabolism, C4-dicarboxylates can be generated via multiple pathways including amino acid deamination and lipolysis.

Two of the intracellular pathogens, APE and CFT, possess an arginine/agmatine antiporter (2.A.3.2.7), which allows these cells to survive under acidic conditions, for example, as exist inside lysosomes [86]. These intracellular pathogens also possess a probable antimonite/arsenite resistance exporter (2.A.47.4.2). Arsenite can be passively taken up by host cells via aquaglyceroporins (TC#1.A.8) [87]. Additionally, an ATP-dependent nucleoside group translocator (4.B.1.1.1) that synthesizes NMN, an intermediate in NAD synthesis, and a thiamin porter (4.B.1.1.4), involved in the synthesis of thiamin pyrophosphate (TPP), were found in these strains but not in the extracellular pathogens (Table 8).

Surprisingly, the intracellular pathogens possess a glucuronate/galacturonate porter (2.A.1.14.2), which is absent in the extracellular pathogens (Table 8). These metabolic products have been shown to be generated intracellularly when exogenous sugars such as lactose are metabolized by *E. coli* [88]. These compounds are present intracellularly in numerous organs of animals, and these *E. coli* strains utilize cytoplasmic UDP-glucuronyltransferases for mucopolysaccharide biosynthesis [89]. Additionally, a sialic acid uptake porter (2.A.56.1.6) is found exclusively in the intracellular pathogens. Although sialic acids are found in abundance in cell surface glycolipids and extracellular glycoproteins, *N*-acetylneuraminic acid, the main mammalian sialic acid, can be found intracellularly where it participates in glycolipid and glycoprotein biosynthesis [90]. Sialic acid-containing polysaccharide capsules are virulence factors in *E. coli* urinary tract infections and contribute to biofilm formation [91].

Intracellular *E. coli* strains lack various transporters present in extracellular pathogens that take up metabolites normally found extracellularly. For example, the latter, but not the former strains possess a rhamnose oligosaccharide porter (3.A.1.5.12), a melibiose permease (2.A.2.1.1) and a 2-O- α -mannosyl-D-glycerate porter (4.A.2.1.3). These first two oligosaccharides are constituents of glycolipid and glycoproteins found on the external surfaces of intestinal villus cells, while the last compound is a known intracellular osmolyte in some bacteria [92]. The extracellular strains, but not the intracellular strains, also have a xylose:H⁺ symporter (2.A.1.1.3), a mannose PTS porter (4.A.2.1.6), a glucitol PTS transporter (4.A.4.1.2), and a galactitol PTS permease (4.A.5.1.3). The former two sugars are found in abundance in cell surface polysaccharides [93,94] while the hexitols are common osmolytes in animals [95]. The α -glucoside-specific IICB permease of the PTS (4.A.1.1.10), which transports many glucosides is similarly found in the extracellular pathogens but lacking in intracellular strains (Table 8).

Other transporters lacking in intracellular but present in extracellular pathogens include an uptake system for the folate catabolite, p-aminobenzoyl-glutamate (2.A.68.1.1), and a cyanate/thiocyanate porter (2.A.1.17.1) ([96,97]). Thiocyanate and cyanate are found in extracellular environments, and although toxic, they can be used as a nitrogen source following destruction by cyanase, CynS [96]. *E. coli* cells lacking the *cyn* operon are more sensitive to the toxic effects of cyanate and thiocyanate than strains that possess it [98].

A putrescine uptake porter (2.A.3.1.13) was found to be present only in extracellular strains. Putrescine, like other positively charged polyamines, stabilizes the negatively charged DNA during replication, but it is also found in abundance in extracellular environments in mammals such as seminal fluid and urine, especially during episodes of protein breakdown [99,100]. Several MDR and iron-siderophore transporters are found exclusively in intracellular pathogens, while others are present in extracellular pathogens exclusively. These facts may reflect the natural cellular substrates of MDR pumps currently not recognized.

3.3. Proteins with e-values between 0.1 and 0.0001

Several proteins were identified that gave poor scores in the G-BLAST search, but which nevertheless proved to be members of TC families. Some of these are presented here in order of their TC numbers. (1) A putative invasins with similarity to proteins in the OmpA family (TC#1.B.6) was identified and given the TC number 1.B.6.2.13. (2) A homolog of pore-forming phage coat protein A was identified and assigned TC#1.B.53.1.4. (3) Several proteins proved to be homologous to the cytotoxic pore-forming oligomeric fimbrial subunit, MrxA. One such protein has been entered into TCDB under TC#1.C.80.1.2. (4) A holin of 108 aas was identified and entered into TCDB with TC#1.E.2.1.10. This protein was encoded by a gene within a prophage segment of the genome of UMN with two adjacent genes, a lysozyme inhibitor, and a lytic protein, probably a lysozyme. (5) An ABC porter, YnjBCD, was assigned TC#3.A.1.19.4. Although it is annotated as a sulfate/thiosulfate transporter, other members of this family transport vitamins. (6) Another protein designated YjiG proved to be a distant member of the FeoB family (TC#9.A.8). A nucleoside-recognition domain may be present in some of these homologs. YjiG was entered into TCDB with TC#9.B.156.1.1.

3.4. Unique characteristics of enterohaemorrhagic (EHEC) strain, O15

Strain O15 exhibited a number of characteristics that distinguished it from the other six pathogenic strains of *E. coli* examined. It has two-partner secretion (TPS) systems corresponding to TC#1.B.20.1.3 and 1.B.20.1.4. The former system plays a role in contact inhibition of growth [59] while the latter protein is a ShlB-type protein that contains a domain critical for substrate recognition and folding [101]. It probably secretes a hemolysin-like virulence factor [102] and has been entered into the database under 1.B.20.1.7.

This strain uniquely has an AT-2 type adhesin, most closely related to TC#1.B.40.1.3, as well as an intimin, most closely related to TC#1.B.54.1.1. These proteins were found in no other *E. coli* strain examined. Most surprisingly, we identified 14 hits of 1.B.6.2.1 and 1.B.6.2.2, all related to OmpX of the OmpA family. These proteins are in the Ail/Lom family, which in *Yersinia* species are multifunctional virulence factors that protect against complement [103]. Equally surprising was the unique discovery of 12 putative holins corresponding to TC#1.E.1.1.3. These holins may be related to phage-encoded shiga-toxins [104].

4. Discussion

4.1. Iron siderophore receptors and transporters contribute to *E. coli* pathogenesis in a host organism

E. coli is a facultative anaerobe that utilizes iron primarily for electron transport and cellular respiration. Succinate dehydrogenase and various cytochromes as well as NADH and ferric oxidase activities are diminished with an iron deficiency [105]. In humans, iron sequestration plays an important role in host defense against pathogens. Certain conserved eukaryotic genes encode siderophore/iron-uptake complexes that allow the host to upregulate iron sequestration in response to inflammatory signals accompanying infection [106]. Some of these genes play pivotal roles in host defense in Crohn's disease, for their upregulation allows the host intestinal epithelial cells to sequester more iron [107]. In our study, the pathogenic *E. coli* strains examined proved to have greater numbers of iron-siderophore receptor-encoding genes than the nonpathogenic K12 strain.

Certain iron-siderophore receptors and ABC iron uptake systems are abundant only in pathovars (Tables 6 and 7). These receptors include a Fe³⁺ catecholate receptor (1.B.14.1.4) and the ferric yersiniabactin uptake receptor (1.B.14.7.2). The ferric yersiniabactin uptake receptor is known to be required for biofilm production in UPEC strains [108], possibly relevant to host inflammatory signaling. Ferri-enterobactin receptors (1.B.14.1.3) and ferrichrome receptors (1.B.14.1.15) were identified exclusively in UPEC strains (ABU and CFT). These receptors may serve specific functions in UPEC pathogenicity.

The increased numbers of iron-complex receptors in pathovars indicate targets for combating *E. coli* pathogenicity. Increases in siderophore receptor complexes may serve as compensatory mechanisms in response to host defense. Increased ferrichrome sequestration undoubtedly allows these pathovars to take up iron more readily, leading to increased survival in the host.

As shown in Fig. 2, increases in numbers of iron-sequestering outer membrane receptors correlate with increases in inner membrane iron ABC transport systems. The UPEC strains (ABU, CFT and UMN) have larger numbers of both iron-siderophore OMRs and inner membrane transporters. We speculate that because UPEC strains must survive in both the gastrointestinal and the urogenital tracts, they must possess a greater number of iron-siderophore transporters for cellular respiration and survival.

It has been shown that ABC porters (3.A.1) in general possess higher affinities for their substrates than secondary carriers (TC#2.A). Secondary carriers transporting metal cations typically exhibit Km values of ~20–800 μM [109] while primary active transporters may exhibit Km values 1 μM [110]. Interestingly, the majority of ABC iron transporters were found either exclusively in pathogenic strains or in increased numbers (Table 7), and the same strains possess the largest numbers of iron-siderophore OMRs. TC subclass 2.A iron uptake secondary carriers appear to be present in invariant numbers in all eight *E. coli* strains although the ABC iron-specific transporters differ greatly among the different serovars (Table 7). This suggests that the low affinity secondary iron carriers serve housekeeping functions outside of the body, where iron concentrations are relatively high,

while the ABC transporters function in pathogenesis in the *in vivo* environment, where competition with the host is an important factor.

4.2. Pore-forming toxins

EHEC and EPEC pathovars typically operate by attaching and effacing (A/E) mechanisms. This type of invasion includes utilizing a Type III secretion system to inject effector proteins into host cells. One effector, EspF, plays an important role in tight junction disruption as well as inhibition of sodium and water uptake [111]. The EspB/D complex was identified in UMN (UPEC) and O15 (EHEC) and serves to initiate pore formation in the host cell plasma membrane as well as translocation of EspF into the cell. UPEC strains typically utilize Type I Secretion Systems for adhesion and entry through manipulating the host cell cytoskeleton using effectors that stimulate Rho-family GTPases and kinases [112]. Nevertheless, a complete T3SS was identified in UMN, suggesting that an A/E mechanism of invasion may occur in this pathovar.

The identification of Hemolysin A (HlyA) exclusively in the UPEC strains supports a previous finding that these strains can utilize haemolysins to inhibit protein kinase B signaling and lead to apoptosis of host cells [35]. Similarly, the exclusive presence of shiga toxin in O15 supports previous findings of EHEC pathogenicity [113]. The Stx pathway may provide an alternative pathway by which the EHEC strain can inhibit chemical signaling and immune responses in the host cell.

Homologs of the clostridial cytotoxin (CCT) form channels and inactivate Rho-type GTPases, leading to manipulation of the host cytoskeleton [39]. Predictably, such a toxin was identified in the UPEC strain, ABU, but more surprisingly, it was also found in O15. This may suggest an alternative mechanism by which EHEC strains manipulate the host's cytoskeletal machinery since these pathovars typically utilize the mitochondrial-associated protein [69] to inhibit the host cell control protein 42 (CDC42), which provides regulatory functions in host cell actin dynamic control [114]. Since the homologs in ABU and O15 are 85% identical, the same mechanism is likely to be operative.

AIEC strains such as APE, have been cited in 36% of Crohn's disease. These pathovars operate through necrosis factors that impair host immune responses, allowing the bacteria to colonize the ileal mucosa and lamina propria [115]. We identified the active subunit of Cytolethal Distending Toxin (CdtB) only in APE. The CdtB active subunit induces DNA double-strand breakage, leading to host cell cycle arrest in the G2/M phase [116]. This is preferable in the AIEC pathotype, presumably because pauses and irregularities in host cell cycle give the bacteria time to proliferate and colonize host cells. Rapid proliferation of these AIEC cells can then induce secretion of Tumor Necrosis Factor Alpha (TNF- α) that causes inflammation in host cells.

Serratia-type pore-forming toxins (S-PFT) were identified in ABU (UPEC), CFT (UPEC) and O15 (EHEC). These toxins exhibit properties vastly different from those of RTX pore-forming haemolysins, which are exported via two-partner secretion systems and are much larger in size [40]. The presence of two-partner secretion system(s) in both ABU and O15

confirmed the possibility of a novel *Serratia*-type Pore-forming Toxin (S-PFT) in both UPEC and EHEC strains (see Table 3).

4.3. Outer membrane protein secretion systems

Many Autotransporter-1 systems were identified in the seven pathovars examined. Examples include the fibronectin binding protein (1.B.12.1.3), a tracheal colonization factor (1.B.12.2.2), a firmicute homolog (1.B.12.2.4), and a *Salmonella* BigA homolog (1.B.12.5.5) (see Table 5). Fibronectin binding protein is known to assist in host cell adhesion by *Salmonella typhimurium* [117], providing evidence that a similar mechanism of adhesion may be utilized by UPEC, AIEC, ETEC and EAEC strains for host cell targeting. Interestingly, homologs of the *Bordetella pertussis* tracheal colonization factor were identified in all pathovars except for O15, providing clues as to how such strains target cilia-rich epithelia in the intestines.

Several Autotransporter-2 systems were found exclusively in the pathovars. Homologs of the membrane-anchored hemagglutinin, identified in UMN and O15, may play roles in bacterium-host cell adhesion [18]. Trimeric adhesins such as Cha (1.B.40.1.5) and UpaG (1.B.40.2.3) were also identified exclusively in pathovars, and they are known to function in adhesion to the host extracellular matrix [118]. YadB homologs (1.B.40.1.3) function by a similar mechanism, and one was identified in O15, suggesting that it may function specifically in EHEC strains.

EHEC and EPEC strains have been shown to use invasins/intimins to target translocated intimin receptors (Tir) and β -1 integrins on host extracellular matrices for adhesion purposes followed by stimulation of actin rearrangements via the N-Wasp pathway ([119,120]). UPEC strains, however, utilize type I pili to bind β -1 integrin on host cell extracellular matrices to initiate actin rearrangements [112]. The presence of invasins/intimins to target β -1 integrins reveals a potential novel mechanism of cell-adhesion and actin rearrangement utilized by these UPEC pathovars.

The MdtP outer membrane factor (OMF; TC#1.B.17.3.9) functions with the MdtO aromatic acid/drug exporter (2.A.85.6.1) and the auxiliary MFP protein, MdtN (8.A.1.1.3). This OMF was found in all pathovars but not in K12. This may indicate a multi-drug resistance mechanism found exclusively in the pathogenic strains that contributes to their resistance to antibiotics.

Fimbrial usher proteins (FUPs) serve important functions in the assembly of fimbriae [67]. As expected, these proteins were found ubiquitously in all strains including K12. However, greater numbers of the encoding genes were observed in all pathovars compared to K12, presumably contributing to pathogenesis by promoting bacterium-host cell adhesion [70].

4.4. Multiple pathovars contain T6SS constituents

Potentially complete VasA-L T6SSs were identified in multiple pathovars. Protein products of the *icmF*, *hlc* and *clpV* genes play essential roles in biofilm assembly and general motility [121]. In recent studies, the T6SS present in *Pseudomonas aeruginosa* and other bacterial

cells were shown to engage in “T6SS dueling” with surrounding bacteria in competition for survival in the microbiome [122,123].

With the discovery of T6SSs in *E. coli* pathovars, we speculate that *E. coli* cells engage in T6SS-mediated competition in the human body. It is possible that pathovars of *E. coli* can utilize T6SSs both for offense and for defense in the host microbiome. Such a mechanism may prove valuable during invasion of the colon, where microbes are densely packed with other types of facultative/obligate anaerobes competing for limited metabolic resources.

4.5. Transporters in intracellular vs. extracellular pathogens

An interesting pattern revealed by our studies was the existence of different sets of nutrient uptake transporters, depending on the organismal site of proliferation. Intracellular vs. extracellular organisms were distinguishable on the basis of the nature of the substrates transported. In general, the intracellular pathovar-specific strains take up metabolites expected to be found inside host cells while the extracellular pathovars transport substrates that are normally found in abundance outside of host cells. Examples of this can be seen in transporters for glycolytic and Krebs cycle intermediates found only in intracellular pathogens as well as transporters for oligosaccharides found only in extracellular pathogens (Table 8). These facts indicate that these organisms have acquired transport systems through the frequent gain and loss of the encoding genes probably via horizontal transfer, to take advantage of nutrients available in their specific environments. Since intracellularity may be a relatively recent development, this suggests that the gain and loss of genes may have occurred with high frequency in response to immediate need. It seems probable that such characteristics (e.g., predilection for certain types of nutrient transporters) can be used for diagnostic purposes.

4.6. Unique characteristics of O15

In screening the data, we noticed that each strain examined has characteristics that distinguish it from all others. Particularly noteworthy was strain O15, which had numerous characteristics lacking in the other seven strains. One such example includes extra two-partner secretion systems as well as a unique AT-2 adhesin and an intimin. More striking was the occurrence of large numbers of OmpA-like Ail/Lom family proteins that probably serve multiple virulence functions, including protection against complement as has been documented in *Yersinia* species [103]. Additionally, this strain possessed numerous holins, all homologous to 1.E.1.1.3, a unique characteristic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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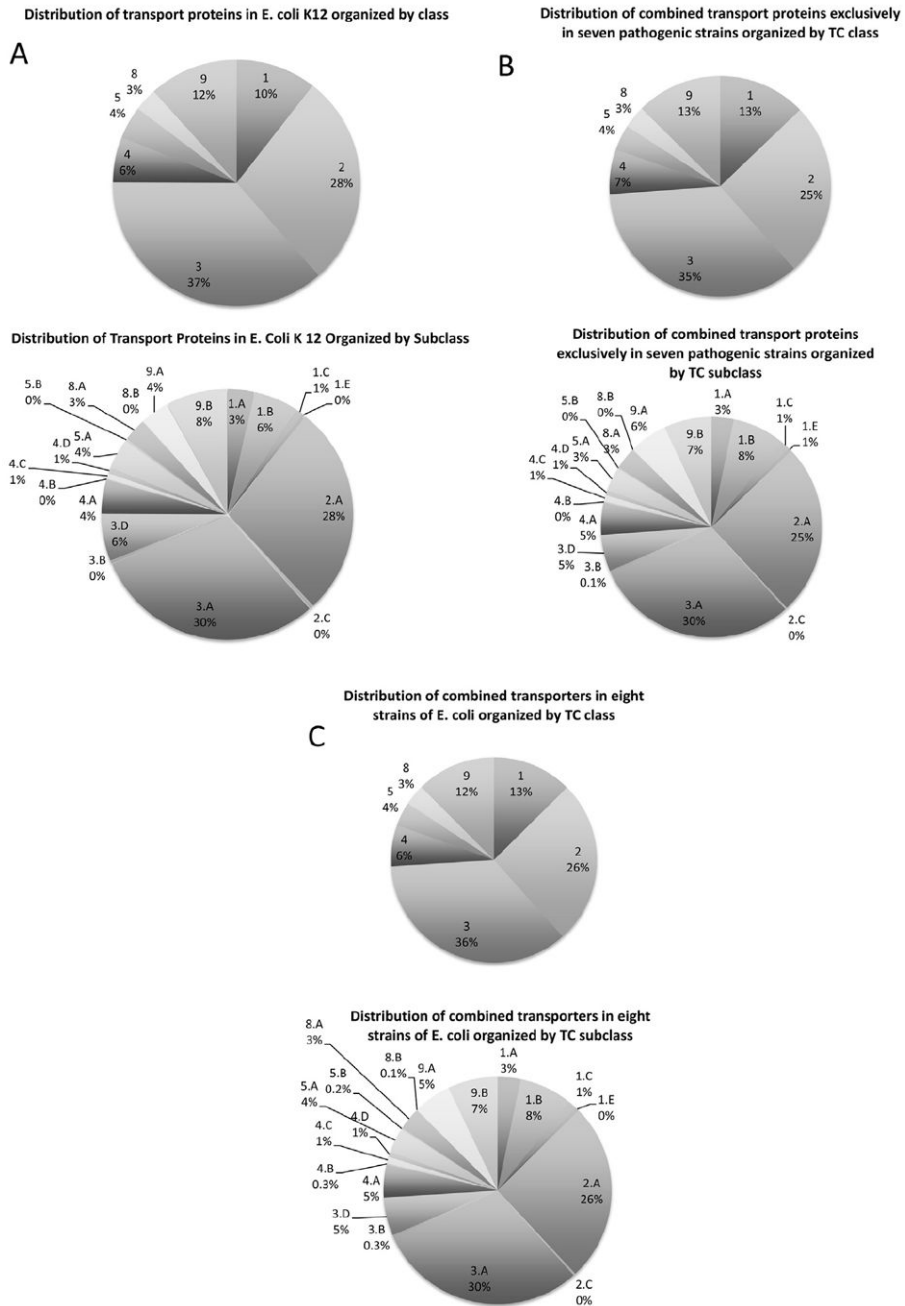


Fig. 1. Distribution of transport proteins according to TC class (top) & TC subclass (bottom) for *E. coli* K12 str. MG1655 (top) and the seven pathogenic strains combined (bottom). Similar distributions for the eight *E. coli* strains individually and combined are presented in Supplementary Figs. S1A–S1I.

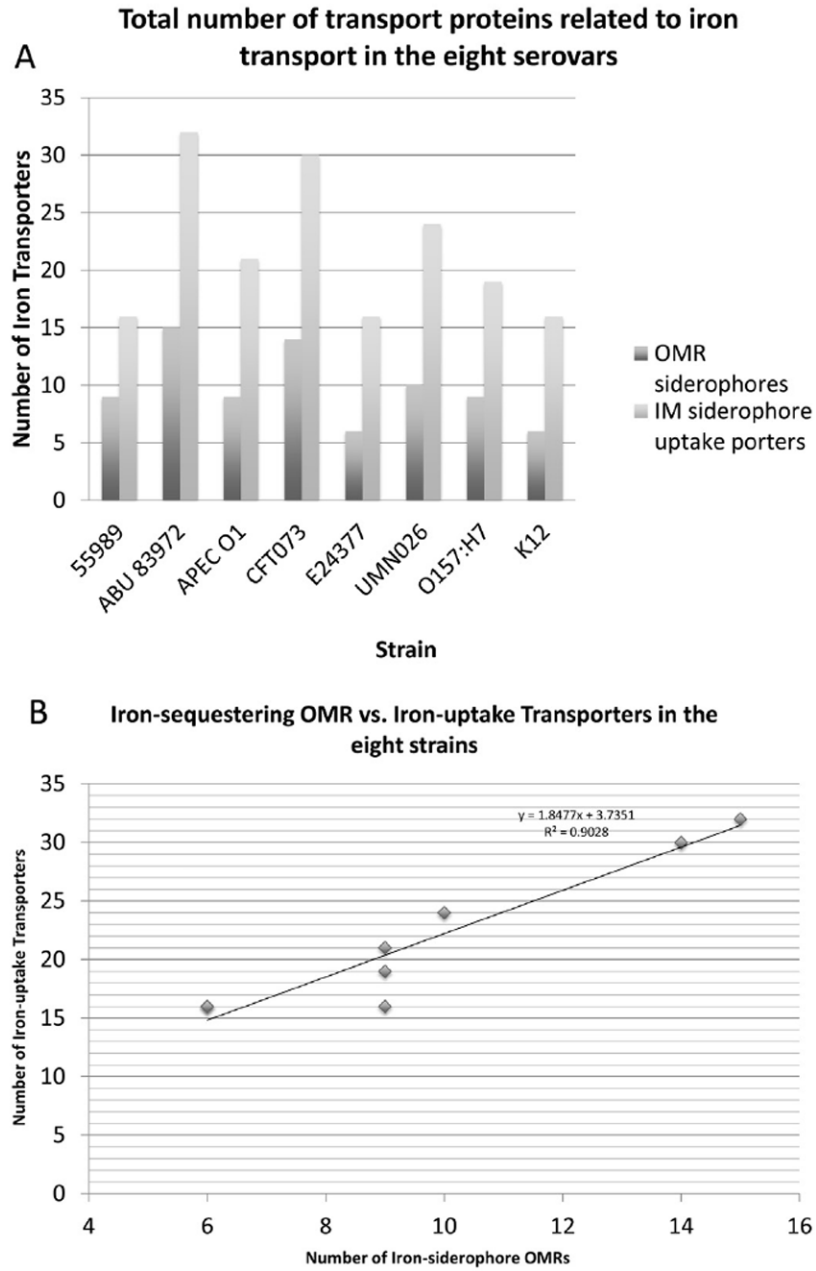


Fig. 2. Total number of proteins associated with iron transport in the eight *E. coli* strains in which (A) shows the relationship between the serovars (*x*-axis) and number of iron-siderophore transporters (*y*-axis). The OMR-type transporters are represented in dark gray while the ABC iron-siderophore uptake porters are presented in light gray. (B) Correlation between iron-sequestering OMRs (*x*-axis) and ABC iron-uptake transporters (*y*-axis) for the eight strains of *E. coli* examined. The best-fit line has a slope of 1.85 and a R^2 value is 0.90.

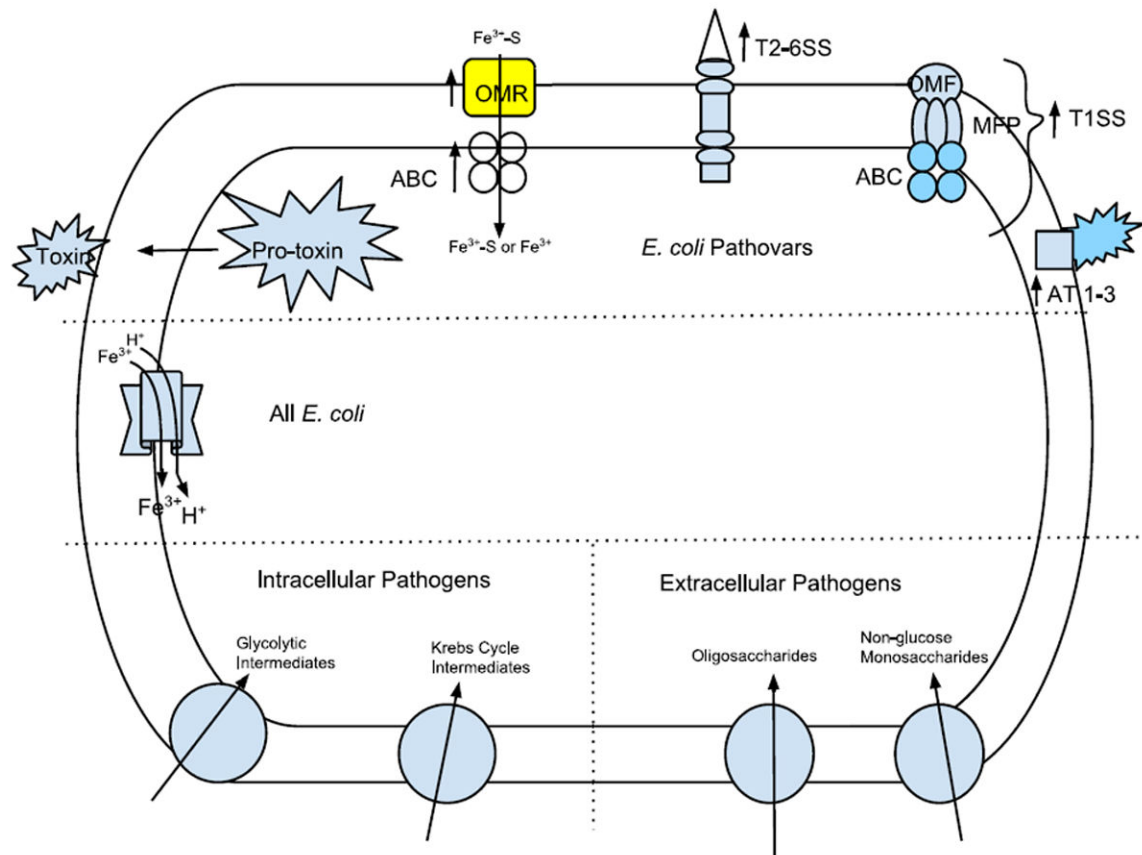


Fig. 3. Schematic view of an *E. coli* cell showing transport proteins of particular importance for pathogenesis. All *E. coli* cells have the same secondary carriers for iron uptake which therefore do not play a specific role in pathogenesis (center left). Systems preferentially present in pathogens (top) often lacking in *E. coli* K12, include pro-toxins, ABC-type iron uptake systems which are proposed to function with their iron-specific outer membrane receptors (OMRs), transenvelope protein secretion systems types 1–6 and autotransporters of the types AT1-3 transporters specifically found in extracellular vs. intracellular pathogens are illustrated in the bottom right and left, respectively. See text for details.

Table 1

Seven *E. coli* pathovars, the non-virulent *E. coli* K12 strain, and their basic traits.

Strain	Abbr.	Genome size (Mbp)	# of proteins	Host location	Pathological condition	Pathovar type
55989	559	5.15	4759	Extracellular	Diarrhea	EAEC
ABU 83972	ABU	5.13	4796	Intracellular	Bacteremia	UPEC
APEC O1	APE	5.50	4853	Intracellular	Respiratory infection (avians)	AIEC
CFT073	CFT	5.23	5369	Intracellular	Urinary tract infection & pyelonephritis	UPEC
E24377A	E24	5.25	4991	Extracellular	Enterotoxicity	ETEC
UMN026	UMN	5.36	5014	Extra/intracellular	Urinary tract infection	UPEC
O157:H7	O15	5.70	5477	Extracellular	Haemorrhagic colitis	EHEC
K12.MG1655	K12	4.64	4290	Extracellular	None	Non-virulent

Table 2

Numbers of proteins (even numbered columns) and percent distribution of subclasses by TC number (odd numbered columns) for all eight strains of *E. coli* as well as numbers of transport proteins per strain (bottom) and all strains combined, with and without K12 (last four columns).

Subclass	559	%	CFT	%	ABU	%	APE	%	E24	%	UMN	%	O15	%	K12	%	All	%	All-K12	%
1.A	42	3.4	37	2.9	41	3.2	41	3.3	42	3.5	39	3.1	42	3.47	39	3.5	323	3.3	284	3.3
1.B	94	7.6	103	8.0	108	8.4	91	7.3	89	7.4	95	7.5	96	7.93	67	5.9	743	7.5	676	7.7
1.C	15	1.2	18	1.4	20	1.6	15	1.2	11	0.9	15	1.2	21	1.74	9	0.8	124	1.3	115	1.3
1.E	5	0.4	6	0.5	4	0.3	6	0.5	3	0.2	7	0.6	14	1.16	4	0.4	49	0.5	45	0.5
2.A	318	25.6	325	25.4	319	24.8	315	25.3	309	25.7	319	25.2	299	24.71	311	27.6	2515	25.5	2204	25.2
2.C	5	0.4	5	0.4	4	0.3	4	0.3	3	0.2	4	0.3	3	0.25	4	0.4	32	0.3	28	0.3
3.A	358	28.8	378	29.5	386	30.0	369	29.7	353	29.4	390	30.8	369	30.50	340	30.1	2943	29.8	2603	29.8
3.B	4	0.3	3	0.2	5	0.4	5	0.4	5	0.4	4	0.3	4	0.33	4	0.4	34	0.3	30	0.3
3.D	69	5.5	66	5.2	64	5.0	59	4.7	69	5.7	72	5.7	68	5.62	69	6.1	536	5.4	467	5.3
4.A	56	4.5	62	4.8	62	4.8	61	4.9	55	4.6	61	4.8	51	4.21	49	4.3	457	4.6	408	4.7
4.B	2	0.2	4	0.3	4	0.3	4	0.3	2	0.2	2	0.2	2	0.17	2	0.2	22	0.2	20	0.2
4.C	10	0.8	17	1.3	16	1.2	11	0.9	9	0.7	11	0.9	7	0.58	8	0.7	89	0.9	81	0.9
4.D	9	0.7	10	0.8	12	0.9	9	0.7	8	0.7	7	0.6	9	0.74	8	0.7	72	0.7	64	0.7
5.A	43	3.5	44	3.4	44	3.4	45	3.6	42	3.5	43	3.4	43	3.55	45	4.0	349	3.5	304	3.5
5.B	3	0.2	2	0.2	2	0.2	2	0.2	2	0.2	4	0.3	3	0.25	3	0.3	21	0.2	18	0.2
8.A	37	3.0	45	3.5	43	3.3	40	3.2	39	3.2	42	3.3	37	3.06	33	2.9	316	3.2	283	3.2
8.B	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.08	1	0.1	8	0.1	7	0.1
9.A	87	7.0	64	5.0	63	4.9	77	6.2	71	5.9	66	5.2	60	4.96	42	3.7	530	5.4	488	5.6
9.B	86	6.9	91	7.1	89	6.9	88	7.1	89	7.4	85	6.7	81	6.69	90	8.0	699	7.1	609	7.0
total	1244	100	1281	100	1287	100	1243	100	1202	100	1267	100	1210	100	1128	100	9862	100	8734	100

Table 3

Toxins identified in *E. coli* pathovars and K12.

Family	TC ID	Function	559	ABU	APE	CFT	E24	UMN	O15	K12
HlyE	1.C.10.1.1	Pore-forming hemolysin, HlyE	0	1	0	1	0	1	1	1
RTX toxin	1.C.11.1.3	Pore-forming hemolysin, HlyA	0	2	0	1	0	0	0	0
IIITCP	1.C.36.1.1	T3SS: pore-forming complex, EspB/D	0	0	0	0	0	0	2	0
IIITCP	1.C.36.3.1 and 3.2	T3SS: pore-forming complex, SipBD	0	0	0	0	0	2	0	0
IIITCP	1.C.36.5.1	T3SS: pore-forming complex, SseBCD	0	0	0	0	0	0	1	0
Shiga toxin B	1.C.54.1.1	Shiga toxin B, St-B	0	0	0	0	0	0	2	0
Clostridial cytotoxin	1.C.57.3.2 and 3.3	Pore formation; necrosis in host, Cnf	0	1	0	0	0	0	1	0
S-PFT	1.C.75.1.1	Pore-forming hemolysin, ShlA	0	1	0	1	0	0	1	0
Cytotoxic major fimbrial subunit (Mrx-A)	1.C.80.1.1	Adhesive fimbriae (pore formation)	2	4	3	5	2	2	4	2
Cytolethal distending toxin	1.C.98.1.1	Cell cycle arrest/apoptosis, CdtABC	0	0	3	0	0	0	0	0

Table 4

Pathogenicity-related transenvelope protein secretion systems.

Family	TC ID	Function	559	ABU	APE	CFT	E24	UMN	O15	K12
T1SS	3.A.1.105.3 and 105.4	Drug exporter 1	8	8	8	8	7	8	8	8
	3.A.1.109.4	Protein exporter 1	0	4	0	4	0	2	2	0
	3.A.1.110.1	Protein exporter 2	0	3	0	4	0	0	0	0
	3.A.1.113.3	Peptide 3 exporter	1	1	1	1	1	1	1	1
T2SS	3.A.1.134.7	Peptide 7 exporter	15	14	13	14	13	13	14	13
	3.A.5.1.1	SECeSRP complex	7	7	7	7	7	7	7	7
GSP										
MTB	3.A.15.1.1	Pullulanase secretion system	8	13	14	8	8	7	0	8
	3.A.15.2.1	Pilin secretion/fimbrial assembly system	5	4	4	3	4	5	4	4
	3.A.15.3.1	LSP/fimbrial assembly system	0	0	0	1	0	1	0	0
T3SS	3.A.6.1.1	Type III secretion system complex	4	1	1	1	6	8	13	1
	3.A.6.2.1	Flagellar protein export complex	11	11	11	11	11	17	11	10
T4SS	3.A.7.3.1	Pertussis toxin exporter	0	0	0	0	0	4	0	0
	3.A.7.7.1	Trs DNA transfer protein complex	2	2	2	2	2	2	2	2
	3.A.7.11.1	Type IV beta-proteobacterial DNA secretion system	2	1	4	1	2	2	3	1
	3.A.7.13.2	Plasmid pLS20 conjugation system	1	2	2	1	1	2	1	1
T6SS	3.A.7.14.2	Conjugation system plasmid DNA IntP/TcpA-J	5	4	3	5	5	4	5	5
	3.A.23.1.1	T6SS VasA-L	19	8	18	7	17	13	13	0
	3.A.23.2.1	T6SS EypA-P	19	5	6	5	6	2	2	1

Table 5

Outer membrane protein secretion systems.

Family	TC ID	Function	559	ABU	APE	CFT	E24	UMN	O15	K12
FUP	1.B.11.2.1 and 2.2	Type π fimbrial usher protein, PapC and YbgQ	3	3	3	4	3	3	2	1
	1.B.11.3.2, 3.3, 3.5, 3.6 and 3.9	Type γ fimbrial usher protein, MrkC, EepC, YcbS, YraI, and FimD	8	8	5	6	6	8	7	6
	1.B.11.4.1	Type α fimbrial usher protein, CfaC	2	1	1	1	2	1	1	1
AT-1	1.B.12.1.1	Adhesin, AidA	1	1	1	1	1	1	1	2
	1.B.12.1.2	Virulence factor, IcsA (VirG)	2	2	0	2	2	0	1	0
AT-1	1.B.12.1.3	Fibronectin binding protein, MisL	1	0	1	1	1	2	1	0
	1.B.12.2.1	Pertactin, Ptt	1	0	0	0	1	1	0	1
AT-1	1.B.12.2.2	Tracheal colonization factor, TcfA	1	1	1	1	1	1	0	0
	1.B.12.2.3	Resistance to killing proteins, BrkA	0	0	1	0	0	0	0	0
AT-1	1.B.12.2.4	Putative adhesin/protease/pertactin	2	3	1	3	0	1	0	0
	1.B.12.4.2 and 4.3	Serine proteases, EspP and Tsh	3	3	1	3	0	1	1	0
AT-1	1.B.12.5.5	Virulence factor, BigA	0	1	1	0	0	1	0	0
	1.B.12.8.2	Auto-aggregation adhesin, Flu	1	3	1	3	2	1	1	1
AT-1	1.B.12.8.3	Adhesin, TibA	2	2	2	1	2	3	3	1
	1.B.17.1.1 and 1.4	Hemolysin, drug and siderophore export protein, TolC	1	1	1	1	1	2	2	1
AT-1	1.B.17.3.9	Multidrug resistance outer membrane export protein, MdtP	1	1	1	1	1	1	1	0
	1.B.20.1.3 and 1.4	Two-partner secretion exporter, CdiB and OptB	0	1	0	0	0	0	2	0
Secretin	1.B.22.1.1	PuID secretin	1	2	2	1	1	1	0	1
	1.B.22.3.3	YscC secretin	0	0	0	0	0	1	2	0
Secretin	1.B.22.4.2	HofQ secretin	1	1	1	1	1	1	1	1
	1.B.22.5.1	Phage gene IV protein secretin	0	0	0	0	1	0	0	0
OMIP	1.B.33.1.3	Outer membrane insertion complex	6	6	6	6	6	6	6	6
	1.B.40.1.2	Hemagglutinin	0	0	0	0	0	1	1	0
AT-2	1.B.40.1.3	Adhesin, YadB	0	0	0	0	0	0	1	0
	1.B.40.1.5	Adhesin, Cha	0	0	1	0	0	1	0	0
AT-2	1.B.40.2.3	Adhesin, UpaG	1	1	1	1	1	0	1	0
	1.B.48.1.1	Curli fiber subunit, CsgA	1	1	1	1	1	1	1	1
AT-3	1.B.54.1.1	Intimin/Invasin (putative Autotransporter-3), Eae	2	5	4	4	4	5	4	2

Table 6

Outer membrane iron-siderophore receptors.

Family	TC ID	Receptor specificity	559	ABU	APE	CFT	E24	UMIN	O15	K12
OMR	I.B.14.1.1	Ferric coprogen, FhuE	1	1	1	1	1	1	1	1
	I.B.14.1.2	Ferrichrome, FhuA	1	1	1	1	1	1	1	1
	I.B.14.1.3	Ferric enterobactin, Iron	0	1	0	1	0	0	0	0
	I.B.14.1.4	Catecholate, CirA	1	2	2	3	1	1	2	1
	I.B.14.1.9	Catecholate, Flu	1	1	1	1	1	1	1	1
	I.B.14.1.10	Ferroxamine, FoxA	0	1	1	1	0	0	1	0
	I.B.14.1.12	Ferric citrate, FecA	1	1	0	0	1	1	0	1
	I.B.14.1.13	Ferric receptor, CfrA	1	1	0	1	0	1	1	0
	I.B.14.1.15	Ferrichrome, FcuA	0	1	0	1	0	0	0	0
	I.B.14.2.2	Heme, Hema	0	2	1	2	0	1	1	0
	I.B.14.3.1	Cobalamin, BtuB	1	1	1	1	1	1	1	1
	I.B.14.4.1	Cupric ion, OprC	0	1	1	1	0	1	0	0
	I.B.14.7.2	Ferric-yersiniabactin, FyuA	2	2	2	1	1	2	1	1
	I.B.14.9.3	Ferrichrome/aerobactin, IutA	1	1	0	1	0	1	0	0
	2.C.1.1.1	TonB	1	2	2	2	1	2	1	1
	2.C.1.2.1	TolA	3	2	2	1	2	2	2	1

Table 7

Inner membrane iron uptake systems.

Family	TC ID	Function	559	ABU	APE	CFT	E24	UMN	O15	K12
CorA	1.A.35.2.1	Divalent cation transport	2	2	2	2	2	2	2	2
ZIP	2.A.5.5.1	Pmf-mediated M ²⁺ uptake	1	1	1	1	1	1	1	1
Nraup	2.A.55.3.1	Divalent cation:H ⁺ symport	1	1	1	1	1	1	1	1
FeoB	9.A.8.1.1 and 1.9	GTPase-coupled ferrous iron uptake	2	2	2	2	2	2	2	2
FeT	3.A.1.10.3	Ferric iron uptake	1	1	1	1	1	1	1	1
FeCT	3.A.1.14.1	Ferric iron-dictrate uptake	4	4	0	0	4	4	0	4
	3.A.1.14.2	Ferric iron-enterobactin uptake	4	8	4	8	4	4	4	4
	3.A.1.14.3	Ferric iron-hydroxamate uptake	3	3	3	3	3	3	3	3
	3.A.1.14.8	Ferric-vibrioferrin uptake	0	2	2	2	0	1	1	0
	3.A.1.14.18	Heme uptake	0	3	3	3	0	3	3	0
	3.A.1.14.21	Heme uptake	0	2	0	2	0	0	0	0
MZT	3.A.1.15.7	Mn ²⁺ and Fe ²⁺ uptake	0	5	4	5	0	4	0	0
BIT	3.A.1.20.1	Iron uptake complex	0	0	0	0	0	0	0	0
Total			16	32	21	30	16	24	19	16

Table 8

Metabolite transporters unique to intracellular vs. extracellular pathogens.

Family	TCID	Function	Exclusively in 2 of 3 intracellular pathogens	Exclusively in all 3 intracellular pathogens	Exclusively in extracellular pathogens
E-CIC	I.A.13.2.3	Chloride efflux	x		
TEGT	I.A.14.2.2	Acetate transport	x		
FNT	I.A.16.1.2	Formate transport			x
OMF porin	I.B.6.1.2	Solute diffusion	x		
T _{ss}	I.B.10.1.1	Nucleoside-specific channel	x		
FUP	I.B.11.2.1	Type π fimbrial export	x		
	I.B.11.2.2	Putative fimbrial export	x		
	I.B.11.3.3	Type γ fimbrial export	x		
	I.B.11.3.5	Type γ fimbrial export	x		
AT-1	I.B.12.1.1	Adhesin export	x		
	I.B.12.2.1	Pertactin (adhesin) export			x
	I.B.12.2.2	Tracheal colonization factor export	x		
	I.B.12.4.1	Protease export	x		
	I.B.12.8.2	Adhesin export			x
	I.B.12.8.3	Adhesin/invasin export			x
OMF	I.B.17.3.4	Metal cation (Ag ⁺) export	x		
OMA	I.B.18.1.2	Polysaccharide export			x
Gene IV secretin (OMS)	I.B.22.5.1	Phage export			x
OmpL porin	I.B.35.2.2	Small molecule transport			x
AT-2	I.B.40.1.2	Hemagglutinin export			x
	I.B.40.1.3	Adhesin (YadB) export			x
AT-3 (Int/Inv)	I.B.54.1.1	γ -Intimin export			x
	I.B.54.1.2	Invasin export			x
IIITCP (EspB/D)	I.C.36.1.1	Host pore for T3SS	x		
ST-B	I.C.54.1.1	STX pore-forming virulence factor			x
Cnt2	I.C.57.3.3	Pore-forming virulence factor			x
Lambda holin	I.E.2.1.4	Holin; autolysis			x

Family	TCID	Function	Exclusively in 2 of 3 intracellular pathovars	Exclusively in all 3 intracellular pathovars	Exclusively in extracellular pathovars
OMR	1.E.14.1.9	Holin; autolysis		x	
MFS; SP	2.A.1.1.3	Monosaccharide uptake			x
MFS; DHA1	2.A.1.2.7	Drug efflux		x	
MFS; DHA1	2.A.1.2.52	Drug efflux			x
MFS; DHA2	2.A.1.3.18	Efflux of signaling molecules	x		
MFS; OPA	2.A.1.4.2	Glycolytic intermediate uptake	x		
MFS; MHS	2.A.1.6.1	Tricarboxylate uptake		x	
	2.A.1.6.9	Metabolite uptake		x	
MFS; ACS	2.A.1.14.2	Hexuronate uptake		x	
MFS; AAHS	2.A.1.15.2	Aromatic acid uptake			x
MFS; CP	2.A.1.17.1	Cyanate uptake			x
GPH	2.A.2.1.1	Disaccharide uptake			x
	2.A.2.3.9	Glycoside uptake			x
APC	2.A.3.1.13	Putrescine (polyamine) uptake			x
	2.A.3.2.7	Arginine uptake; acid homeostasis	x		
	2.A.3.7.2	Glutamate uptake; acid homeostasis			x
	2.A.3.8.17	Amino acid derivative uptake			x
RND	2.A.6.2.19	Drug export		x	
	2.A.6.2.6	Drug/autoinducer efflux			x
Betaine/carnitine/choline symporter	2.A.15.2.3	Organosamine uptake	x		
CPAI	2.A.36.3.1	Na ⁺ :H ⁺ antiport		x	
CNT	2.A.41.2.10	Nucleoside uptake			x
DASS	2.A.47.3.2	Citrate uptake; succinate export		x	
	2.A.47.4.2	Arsenite/antimonite export		x	
SuIP	2.A.53.3.8	Bicarbonate uptake			x
TRAP-T	2.A.56.1.5	Rhamnogalacturonide uptake		x	
	2.A.56.1.6	Sialic acid uptake		x	
DeuC	2.A.61.1.2	Dicarboxylate antiport			x
PST	2.A.66.2.10	Lipopolysaccharide precursor export	x		
AbgT	2.A.68.1.1	p-Aminobenzoyl-L-glutamate uptake			x

Family	TCID	Function	Exclusively in 2 of 3 intracellular pathovars	Exclusively in all 3 intracellular pathovars	Exclusively in extracellular pathovars
TTT	2.A.80.1.1	Tricarboxylate uptake		x	
ABC; CUT2	3.A.1.2.1	Ribose uptake		x	
	3.A.1.2.8	Sugar uptake			x
	3.A.1.2.11	Sugar alcohol uptake		x	
ABC; PepT	3.A.1.2.13	Autoinducer-2 uptake		x	
	3.A.1.5.12	Rhamnose oligosaccharide uptake			x
	3.A.1.5.20	Oligopeptide uptake		x	
ABC; LPT	3.A.1.125.2	Lipoprotein export			x
ArsAB	3.A.4.1.1	Arsenite export			x
NDH	3.D.1.9.1	H ⁺ /K ⁺ exchange (hydrogenase)			x
NFO	3.D.6.1.2	H ⁺ export. NADH:ferredoxin oxido-reductase			x
PTS; Glc	4.A.1.1.3	Maltose uptake		x	
	4.A.1.1.9	Glucose uptake		x	
PTS; Fru	4.A.1.1.10	Glucose uptake			x
	4.A.2.1.3	2-O- α -Mannosyl-D-glycerate uptake			x
	4.A.2.1.6	Mannose uptake			x
PTS; Gut	4.A.2.1.9	Keto sugar uptake		x	
	4.A.4.1.2	Sugar alcohol (glucitol) uptake			x
PTS; Gat	4.A.5.1.3	Sugar alcohol (galactitol) uptake			x
	4.B.1.1.4	Coenzyme precursor (thiamin) uptake		x	
DUF318	9.B.28.1.1	Unknown			x
YiaW; DUF3302	9.B.32.1.2	Unknown			x
	9.B.50.1.2	Possible dicarboxylate uptake		x	
UIT3	9.B.124.1.1	Unknown			x
DUF805	9.B.124.1.4	Unknown		x	