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Dr Adam P. Dale, Neil Woodford

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Extra-Intestinal Pathogenic *Escherichia coli* (ExPEC): *Disease, Carriage and Clones*

Adam P. Dale[a*] & Neil Woodford[b,c]

[a]*Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, South Academic Block, Tremona Road, Southampton, SO16 6YD, UK;* [b]*Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, Reference Microbiology Services, Public Health England, London NW9 5EQ, UK;* [c]*The NIHR Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance at Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0HS, UK*

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*Correspondence: Dr Adam P. Dale, *Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, South Academic Block, Tremona Road, Southampton, SO16 6YD, UK. Email: A.P.Dale@Soton.ac.uk. Tel: 023 81206455*

Abstract

Extra-intestinal pathogenic *E. coli* (ExPEC) have a complex phylogeny, broad virulence factor (VF) armament and significant genomic plasticity, and are associated with a spectrum of host infective syndromes ranging from simple urinary tract infection to life-threatening bacteraemia. Their importance as pathogens has come to the fore in recent years, particularly in the context of the global emergence of hyper-virulent and antibiotic resistant strains. Despite this, the mechanisms underlying ExPEC transmission dynamics and clonal selection remain poorly understood. Large-scale epidemiological and clinical studies are urgently required to ascertain the mechanisms underlying these processes to enable the development of novel evidence-based preventative and therapeutic strategies. In the current review, we provide a concise summary of the methods utilised for ExPEC phylogenetic genetic delineation before exploring in detail the associations between ExPEC VFs and site-specific disease. We then consider the role of ExPEC as an intestinal colonist before outlining known associations between ExPEC clonal variation, specific disease syndromes and antibiotic resistance.

1. Introduction

Escherichia coli is a genetically diverse species comprising non-pathogenic gut commensals and strains responsible for intestinal and extra-intestinal disease. While awareness amongst healthcare professionals and the public relating to *E. coli* strains associated with intestinal disease, *e.g.* enterohaemorrhagic *E. coli* (serogroup O157:H7), is high, the same has not always been true for strains associated with extra-intestinal disease (1). In the context of rapidly increasing multidrug-resistance worldwide and a diminishingly effective antimicrobial arsenal to tackle resistant strains, extra-intestinal *E. coli* infections are now a serious international public health concern associated with a significant economic impact. Consequently, there is an increasing awareness of the importance of extra-intestinal *E. coli* amongst both healthcare professionals and the general public alike (2,3).

Historically, extra-intestinal *E. coli* isolates were separated into groups determined by disease-association, including uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), and sepsis-causing *E. coli* (SEPEC). These terms were subsequently deemed out-dated following the observation that *E. coli* isolates assigned to specific groups were capable of causing infection at multiple anatomical sites. In 2000, Russo and Johnson suggested the term extra-intestinal pathogenic *E. coli* (ExPEC) as an alternative descriptor for all non-commensal *E. coli* isolates capable of causing extra-intestinal disease (4). Unlike commensal *E. coli*, ExPEC have the ability to cause disease once outside the host gut reservoir due to the possession of pathogenic virulence factors. From a molecular viewpoint, Peirano *et al.* (5) defined ExPEC as isolates containing at least two of the following virulence factors (VFs) within their genome: *papA and/or papC, sfa/foc, afa/draBC, kpsM II and iutA* (see table 1).

ExPEC are most frequently implicated as urinary pathogens and are isolated as the infective agent in up to 90% of both simple community-acquired urinary tract infections (UTIs) and pyelonephritis cases. Other infections of the urinary tract, including prostatitis and catheter-associated UTIs, are also frequently caused by ExPEC (6–8). ExPEC are frequently implicated in infections originating from abdominal and pelvic sources including, but not limited to, biliary infections, infective peritonitis, and pelvic inflammatory disease (9,10). Less frequently, they are associated with skin and soft tissue infections, neonatal meningitis and hospital-acquired pneumonia (11,12). Haematogenous invasion of ExPEC from the initial infective focus results in the sepsis syndrome which, in the absence of timely management, may result in death.

Following a brief overview of the methods used for ExPEC phylogenetic delineation, the current review will consider the role of ExPEC as a potential intestinal commensal and detail the associations between ExPEC strains, their virulence factor profiles, and host disease.

2. ExPEC Lineage Determination; *A Brief History*

Methodologies used to define and understand ExPEC lineages and *E. coli* phylogeny are numerous and have evolved in parallel with the availability of new technologies (see table 2). In-depth analyses are available elsewhere (13,14). Briefly, in the early 1970s, O antigen-based serotyping, followed later by the addition of H and K antigen serotyping, was first utilised to delineate *E. coli* isolates from humans and other animals and allowed the identification of some of the *E. coli* strains we now refer to as ExPEC (15,16). In 1984, the pioneering work of Ochman and Selander led to the establishment of the *E. coli* reference strain collection (ECOR) comprising 72 isolates from human and other mammalian hosts (17). Multi-locus enzyme electrophoresis (MLEE) separated the isolates into five key phylogenetic groups (phylogroups), namely A, B1, B2, D and E. The distribution of ExPEC isolates within these phylogroups will be discussed in detail later, however they are mainly limited to groups B2 and D (see figure 1).

In 2000, a triplex polymerase chain reaction (PCR)-based method was devised by Clermont *et al.* and enabled rapid *E. coli* phylogroup assignment (18). This methodology was much faster than MLEE, was simple and inexpensive, and allowed *E. coli* isolates to be separated into four main phylogroups (A, B1, B2, D). An updated multiplex PCR method was more recently devised by Doumith *et al.* (19). An independent study by Turrientes *et al.* subsequently compared both methods against a multi-locus sequence typing standard and demonstrated superiority of Doumith's method with regards to accuracy of phylogroup assignment (20).

Since 2000, the understanding of *E. coli* phylogeny has improved significantly. Eight *E. coli* phylogroups are currently recognised (A, B1, B2, D, E, F, G, and Clade 1) and a new PCR approach enables isolates to be assigned to one of these phylogroups (21).

As DNA sequencing methods became more widely available, they superseded MLEE as the preferred technologies for phylogenetic analysis given their superior discriminative ability. Multi-locus Sequence Typing (MLST) involves sequencing of selected (often seven) bacterial housekeeping genes and, due to its standardised approach and greater resolution as compared with 'phylogrouping', has allowed more detailed analysis of ExPEC lineages (22,23). It separates the isolates into distinct sequence types (STs), which are defined as isolates with

identical allelic profiles, and into broader clonal complexes (CCs), which are defined as a group of at least three STs each differing from the others by no more than 1 of 7 alleles (24). Of the three MLST schemes available for *E. coli*, the Achtman scheme (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) is most commonly used (14). *E. coli* sequence type (ST) data presented in the current review are derived from the Achtman scheme unless otherwise stated.

Although MLST is the preferred method for determining phylogenetic relationships in ExPEC, the discriminatory power of this technique is limited. Isolates belonging to the same ST can be genetically distinct and may be associated with variable pathotypic behaviours. In 2012, Weissman *et al.* (25) described a new method, CH typing, which derives its name from *fumC* and *fimH* gene analysis. They demonstrated that this approach was able not only to predict the respective MLST-based profile with up to 95% accuracy, but that it also enabled large STs to be split into a number of smaller clonal sub-groups (25). Although CH typing will not replace MLST as a tool for phylogenetic studies, there are clear advantages of this technique, particularly relating to delineation of clones within STs and reduced costs when performing preliminary evaluations on larger clinical specimen collections (25,26).

Ultimately, techniques such as pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) provide the greatest resolution for the purposes of ExPEC phylogenetic analysis, such as may be needed for outbreak investigation. Although internationally-standardised approaches, *e.g.* PulseNet (hosted by the Centre for Disease Control and Prevention), do allow for cross comparison of bacterial isolate PFGE results, the nature of any observed banding differences usually remains undefined. As a consequence of these factors, and as a result of the work-intensive nature of this method, PFGE is fast becoming an out-dated method. By contrast, WGS offers definitive and semi-quantitative comparison between isolates, *e.g.* through comparative single nucleotide polymorphism (SNPs) studies. As the cost of WGS falls it is likely to be used increasingly for surveillance and routinely in clinical practice. However, for reasons relating to standardisation, the ST nomenclature derived from well-established MLST schemes seems likely to be retained, even if the PCR-based methodologies themselves are replaced and ST data are, in future, largely derived from whole genome analysis (14,27).

3. ExPEC Virulence Factors

Virulence factors (VFs) associated with ExPEC pathogenicity are numerous and broad in scope, ranging from those associated with colonisation and bacterial fitness, to those that are

more traditionally associated with the term virulence, *e.g* VFs associated with ExPEC host invasion. ExPEC VFs are encoded on the bacterial chromosome, where they are usually located within pathogenicity islands (PAIs), or on plasmids, and are separated into five main groups including: 1. adhesins; 2. toxins; 3. iron acquisition systems; 4. capsule production and; 5. protectins and invasins (table 1). Current evidence suggests that no single VF renders an ExPEC isolate capable of causing site-specific disease. Instead, site-specific ExPEC pathogenicity is likely related to the timely expression of a whole complement of VF genes. An ever-expanding body of evidence, derived mainly from epidemiological and *in vitro* studies, continues to explore the associations between particular ExPEC VFs and site-specific disease.

3.1 Urinary Tract Infection-associated VFs

UTI-associated ExPEC originate mainly from the host's own faecal reservoir. Following urethral colonisation, ExPEC migrate proximally through the urinary tract to reach the bladder and kidneys where tissue invasion results in the clinical syndromes of cystitis and pyelonephritis, respectively (28,29).

Proteins expressed on the external surface of ExPEC ensure successful urinary tract colonisation by binding uroepithelial cells. Type 1 fimbriae, assembled by the chaperon-usher secretion pathway, bind to uroepithelial-associated α-D-mannosylated proteins, *e.g.* uroplakins, via the distally-located fimbrial adhesin H (FimH) and represent a key mechanism underlying ExPEC urothelial adhesion (30). *In vitro* studies have demonstrated that conformational changes in the FimH adhesin, and the formation of catch bonds, occur as consequence of rising shear stress and result in increased tissue binding affinity (31,32). Although these are *in vitro* findings, they provide insight into the potential mechanisms that may facilitate continued colonisation in the presence of variable hydrostatic forces resulting from urinary flow observed within the urinary tract (30). Other VFs associated with uroepithelial adhesion include Dr binding adhesion (33), S fimbriae/F1C fimbriae (30,33) and P fimbriae (34).

An array of VFs allows ExPEC to acquire ferric iron (Fe^{3+}), which is required for optimal growth and continued persistence within the urinary tract. Siderophores including yersiniabactin, salmochelin and aerobactin enable ExPEC to internalise and utilise distantly scavenged Fe^{3+} for growth (35). Many ExPEC also carry genes encoding haem receptors, which enable direct haemin uptake, while others produce toxins including α-haemolysin,

which cause host uroepithelial injury, cell lysis, and the subsequent release of intra-cellular micronutrients (36–38).

ExPEC are able to subvert the host immune response within the urinary tract through the production of conjugal transfer surface exclusion protein (TraT), an outer membrane protein associated with serum resistance (39). Furthermore, following uroepithelial cell invasion, ExPEC form intracellular bacterial communities (IBCs) that exhibit biofilm-like properties, protect bacteria from the host immune response, and are postulated to play a role in chronic infection and re-infection (40,41).

Studies in humans provide insight into the likely importance of known ExPEC virulence factors and their association with UTI syndromes. While some VF-encoding genes are evenly distributed amongst UTI-associated ExPEC, others have a skewed distribution, favouring isolates associated with particular UTI syndromes. Presence of the *pap* operon, encoding P fimbriae, for example, has been associated with isolates obtained from patients with pyelonephritis (42). A recent study by Firoozeh *et al.* (43) compared the prevalence of VF-encoding genes in 150 ExPEC isolates associated with UTI and demonstrated that *pap*, *traT*, *aer* and *hly* were more prevalent amongst patients with pyelonephritis compared with those suffering from cystitis. While these associations must be interpreted with caution, they do provide evidence to support the theory that VF profiles are likely to be associated with the propensity of ExPEC to cause site-specific disease within the urinary tract.

3.2 Neonatal Meningitis-Associated VFs.

ExPEC-related neonatal meningitis is associated with mortality rates approaching 40% and, in survivors, up to 50% develop significant neurological sequelae including seizures, hydrocephalus, encephalopathy and neurodevelopmental delay (44–46). The definitive mechanisms relating to blood brain barrier (BBB) invasion by ExPEC have yet to be completely elucidated, however the evidence base exploring these mechanisms comprising laboratory, animal and human studies continues to grow.

High-level bacteraemia precedes the development of ExPEC neonatal meningitis (47,48). VFs that contribute to the ability of ExPEC to maintain high-level bacteraemia include those that allow it subvert the host immune response, *e.g.* the anti-phagocytic external capsule and serum-resistance-associated outer membrane protein A (OmpA) (49,50). As well as penetrating the BBB via transcellular and paracellular mechanisms, meningitis-associated pathogens may also be carried across the BBB as intracellular residents within phagocytes.

ExPEC VFs associated with BBB invasion include invasion of brain endothelium (*ibe*) proteins, OmpA, FimH, the bacterial capsule, and cytotoxic necrotising factor 1 (CNF1) (48,50). The molecular mechanisms underlying BBB invasion and the involvement of these VFs is discussed in detail elsewhere (12,50,51).

3.3 VFs Associated with Bacteraemia and Gut Translocation

The VF profiles of ExPEC strains from bacteraemia are highly variable. Iron-acquisition systems are essential for bacteraemia strains and include aerobactin and yersiniabactin siderophores, iron uptake systems, *e.g.* IroN and SitA systems, and ChuA haemin receptors (52). Serum resistance mechanisms, which subvert complement-mediated killing, are also essential in bacteraemia ExPEC strains and include capsular components, lipopolysaccharide, and genes encoding proteins involved in serum resistance, *e.g. iss* and *traT* (52,53).

In vitro studies suggest that the ability of ExPEC to translocate across the intestinal mucosa is strain-specific and related to localised inflammatory and metabolic stressors (54). A collection of small association studies have attempted to establish the role of particular VFs associated with intestinal translocation and have all demonstrated that a collection, as opposed to individual VFs, are likely to be related to these events (55–57). In a recent study, Krawczyk *et al.* (57) assessed VF profiles of ExPEC bacteraemia isolates derived from bowel translocation events in patients with haematological malignancy and concluded that the co-occurrence of multiple virulence genes was associated with an increased likelihood of intestinal translocation events.

4. ExPEC as an Intestinal Colonist

Over the last decade, a number of authors have sought to establish the prevalence of *E. coli* with ExPEC potential colonising the gut by performing microbiological screening studies on the stool of healthy individuals that delineate resident *E. coli* strains based on their phylogroup (58–63). Results of these studies have demonstrated that 11-48% of healthy individuals are colonised with *E. coli* belonging to phylogroup B2, which is traditionally associated with virulence and ExPEC infections. The observed variation in the prevalence of asymptomatic gut carriage of strains belonging to phylogroup B2 is multifactorial and likely represents differences in geographical, population, and sampling-related factors.

Although it is clear that colonisation with *E. coli* isolates from phylogroup B2 is common, what is less clear is the associated virulence potential of phylogroup B2 isolates carried by asymptomatic individuals as determined by VF profile analysis. A recent review by Erjavec *et al.* (64) systematically assessed published studies that performed genomic VF profiling on gut colonising *E. coli* isolates in asymptomatic individuals. Their analysis demonstrated that, of 26 well-established ExPEC-associated VFs, molecular screening of only 7 (Afa, CNF-1, Fim, HlyA, Iuc, P fimbriae, and S/F1C fimbriae) was performed in over half of the 21 studies identified. This review highlights the lack of knowledge in this area and future studies are required to further our understanding of the full complement of VFs carried by *E. coli* strains belonging to all phylogroups, but particularly to those belonging to phylogroups B2 and D. Despite the aforementioned limitations, the analysis by Erjavec *et al.* demonstrated that ExPEC-associated VF genes were carried by ≥10% of colonising *E. coli* strains. This data supports the theory that, in a large proportion of healthy individuals, the intestine is a potential reservoir for ExPEC.

Following the observation that a number of ExPEC VFs were associated with intestinal persistence (65–67), including genes encoding P fimbriae, type 1 fimbriae, haemolysin and aerobactin, Nowrouzian *et al.* (65) postulated that these factors may first and foremost be fitness-associated, with uropathogenicity being a side-effect. A subsequent study by the same authors assessed the changes in the commensal faecal *E. coli* population in 70 infants over the period of a year (59); *E. coli* isolates belonging to phylogroup B2 were far more likely to be resident as opposed to transient faecal strains over the study period (p=0.004), while isolates belonging to phylogroups A, B1 or D were not. Interestingly, the prospect of undiscovered adherence-related factors was postulated following results of multivariate logistic regression analysis on the same dataset, which demonstrated that phylogroup B2 faecal *E. coli* residency was independent of all identified VFs (59).

The evidence discussed provides insight into the proportion of individuals carrying potentially pathogenic *E. coli* within their normal intestinal flora. However, despite harbouring ExPEC with varied VF profiles, infection does not develop in many individuals. This finding highlights the importance of non-pathogen-related factors, *e.g.* host factors, and their associations with susceptibility to and severity of infection. In relation to UTI, for example, host factors associated with an increased risk of infection include being female (shorter urethral length), anatomical abnormalities of the urinary tract, a pre-existing diagnosis of diabetes mellitus, and the presence of an indwelling urinary catheter (7,39,68). Advanced age has also been associated with increasing severity of infection (69) and, in a recent retrospective study by Wester *et al.*, poorer outcomes were associated with age ≥65

years (p=0.042) and the presence of ≥1 medical co-morbidity (p=0.003) in a cohort of 212 patients admitted from the community with ExPEC bacteraemia secondary to UTI, respiratory tract infection, gastro-intestinal infection or infection of unknown origin (70).

Colonising *E. coli* lacking ExPEC-associated VFs, hypothetically belonging to any phylogroup, may become potentially virulent organisms as a result of horizontal transfer of virulence genes from other Enterobacteriaceae (64). Furthermore, *E. coli* strains are frequently shared among family members, including pets (71). These abilities make for a dynamic ecology and have the potential to result in the widespread distribution of potentially pathogenic ExPEC strains amongst a broad population. Large pan-genomic studies are warranted to assess the distribution and prevalence of virulence genes across diverse *E. coli* STs and to understand this dynamic process better.

5. ExPEC Clonal Variation and Associations with Disease and Antibiotic Resistance

5.1 Clonal Variation

MLEE analysis demonstrates that the majority of ExPEC strains from immunocompetent individuals belong to phylogroup B2 or, less commonly, phylogroup D. In contrast, commensal *E. coli* strains and those associated with enteric disease are generally derived from phylogroups A/B1 or A/B1/D, respectively (72). However, these delineations are not absolute and isolates from non-B2/D groups have been associated with extra-intestinal infection. More recently, MLST analysis of ExPEC strains has provided greater resolution within these phylogroups and has revealed associations between specific ExPEC STs and host disease syndromes.

Data from international studies reporting the distribution of ExPEC STs based on the Achtman MLST scheme isolated from individuals with ExPEC infection syndromes demonstrate the extent of ExPEC diversity. However, the world-wide predominance of STs 69, 73, 95 and 131 is clearly evident (26,73,74). In a study based in Minnesota, USA, Banerjee *et al.* identified 47 ExPEC STs from 299 community and healthcare-associated isolates (90% of isolates from urine), the most common being ST131 (27%), ST95 (11%), ST73 (8%), ST127 (6%) and ST69 (5%) (26). In a further American study including only patients from one hospital in San Francisco, 246 consecutive ExPEC bacteraemia isolates were analysed over a 3-year period. ST12 CC, ST69, ST73 CC, ST95 and ST131 accounted for 66% of isolates (74). Data from the UK also demonstrate similar findings. For example, a recent study by Horner *et al.* revealed that ExPEC STs 69, 73, 95 and 131 accounted for 55%

of 770 ExPEC bacteraemia isolates studied in a surveillance programme in northern England over 2 years (75). A further study by Gibreel *et al.* carried out in the north west of England, UK, demonstrated that among 300 ExPEC isolates responsible for UTI, the most common STs were ST73 (16.6%), ST131 (12.3%), ST69 (9%), ST95 (6.3%), ST10 (4.3%) and ST127 (3.6%)(76).

5.2 Disease Associations

Studies outlining associations between ExPEC STs and specific disease syndromes report variable results. In a study comparing men with ExPEC pyelonephritis (n=101) versus cystitis (n=153), prevalence of ST131 was significantly higher in the pyelonephritis group (36% vs. 8% p<0.001) (77). A further study associated the ST131 clonal subgroup, ST131 H30 (as determined by CH typing), with the propensity to cause persistent or recurrent UTI, and sepsis (78). However, in a further study by Chung *et al.*, which assessed clinical outcomes in 122 patients with extended-spectrum beta-lactamase (ESBL) *E. coli* bacteraemia, 14- and 28-day mortality rates were similar in patients with infections caused by ST131 versus non-ST131 ExPEC STs (79). Other authors have also associated ExPEC STs 127 and 73 with pyelonephritis (25). From the non-infectious viewpoint, *E. coli* belonging to phylogroup B2 have recently been associated with inflammatory bowel disease in a systematic review and meta-analysis by Petersen *et al*. (80). Although these findings are interesting, further studies are required to understand whether IBD is a consequence of, or a cause of, intestinal colonisation with *E. coli* belonging to phylogroup B2.

Following on from their early work, which demonstrated an association with phylogroup B2 *E. coli* isolates and urosepsis (81), Jaureguy *et al.* published a comprehensive study assessing associations between 161 consecutively collected *E. coli* bacteraemia isolates, infective source, and clinical outcomes (73). Eighty-seven STs were identified, using the Pasteur MLST scheme rather than the Achtman scheme, and categorised into 19 CCs, 11 of which comprised ≥5 isolates. There was a significant association between CC1 and CC4 (the two major clones identified from phylogroup B2) and urinary tract infection as a source of bacteraemia when compared with other B2 genotypes (UTI-associated bacteraemia accounted for 12/17 and 11/17 of isolates belonging to CC1 [p=0.017] and CC4 [p=0.041], respectively). Conversely, a further phylogroup B2 clone, CC32, was associated with non-urinary tract source of bacteraemia (p=0.023). No association between CCs and infection severity or clinical outcome was demonstrated. This study confirmed that clonal groups rather than whole phylogroups were more appropriate phylogenetic delineators to be utilised for the purpose of association studies.

Although MLST allows greater discrimination than MLEE, strains within specific STs may possess varied genotypes affecting pathogenicity. For example, ExPEC ST73 includes two probiotic *E. coli* strains, Nissle 1917 and the asymptomatic bacteriuria (ABU) strain 83972, as well as the highly virulent uropathogenic CFT073 strain. Vejborg *et al.* utilised a comparative genomic hybridisation (CGH) microarray analysis to compare the genomes of Nissle 1917, ABU 83972 and CFT073 *E. coli* strains. (82). Their results revealed only minor genetic differences between the three strains and suggested that they likely originated from a common ancestor. Almost all known CFT0730-associated VFs were present in Nissle 1917 and ABU 83972 and, interestingly, ABU 83972 contained additional PAI-associated regions when compared with CFT0730. The authors suggested that the observed differences in pathogenicity between the three strains seemed likely to be a consequence of differential gene expression. However, they also noted that genetic mutations (deletions or point mutations) were present in both Nissle 1917 and ABU 83972 that may have directly altered pathogenicity by altering expression of type 1, P and F1C fimbriae as well as PAIs associated with haemolysis and haemagglutination. These findings highlight the potential pitfalls of epidemiological research solely assessing the presence of VF genes and emphasise the importance of considering phenotypic bacterial properties. A spectrum of clinical disease syndromes, ranging from neonatal meningitis to urinary sepsis, has also been observed for ExPEC strains belonging to ST95 (25,82,83).

Pathotypic variations observed within members of the same ST demonstrate the potential limitations of MLST, particularly when it is used in the context of clinical association studies. WGS and PFGE provide greater phylogenetic resolution and are both valuable in the context of surveillance and outbreak analysis. PFGE is still widely utilised and international standardisation does allow for profile cross-comparison. However, as previously mentioned, this method is particularly labour-intensive and the cause of any identified banding differences cannot be easily established. Conversely, WGS provides for full standardisation and easy comparison of results and, if necessary, MLST profiles can be determined retrospectively. As a consequence of these features, and given that in the future the technology will become increasingly automated, WGS will likely become widely used. As a consequence, the use of PFGE will in the main become redundant.

Although WGS provides superior phylogenetic resolution, this technology is not yet widely utilised and is currently associated with significant cost. As a consequence, in the setting of an outbreak where phylogenetic information may significantly alter the subsequent management strategy, the delivery of real-time information using WGS is still currently limited in most hospitals. These observations led Weissman *et al*. (25) to develop ‘CH

typing', a two-locus typing method, which utilises a 489-bp internal fragment of the type 1 fimbria adhesin gene, *fimH*, and a 169-bp internal fragment of the MLST locus, *fumC*. When evaluated on ExPEC isolates, CH typing predicted STs with 95% accuracy, while providing greater phylogenetic resolution than MLST. Of particular importance, CH typing enabled dominant ExPEC STs, accounting for large proportions of isolates *e.g.* ST131, ST73, ST127, to be divided into smaller clonal groups. The authors commented that, although CH typing will not replace traditional MLST or WGS/PFGE for detailed phylogenetic studies, it may have value as a rapid screening test in the setting of an outbreak, or where sub-ST clonal diversity may be important, *e.g.* in determining antibiotic resistance patterns associated with ST-specific clones, where time and financial constraints limit immediate use of methodologies with higher resolving power (25).

5.3 Antibiotic Resistance Associations

The importance of rapid, high-throughput typing methods becomes apparent on considering the variation in antimicrobial resistance profiles observed between ExPEC STs. In the UK, ExPEC ST131 isolates now account for the majority of multidrug-resistant ExPEC strains, and, as well as being associated with fluoroquinolone and aminoglycoside resistance, often carry ESBLs, particularly of the CTX-M-type (76,84). By contrast, ExPEC STs 69, 73 and 95 are less often associated with multi-resistance and generally remain susceptible to extended-spectrum cephalosporins, although the reasons for this are poorly understood. From the clinical viewpoint, establishing the antimicrobial susceptibility profile rapidly in patients with ExPEC infections is paramount and will guide subsequent antimicrobial therapy. With this in mind, Doumith *et al*. (84) recently devised a novel multiplex PCR technique that enables the rapid identification of ExPEC STs 69, 73, 95 and 131. Results of validation studies against MLST demonstrated that the new multiplex PCR assay was 100% sensitive and 99.5% specific. It is predicted that technologies such as this will be highly valuable where rapid results are required locally, *e.g.* in the hospital clinical microbiology setting.

ExPEC ST131 was first reported in 2008 and is now considered a pathogen of global importance (85). Recent molecular epidemiological studies have revealed a number of ST131 clonal subgroups associated with variable antimicrobial resistance patterns, the most prevalent being ST131 clonal subgroup H30 (contains the H30 variant of *fimH*) (86–88). A number of ST131 H30 subgroups, mainly H30-R (encodes fluoroquinolone resistance), H30(non-R) and H30-Rx (encodes fluoroquinolone resistance and produces CTX-M-15 ESBL), have subsequently evolved through a process of clonal selection from a common

ST131 H30 ancestor (see figure 2) (88–90). The rapid emergence of multidrug-resistant ST131 and its associated subclones has significant clinical consequences, particularly when considering appropriate empirical antimicrobial regimens. CH typing is likely to be beneficial in this setting as it allows for the rapid identification of the ST131 H30 subclone and may be used as an initial high-throughput screening test in the context of an outbreak or to guide initial empirical antimicrobial choice (86,90). An in-depth analysis relating to ExPEC ST131 and its associated clonal subgroups is beyond the scope of this review but is available elsewhere in two comprehensive reviews by Banerjee and Johnson (90) and Mathers *et al*. (85).

6. Conclusions

E. coli is a genetically diverse species with a complex phylogeny, broad VF armament and significant genomic plasticity. In recent years, the importance of ExPEC as pathogens capable of causing a spectrum of host infective syndromes has come to the fore, particularly in the context of the emergence of multidrug-resistance. Particular STs of ExPEC isolates, *e.g.* ST131, ST73 and ST127, have emerged globally as the predominant strains responsible for disease, however the underlying reasons for their success are yet to be fully elucidated. Pathogen-related factors may include increased bacterial fitness resulting in enhanced colonisation ability, increased virulence, and selection pressure resulting from widespread use of broad-spectrum antimicrobial agents. Meanwhile environmental factors including sub-standard infection-control practices and increased international travel are also of likely significance.

Individual VFs or VF profiles have been associated with site-specific infections and broadly belong to one of five groups including adhesins, toxins, iron acquisition systems, capsular components and protectins/invasins. Studies to date tend to assess the virulence of an ExPEC isolate based on the detection of chromosomally-encoded VFs. However throughout this review, evidence has been presented to demonstrate the variable clinical syndromes that may result from infection with genetically similar strains. Hence, although genetic studies are valuable in the context of disease association studies, the potential for significant phenotypic variation must not be ignored.

Host factors and susceptibility to ExPEC infections are of significant importance and must be accounted for when performing genetic association studies. In the context of a growing evidence base relating to a high proportion of asymptomatic intestinal carriage of potentially pathogenic *E. coli*, the consideration of host factors becomes increasingly important.

However to date, the true extent of asymptomatic ExPEC carriage is yet to be fully elucidated. Large-scale pan-genomic carriage studies, ideally of prospective design, are now required to ascertain the true extent of the intestinal ExPEC reservoir and to understand better the complex dynamics of carriage.

Novel strategies to prevent, rapidly diagnose and treat ExPEC infections are needed, particularly in the context of the recently observed clonal expansion of specific hyper-virulent and antibiotic-resistant strains. Large-scale epidemiological and clinical studies are required to ascertain the mechanisms underlying transmission dynamics and clonal selection, and to allow for an evidence base to be established from which new preventative or therapeutic strategies could be devised. Potential strategies might relate to the deployment of targeted infection control measures or the development of targeted national/international antimicrobial prescribing policies. Detailed molecular studies, particularly relating to the mechanisms underlying invasive disease and asymptomatic intestinal carriage, must also be performed to guide the development of both preventative strategies, *e.g.* potential vaccine production, and new treatment strategies, *e.g.* therapeutic strategies targeting novel bacterial factors.

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Conflicts of interest: None

8. References

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Table 1 – ExPEC Virulence Factors

Virulence Factor	Gene(s)	Ref.
Adhesins		
Adhesion siderophore	*iha*	(91)
Dr-binding adhesins	*afa/draBC*	(91)
E. coli common pilus	*ecpA*	(92)
F1C fimbriae	*foc* gene cluster	(93)
Heat-resistant haemagglutinin	*hra*	(91)
M fimbriae	*bmaE*	(94)
N-acetyl D-glucosamine-specific fimbriae	*gaf*	(95)
P fimbriae	*papACEFG*	(91,96)
S fimbriae	*sfa/sfaS*	(96)
Temperature sensitive haemagglutinin		(91)
Type 1 fimbriae	*fimH*	(91)
Iron Acquisition Systems		
Aerobactin receptor	*iutA*	(91)
Peri-plasmic iron binding protein	*SitA*	(95)
Salmochelin receptor	*iroN*	(91)
Siderophore receptor	*ireA*	(91)
Yersiniabactin receptor	*fyuA*	(91)
Protectins and Invasins		
Colicin V	*Cva*	(95)
Conjugal transfer surface exclusion protein	*traT*	(95)
Group 3 capsule	*kpsMT* II	(91)
Increased serum survival	*iss*	(91,95)
Invasion of brain endothelium	*ibeA*	(97)
K1/K2/K5 group 2 capsule variants	K1/K2/K5 genes	(91)
kpsM II group 2 capsule	*kpsM* II	(91)
Outer membrane protease T	*ompT*	(91,95)
Toxins		
α-haemolysin	*hylD*	(91)
Cytolethal distending toxin	*cdtB*	(97)
Cytotoxic necrotising factor	*Cnf1*	(48)
Enteroaggregative *E. coli* toxin	*astA*	(91)
Haemolysin A	*hylA*	(50)
Secreted autotransporter toxin	*sat*	(91)
Serine protease	*pic*	(91)
Vacuolating toxin	*vat*	(91)
Others		
β-glucoronidase	*uidA*	(97)
Colibactin synthesis	*Clb & clbB*	(97)
Uropathogenic-specific protein	*usp*	(91)
Flagellin variant	H7 *fliC*	(91)
Maltose and glucose-specific PTS transporter subunit IICB	*MalX*	(95)
Pathogenicity island marker	*malX*	(91)
D-serine deaminase	*DsdA*	(95)

Table 2 - Strengths and Weaknesses of ExPEC Typing Methods.

Typing Method	Strengths	Weaknesses
Phenotypic		
Multi-locus enzyme electrophoresis (MLEE)	• Majority of strains can be typed. • Easy to interpret results.	• Provides poor phylogenetic resolution compared with newer techniques.
Genetic		
Multiplex-PCR-based techniques	• Allow for rapid determination of phylogroup or ST. • Potential application in clinical setting (*e.g.* in predicting likely antibiogram or as screening tool in the setting of an outbreak • Relatively low cost.	• Only allow for detection of specific genomic targets as determined by primer mix.
Pulse-field Gel Electrophoresis (PFGE)	• Highly discriminative method with excellent phylogenetic resolution and epidemiological concordance. • Standardised systems available to aid in interpretation and cross-comparison with other isolates, *e.g.* Pulsenet. • Highly reproducible.	• Labour-intensive, time-consuming and technically demanding. • Results available in days. • Resolution limited when DNA fragments of similar size. • Although standardisation systems exist, an element of subjectivity remains.
Multi-locus sequence typing (MLST)	• Generated data highly portable compared with those produced by PFGE. • Internationally-standardised system that is highly reproducible.	• Phylogenetic resolution is limited. • Expensive.
Whole Genome Sequencing (WGS)	• Provides the highest level of phylogenetic resolution and discriminatory power. • MLST can be determined from WGS data to allow for cross-comparison. • Lends itself to being automated.	• Currently expensive (although now probably cheaper than MLST) • Requires significant resources, particularly bioinformatics expertise following initial sequencing. • At present results and analysis are time consuming are therefore at present mainly used in research setting.

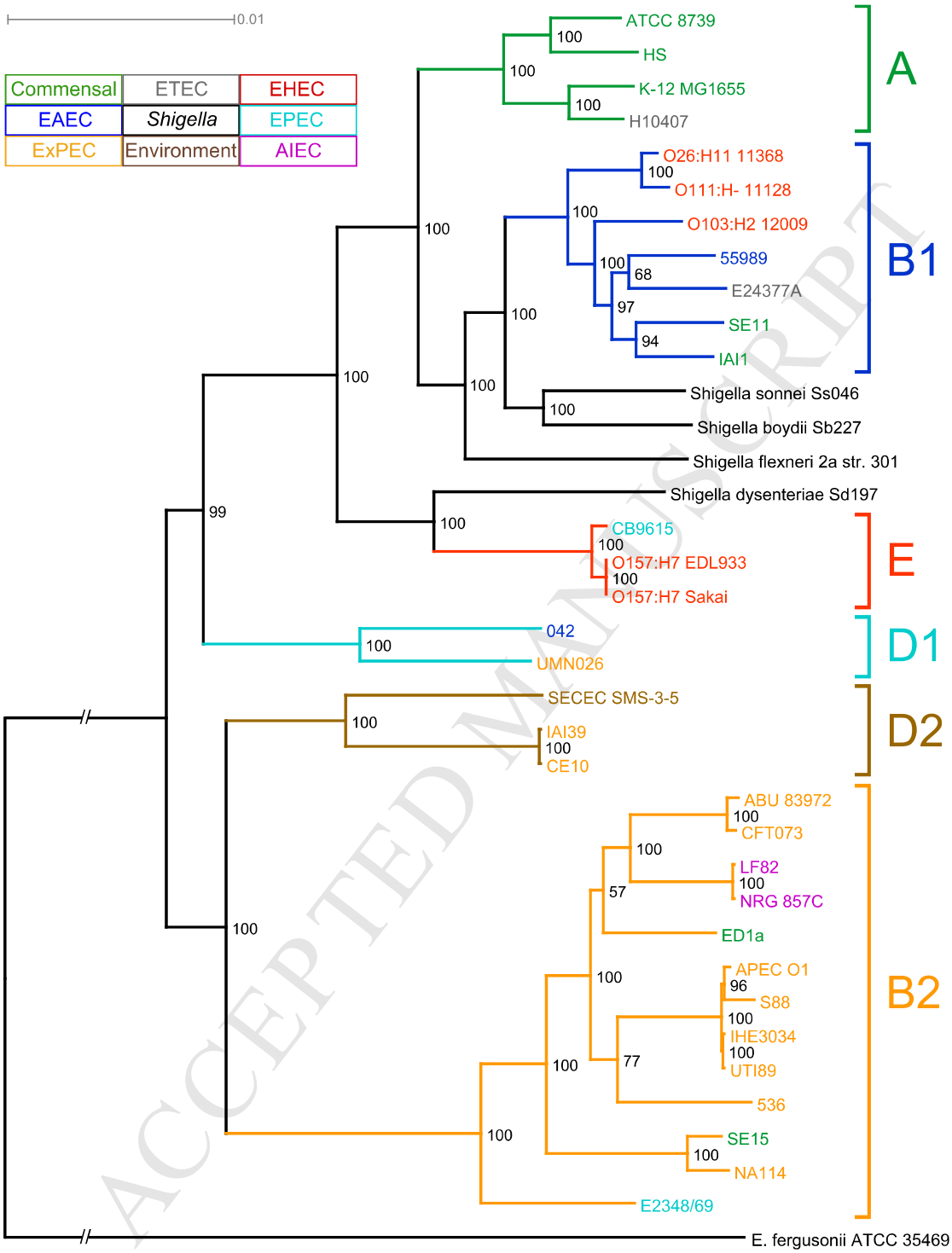

Figure 1 – Phylogeny of a selection of complete *E. coli* genome sequences based on whole genome alignment. Methodology utilised to derive phylogenetic tree outlined in detail elsewhere (61). Figure reproduced with kind permission from Leimbach *et al*. (61) and Spinger Science and Business Media.

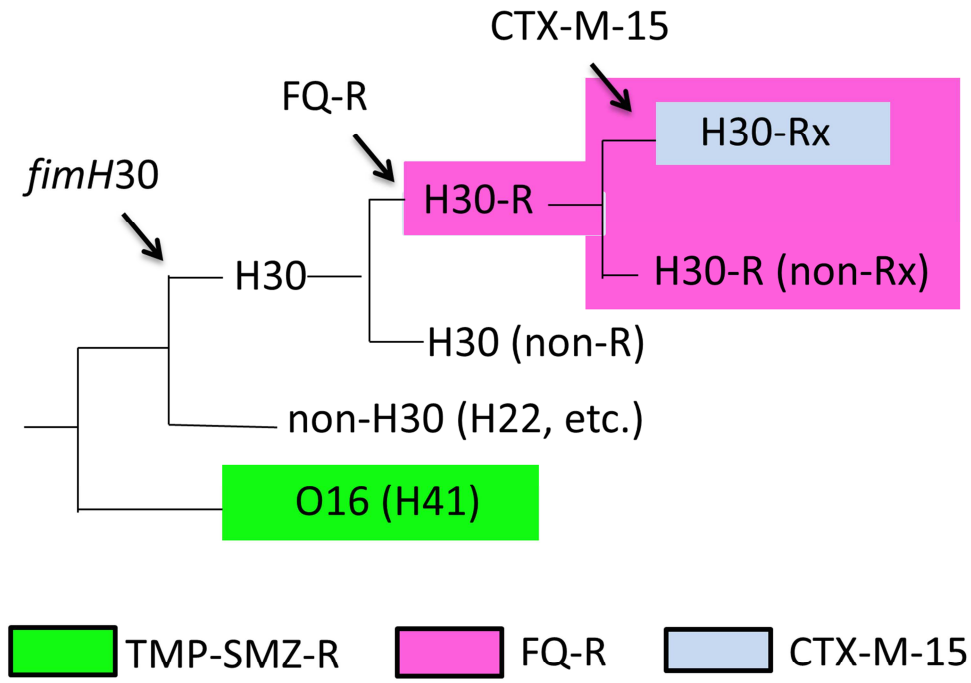

Figure 2 - Schematic dendrogram of ST131 phylogeny reconstructed using whole-genome single nucleotide polymorphism analysis. Arrows indicate emergence of *fimH30* allele, FQ resistance, and CTX-M-15 ESBL. Colors indicate resistance traits significantly associated with (although not confined to) specific lineages. FQ, fluoroquinolone; TMP-SMZ, trimethoprim-sulfamethoxazole; R, resistance. Figure and legend reproduced with kind permission from Banerjee and Johnson (90) and The American Society for Microbiology.