Association between antimicrobial resistance and virulence in Escherichia coli

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Abbreviations: APEC, avian pathogenic E. coli; esp-F, cytotoxic necrotizing factor type 1 gene; eaeA, intimin gene; EHEC, enterohemorrhagic E. coli; ESBLS, extended-spectrum β-lactamases; ETEC, enterotoxigenic E. coli; EPEC, enteropathogenic E. coli; EU, European Union; ExPEC, extraintestinal pathogenic E. coli; FQ, fluoroquinolone; fnmA, yersiniabactin receptor gene; HGT, horizontal gene transfer; hlyA, enterohemolysin A gene; HUS, hemolytic-uremic syndrome; iutA, aerobactin receptor gene; NTEC, necrotoxigenic E. coli; PAI, pathogenicity island; papH, P-pili F13 genes; Q, quinolone; fliA, S-family adhesin gene; ST, sequence typing; stx1 and stx2, Shiga toxins genes; iucD, serum resistance-associated outer membrane protein gene; UPEC, uropathogenic E. coli; ugp gene, uropathogenic-specific protein gene; VFs, virulence factors

Escherichia coli represents a major cause of morbidity and mortality worldwide. The treatment of E. coli infections is now threatened by the emergence of antimicrobial resistance. The dissemination of resistance is associated with genetic mobile elements, such as plasmids, that may also carry virulence determinants. A proficient pathogen should be virulent, resistant to antibiotics, and epidemic. However, the interplay between resistance and virulence is poorly understood. This review aims to critically discuss the association and linked transmission of both resistance and virulence traits in strains from extraintestinal infections in E. coli, and intestinal pathotypes. Despite the numerous controversies on this topic, findings from research published to date indicate that there is a link between resistance and virulence, as illustrated by the successful E. coli ST131 epidemic clone. Perhaps the most commonly accepted view is that resistance to quinolones is linked to a loss of virulence factors. However, the low virulent phylogenetic groups might be more prone to acquire resistance to quinolones. Specific characteristics of the E. coli genome that have yet to be identified may contribute to such genetic linkages. Research based on bacterial populations is sorely needed to help understand the molecular mechanisms underlying the association between resistance and virulence, that, in turn, may help manage the future disseminations of infectious diseases in their entirety.

Introduction

The last decades have witnessed an ever-growing emergence of antimicrobial resistance worldwide. Antimicrobial resistance has been recognized as one of the world’s most pressing public health problems.1,2 The intensive use and, particularly, the misuse of antibiotics have led to the development and selection of resistant bacteria in different settings. Beyond the use of drugs for therapeutic purposes in the human and animal settings, antibiotics are also used extensively as prophylactic agents and as animal growth promoters in agriculture, except in European Union (EU) countries, where their use is banned since 2006 in animal feed.3 Therefore, resistant bacteria are not only confined to the human clinical setting, such as hospitals, where they were first recognized and studied. They have also increased significantly in the community and in both farm and companion animals. Animals may act as reservoirs of resistant bacteria that can be transmitted to humans, or vice versa, by direct contact or indirectly, via the food chain.4,5 Moreover, the growth of global trade and travel allows resistant microorganisms to be spread rapidly to distant countries and continents.6 The consequences of antimicrobial resistance represent a growing threat to human societies. When infections often fail to respond to standard treatments, they result in prolonged illness and greater risk of death, and more chances for the resistant microorganism to spread. Furthermore, the costs associated with the length of hospitalization and the use of last generation antibiotics are significantly increased.6,7 The continuous use of antibiotics in diverse human activities, such as in human/animal medicine, agriculture, livestock, and the improved understanding of the mechanisms of horizontal resistance gene transfer between bacterial species and strains,8 have led to new questions: the association of antimicrobial resistance genes with bacterial virulence determinants. Virulence factors are expressed proteins encoded by genes located in the chromosome or in plasmids. The location of virulence factors in genetic mobile elements is at the core of this epidemiological concern, as it may facilitate the spread of virulence within bacterial communities. For example, the majority of virulence associated plasmids in Escherichia coli belong to the F incompatibility
group carry transfer functions, and often antimicrobial resistance determinants.11

Bacteria can acquire antimicrobial resistance by DNA mutation or by horizontal gene transfer (HGT).12,13 Mutations occur spontaneously, at variable frequency, depending on the antibiotic and the microorganism. Sometimes, bacteria need to accumulate mutations in a stepwise process to develop fully functional clinical resistance, e.g., in the resistance to fluoroquinolones.14 This type of resistance may be secondary to human antimicrobial therapy or may evolve in a non-animal gut when feed is supplemented with sub-minimal inhibitory concentrations of antibiotics for growth promotion.15 HGT plays a key role in the evolution of bacteria and the spread of antimicrobial resistance genes.16,17 It involves the acquisition by the bacterial cell of foreign DNA, a phenomenon that may occur via three mechanisms: transformation (capture of free DNA), transduction (via bacteriophage DNA), or conjugation.18 Resistance traits located in genetic mobile elements like plasmids, transposons or integrons can be transferred to different strains or bacterial species.19 The acquisition of resistance or virulent traits may represent a survival advantage to the microorganism. It is conceivable that virulence genetic determinants, if located on the same genetic platform as antimicrobial resistance genes (plasmids, transposons, integrons) may be co-mobilized under antimicrobial selective pressure.20 Furthermore, stable virulent clones or strains may be perpetuated if they acquire resistance determinants.

The topic on the link on resistance/virulence is complex, considering the diversity of antimicrobial resistance genes, virulence factors, bacterial species and hosts. Most reports on this topic correlate the epidemiology of specific resistance genes with virulence genetic traits, a first step toward understanding whether there is a connection between resistance and virulence. More in depth molecular studies on the genetic between antimicrobial resistance and virulence determinants are sorely needed, to fully understand the interplay of resistance and virulence genes, whether virulence expression is affected by chromosomal mutations leading to specific resistance (e.g., fluoroquinolone resistance), if both determinants are inserted in the same mobile genetic element, like a conjugative plasmid, or the role of the phyletogenetic background of the strain.

E. coli is a microorganism responsible for human and animal infections, whose genetic variability allows it to grow in diverse ecological niches. It is recognized as one of the most frequent bacterial cause of infections, food-borne diarrheal disease, and extraintestinal infections. E. coli is one of the most versatile bacterial species and the diversity of its lifestyles is achieved through a high degree of genomic plasticity, via gene loss or gain, through lateral gene transfer.19 Recent years have seen increasing numbers of reports on the acquisition of antimicrobial resistance by E. coli strains.

The main goal of this review is to critically discuss the association between antimicrobial resistance and virulence in E. coli strains of human and animal origin. A section will address antibiotic resistance in E. coli collected from food products of animal origin. These isolates may represent pathogenic E. coli responsible for gastrointestinal infections in humans.

**Escherichia coli: Infections and Phylogeny**

E. coli is a normal inhabitant of the intestines of various animals, including humans. Although E. coli usually appears to be a harmless commensal, some strains are clearly pathogenic and have been recognized as agents of food-borne enteritis. However, others challenge the threshold between commensal and pathogen, as they most frequently cause extraintestinal disease in hosts where they first asymptomatically colonize the intestine. They do not produce enteric disease, and are phylogenetically and epidemiologically distinct from commensal or enteropathogenic strains.21,22 Therefore, E. coli found in humans can be categorized on basis of genetic and clinical criteria into three main groups: commensal, pathogenic (enteric or diarrheogenic) and extraintestinal pathogenic E. coli (ExPEC).23

Intestinal pathogens induce gastroenteritis when contaminated food or water is ingested by the host, via fecal-oral transmission. They possess diverse mechanisms of pathogenicity according to their virulence traits, which they acquire mostly through transfer of plasmids that have been maintained stable in the host-cell, or by phages. Disease severity depends on microbial and the host factors, and ranges from low or mild gastroenteritis, to severe and life threatening infection.23 The typical diarrheagenic strains include: enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (IEEC), enteropathogenic (EPEC) and enteraggregative (EAECC) E. coli.21,24 The reservoir of some of these strains include animals, such as cattle for EHEC, where they do not cause disease.24

Another two important pathotypes causing intestinal disease in humans need now to be added to this list: Adherent-invasive E. coli associated with inflammatory bowel diseases25 and the AIEC. E. coli (attaching-and-effacing A/E).26 Extraintestinal E. coli infections are frequently associated with infections in the ambulatory or long-term care facilities, and in hospitals. E. coli is capable of causing infection in nearly every organ. However, the urinary tract is the most frequent extraintestinal site, causing 85–95% of cases of uncomplicated cystitis and pyelonephritis in premenopausal women. Abdominal and pelvic infections are other extraintestinal types of infections. In these cases, E. coli can be isolated alone, or mixed with facultative and/or anaerobic members of the intestinal flora. E. coli can also cause pneumonia. Additionally, it can infect surgical sites, cause neonatal meningitis or septicaemia.27

As for all bacteria, E. coli and its subpopulation ExPEC, virulence potential is largely determined by the presence of specialized virulence factors (VFs), such as fimbriae, adhesins, toxins, siderophores, capsules, hemolysins and invasins. These VFs help the microorganism to avoid or subvert host defenses, colonize key anatomical sites, and/or incite a noxious host inflammatory response, thereby causing disease.28,29 Phylogenetin studies have suggested that E. coli may be grouped into four major clusters, where human ExPEC strains belong generally to the more virulent group B2, and to a lesser extent, group D, whereas commensal strains, considered less virulent strains, belong to A or B1 phylogroups.23,30 Historically, the effects of E. coli on morbidity, mortality, and healthcare were not a major source of concern, since these microorganisms were eradicated with antibiotic therapy. Unfortunately, the situation has changed dramatically in the last decades, and
research on the association between resistance and virulence in this ubiquitous bacterium is sorely needed. The extreme genomic plasticity of E. coli further underlines the importance of the topic.

Resistance and Virulence in ExPEC of Human Origin

The following section will address the association of specific resistance and virulence traits found to date in E. coli strains isolated from human specimens. Over the recent years, the emerging resistance seen among human clinical isolates to early antimicrobial agents prompted our increased reliance on broad-spectrum agents, such as extended spectrum cephalosporins and fluoroquinolones. Unfortunately, newly developed resistance now threatens these agents as well. Resistance to β-lactams and fluoroquinolones will be given special attention in this review, in an attempt to illustrate the complexity of this topic.

Resistance to extended-spectrum cephalosporins vs. virulence traits. Since the 1980s, susceptibility patterns of clinical E. coli isolates have been dramatically changed with the introduction of successive extended spectrum cephalosporins. The emergence of strains producing extended-spectrum β-lactamases (ESBLs) has become a daunting challenge to the clinician, since this dramatically reduces the therapeutic choices. This may be devastating to the clinical outcome for the patient if the strain is resistant to other antibiotic classes as well. ESBLs have the incredible capacity of inactivating all β-lactamic antibiotics at variable degree, with the exception of carbapenems. The first ESBLs were derived from a one point mutation of the early penicillinases TEM and SHV. In 1995, a novel β-lactamase, with a higher hydrolytic efficiency against cefotaxime than cefazidime, emerged. This cefotaximase was discovered for the first time in an E. coli strain in Munich, and hence was named CTX-M. Since then, a number of derivatives of the CTX-M-1 have been reported. They replaced the ESBLs identified earlier, the TEM- and SHV-type ESBLs, and now are the most prevalent ESBLs worldwide, with E. coli as their major microbial host. CTX-M enzymes are diverse and can be phylogenetically grouped into five clusters on the basis of sequence homology (www.lahey.org/studies). Over the last decade, CTX-M enzymes which also confer efficient inactivation of the third generation cephalosporin ceftazidime appeared. This trait was acquired by the replacement of only one aminoacid. CTX-M-15 is one example of an emerging enzyme, being successfully spread throughout the world, mostly by E. coli strains. The success of its rapid distribution lies not only in the ubiquity of E. coli, but also in the carriage of the blaCTX-M-15 gene in highly mobile plasmids.

In the clinical management of infectious disease, with respect to multidrug-resistant pathogens, it is frequently assumed that more antimicrobial drug resistance equates with greater virulence, which may not be true. Nevertheless, the link between resistance and virulence remains unclear, and depends on the interactions between the phylogenetic background of the strain and the type of resistance determinant. The phylogenetic classification of ExPEC into four major groups is useful, and may be performed by a simple and inexpensive method. The method relies on the screen and combination of two conserved genes (chuA and yjaE) and the DNA fragment TSSP4 C2, not exposing all the genome beyond. In fact, the phylogeny of E. coli continues to be a topic of intense investigation, further underscoring the complexity, the diversity and the plasticity of the E. coli genome.

Branger et al. reported that SHV-type ESBLs, and to a lesser extent, TEM-type ESBLs have been found preferentially in E. coli belonging to the virulent B2 phylogroup, while CTX-M enzymes were associated to the rare D2 genotype, which tends to have fewer virulence factors. The findings indicate that mobile genetic elements carrying ESBL determinants are not randomly distributed in the diverse genotypes of E. coli strains. The acquisition of ESBLs and their maintenance in the host cell result from a complex interaction between the type of ESBL, the E. coli phylogeny, intrinsic virulence, and the fluoroquinolone resistance of the strains. This observation further underscores the importance of the diversity of the niches submitted to different selective pressures.

Lee et al. compared the occurrence of nine virulence factors with the production of blacTX-M enzymes among pathogenic E. coli strains isolated from human blood and urine. The prevalence of all VFs, except S fimbral adhesion, was more frequent in the phylogenetic group B2. Alpha-hemolysin, yersiniabactin receptor (fimH), serum resistance-associated outer membrane protein (sraT), and aeroabactin receptor (iucA) were found to be independent predictors for pathogenicity. Of these VFs, iucA and traT were significantly more common in strains producing the CTX-M-9 group and the CTX-M-1 group of ESBLs, respectively.

In contrast with the findings of Branger et al., some reports indicate that E. coli CTX-M-producers belong mostly to the virulent phylogenetic groups, mainly the B2 genotype. However, the successful B2 epidemic strains circulating in the United Kingdom carrying CTX-M-15 did not appear to be more virulent, despite iucA and fyuA being more prevalent in these than in non-clonal CTX-M-15 producing isolates. In fact, it seems that there are significant differences in the prevalence of individual VFs among CTX-M producers and non-producers, according to a different study. Pinot et al. found that in general the VFs were more prevalent in CTX-M producers than in non-producers. However, iucA and traT were also found in higher percentages in non-CTX-M producers. The virulence genes were (iron-regulated element), and the 23S rRNA operon (CuIV) were only present in non-CTX-M producers. The sop gene (unpathogenic-specific protein) was detected in CTX-M-15 β-lactamase producers and non-CTX-M producers, but not in E. coli CTX-M-14 producers' isolates.

Another study performed in France focused on the evaluation of the virulence potential of E. coli CTX-M producers collected from urinary tract infections. Fourteen VFs were less prevalent in ESBL isolates than in susceptible E. coli strains. iucA and traT were more prevalent in ESBL isolates. In contrast with the study performed by Lee et al. mentioned above, CTX-M producers had fewer VFs than TEM-producing isolates. When using a Caenorhabditis elegans model for in vivo tests, the ability to kill the nematode correlated with the presence of VFs, with CTX-M producers clearly showing low virulence in vivo.
Another investigation on the association of virulence with antimicrobial resistance was performed on patients with urosepsis. In those patients, *E. coli* B2 isolates expressed P fimbriae and hemolysin, and lacked antimicrobial resistance. In contrast, B1 isolates from urologically impaired hosts characterizedally lacked P fimbrial and hemolysin determinants, and often carried a plasmid-encoded aerobactin system (possibly on multiple antimicrobial resistance plasmids). The prevalence of antimicrobial resistance was lower among strains belonging to B2 phylogenetic group, suggesting a trade-off between resistance and virulence. However, this inversion is not seen in the recently discovered multilocus sequence type (ST) 131 CTX-M-15 producing *E. coli* clone. Indeed, the CTX-M-15-producing B2 isolates were highly virulent in murine models of extraintestinal infection, even though they lack several classical virulence genes, like adhesion genes such as *papC* and *papG*. Compared with a genetically closely related EC7372 *E. coli*, this B2 clone does not adhere avidly to epithelial cells. However, it produces an extensive biofilm, which may contribute to its long persistence in various environments, and to the resistance to antimicrobials. The *E. coli* strains causing recurrent urinary tract infections are more likely to produce biofilms and to produce yersiniabactin (*fyu*) and aerobactin (*aer/iutA*). The property to produce biofilms by the CTX-M-15 producing isolate does not appear to be encoded by the CTX-M plasmid itself. Taken together, the results indicate that these particular isolates carry unidentified genes that may play a crucial role in the successful dissemination of the clone.

The *E. coli* clonal group ST131 producing CTX-M-15 with a high virulence potential has been reported all over the world. It now represents a major public health threat. This clonal group belongs to the B2 phylogenetic group and to the serotype O25b:H4. The *E. coli* O25b:H4-B2-ST131 is characterized by its co-resistance to several classes of antibiotics, and its ability to acquire new resistance mechanisms to antibiotics. β-lactamases other than CTX-M-15 have been described, namely TEM-1, TEM-24, SHV-12, CTX-M-1, -2, -3,-9, -14, -27, -32, -35 and -61, the plasmid mediated AmpC CMY-2, and the gene *aac(6\')-Ib-cr*, a variant of an aminoglycoside-modifying enzyme that is responsible to reduced susceptibility to aminoglycosides, but also to certain fluoroquinolones.

A high prevalence of the O25b-ST131 clone has been identified among fluoroquinolone resistant strains in diverse geographical regions. This clone is now being disseminated among the community as well as through the environment. *E. coli* ST131 is an example of a clonal group that combines resistance and virulence genes, and this property may be linked to its successful dissemination in hospital and community settings worldwide. Studies are needed to better understand the genetic dynamics of ST131, as well as, its sources and transmission pathways.

Together, these studies illustrate the complexity of the interactions between ExPEC virulence and resistance factors via ESBL production. Table 1 shows examples of the complex relationship between virulence and resistance factors.

| Phylogenetic group/clone | ESBL* | VFb prevalence | Resistance prevalence/resistance genes | References |
|--------------------------|-------|---------------|----------------------------------------|------------|
| B2 CTX-M-type            |       | +/−           |                                        | 42, 43, 44 |
| B2 TEM-type              | ++    | +/−           |                                        | 40         |
| B2 SHV-type              | ++    | +/−           |                                        | 40         |
| D2 CTX-M-type            | +/−   | Quinolone resistance |                                   | 41         |
| B2 CTX-M-9               | ++    | iutA prevalent |                                        | 41         |
| CTX-M-1                  | ++    | iutA prevalent |                                        | 41         |
| B2 CTX-M-15              | iutA and *fyu* prevalent | 43       |
| Diverse EIBL             | +/−   | Resistant to diverse antibiotics, including quinolones | 45         |
| B2, D, C, B, B2 (B2 prevalent) TEM-types | +/− | *fyu* and iutA Biofilm producer | 48         |
| B2 CTX-M-15              | +/−   | *fyu* and iutA Biofilm producer | 48         |
| B2, ST131, O25b:H4       | ++    | TEM-types     |                                        | 35, 36, 50–53 |
|                         |       | SHV-12        |                                        |            |
|                         |       | AmpC CMY-2    |                                        |            |
|                         |       | AmpC CMY-2    |                                        |            |

Table 1. Examples of relationship within various phylogenetic groups or clones, of resistance due to extended-spectrum β-lactamases (ESBL), and virulence factors. The virulence factors analyzed vary between studies.

*ESBL, extended-spectrum β-lactamase; *VFb, virulence factors. Prevalence of VFs: +/−, very low; +, low; ++, high.
within the phylogenetic groups or clones, of resistance due to extended-spectrum β-lactamases (ESBL), and virulence factors. The linkage between resistance and virulence may reflect the geographic origin, and genetic background of particular clones. For example, the IncI1 plasmid family has been associated with the spread of some ESBL genes which has been extensively reviewed by Carattoli.22 The IncI1 plasmid carrying blactTXCM-1 has been found in France in E. coli strains isolated from human patients and in healthy poultry, suggesting a potential link between the human and animal settings.19,23 Further studies have described the genetic variability of IncI1 plasmids.49 However, all IncI1 plasmids encode the type IV pilE determinant, known to contribute to the adhesion and invasion of uropathogenic E. coli.50 Hence this virulence factor is encoded in a plasmid family, and its association with epidemic spread and resistance determinants may have helped facilitate its dissemination.

The IncI1 plasmid family is very common in Enterobacteriaceae, and may be considered epidemic, as it has been detected in diverse geographic locations and in bacteria from various sources.46 Sequencing of the IncI1 plasmid pRSB107 revealed the presence of nine different antibiotic-resistance determinants, an aero bacterin iron uptake system, and other putative virulence factors.51,52 Furthermore, this family encodes the TraT virulence protein, which is responsible for serum resistance in E. coli, and reduced susceptibility to phagocytosis by macrophages.53 Additionally, many ESBL genes from the CTXM-M, TEM and SHV groups, are inserted in this plasmid family, as well as genes determining resistance to other antibiotic groups, such as quinolones. These particular examples illustrate how plasmids carrying virulence and resistance determinants can be selected by antimicrobial pressure, and hence allow for virulent traits to be selected in a bacterial population by antimicrobial usage.54

Quinolone resistance and virulence factors. Quinolones are antimicrobial agents with a broad antibacterial spectrum, good tolerance, and are available both in oral and parenteral formulations. They are not considered a first line drug for the treatment of infections. However, the emergence of resistance to the first-line antibiotics in uropathogenic E. coli (UPEC) has made the management of urinary tract infections very problematic, where fluoroquinolones may be used. Resistance to quinolones is largely mediated by point mutations in DNA gyrase and topoisomerases, but it may also be due to the synergistic combination of efflux pumps and plasmid-mediated mechanisms.45

Interestingly, albeit controversial, evidence indicates that an inverse relationship between quinolone resistance and virulence may occur. Spontaneous fluoroquinolone (FQ)-resistant E. coli mutants obtained from hemolytic FQ-susceptible isolates may still produce hemolysin.49 Other studies suggest that nalidixic acid-resistant uropathogenic E. coli strains carry less-frequent virulence factors, such as pilE and pilEF, and show a decreased expression of others, like type 1 fimbriae.55 Also, FQ-resistance maybe linked to a lower expression of β-hemolysin and papEF.56 Further studies suggested that this phenomenon may be particularly frequent among strains of the B2 phylogenetic group. Typically, ExPEC-associated VFs, such as P-fimbriae (pap) and hemolysin (βh), were found to be less prevalent in the genome of quinolone (Q-) and FQ-resistant isolates. This finding may be explained by the acquisition of Q- and/or FQ-resistance by E. coli strains that naturally lack these VFs, and then spread in a clonal fashion.75 However, the different clonal patterns found in this study do not support entirely this hypothesis.76 The mutation at codon 83 in gyrA (topoisomerase II) produces a reduction in the degree of DNA supercoiling, which might have implications in the expression of some virulence genes. Another hypothesis is that during the development of quinolone resistance, the antibiotic can increase the deletion or transposition of some DNA regions containing virulence genes.77,78

Typical VFs of ExPEC such as hemolysin (βh gene), cytotoxic necrotizing factor type 1 (cnf1 gene), P-pili F15 (papH genes), S-family adhesins (fim gene) can form clusters named pathogenicity islands (PAIs). They are usually located in the chromosome, but can be found in plasmids as well. They are composed by large segments of DNA associated with rRNA genes, differentiated in bacterial genome by a different G+C content. PAIs are mobile genetic elements that can be transposed to other DNA elements, and transferred by HGT.79,80 The PAIs constitute a huge benefit to the bacterial fitness by allowing the transmission of a large amount of genes that can benefit the survival of bacteria or its ability to cause disease.81

Partial or total loss of PAIs in the face of possible inhibition of topoisomerases II and IV has been reported.82 Indeed, the driving force behind PAI loss may be a signal to escape a genome which is less fit to replicate. Deletion processes may play a role in certain stages of infection.82-85 The genetic flexibility may be seen has an advantage resulting in a more efficient replication in the host and colonization of other ecological niches.86,87 In an attempt to elucidate the quinolone-induced mechanism leading to the loss of PAIs, which may be linked to the SOS system response (a DNA repair mechanism), studies have found that sub-inhibitory concentrations of quinolones may induce partial or total loss of PAIs in UPEC strains in vitro, via an SOS-dependent or -independent pathway, respectively. Thus, while it appears that quinolone resistance is not necessary for the loss of PAIs (genetic mobile elements), previous contact with these antimicrobials may favor the loss of certain PAIs.75,88

Ongoing projects in our laboratory also found an inverse relationship between FQ-resistance and the content of PAIs in clinical E. coli isolates mostly collected from urine and blood. However, a small percentage of B2 E. coli did not exhibit this inverse relationship, as they were resistant to ciprofloxacin, and yet contained three to five different PAIs in their genome.89-91 Furthermore, exposure of FQ-susceptible B2 E. coli isolates to sub-minimal inhibitory concentrations of nalidixic acid and ciprofloxacin on successive agar plating did not result in the deletion of PAI513 (alpha-hemolysin, CS12 fimbriae and F17-like fimbrial adhesion gene) and PAI515 (alpha-hemolysin and P-related fimbriae genes) (unpublished).

Despite repeated attempts at explaining the biology of the inverse link between FQ-resistance and virulence, the conflicting results found in the literature underline the complexity of the topic. It remains unclear whether drug-refractory E. coli strains are intrinsically less virulent bacteria, or if they become less
virulent following acquisition of the quinolone target-enzymes mutation.

Spontaneous transition from susceptibility to resistance to nalidixic acid and ciprofloxacin may occur during the selective passage on antimicrobial supplemented agar plates, independently of strain source (urine vs. fecal), phylogenetic background and VF content.41 This observation indicates that the low-virulence background and VFs content in Q- and FQ-resistant E. coli are not due to the loss of VFs. The enhanced development of resistance might reflect the genetic background of the bacteria within which resistance initially emerged.81

Resistance to ciprofloxacin in B2 E. coli recovered from women with uncomplicated cystitis was associated with a marked reduction in inferred virulence.82 How then such low-virulence strains still may cause cystitis remains unclear. Studies need to determine whether pathology in such cases is due to unknown VFs and/or to host immune factors. Regardless, these observations lead to the suggestion that in E. coli, the relationship between antimicrobial resistance and virulence traits may vary according to a particular resistance phenotype.81 For ciprofloxacin resistance, the relationship is strongly influenced by the phylogenetic background. What causes a high resistance to ciprofloxacin in groups A and D remains unclear. However, ongoing research tries to determine whether this phenomenon may originate from a possible animal source exposed to antibiotics, followed by food-borne transmission to humans.82,83

Another study84 with uropathogenic E. coli (UPEC) attempted to assess two current theories on a key debate: whether the low frequency of VFs precedes resistance, 82,83 or whether the loss of VFs to assess two current theories on a key debate: whether the low frequency of VFs precedes resistance, 82,83 or whether the loss of resistance and virulence traits may vary according to a particular resistance phenotype.81 For ciprofloxacin resistance, the relationship is strongly influenced by the phylogenetic background. What causes a high resistance to ciprofloxacin in groups A and D remains unclear. However, ongoing research tries to determine whether this phenomenon may originate from a possible animal source exposed to antibiotics, followed by food-borne transmission to humans.82,83

A relationship among this group of isolates may explain the observation of this same mutation. However, as the UPEC isolates were collected during three years and from diverse Japanese hospitals, clonal diversity may indeed have been significant. More research is required to characterize the mechanisms by which mutations in gyrases and topoisomerases may influence the acquisition or loss of expression of VFs.

Animals and Environment: Virulence and Antimicrobial Resistance

Antimicrobial agents are still used at sub-inhibitory concentrations as growth promoters in farm animals in some countries, with exception of EU countries. This provides a selective pressure which eliminates susceptible bacteria in the host and spares resistant ones, eventually inducing new resistance in the intestinal microflora.84 The following paragraphs will focus on the resistance and VFs reported in intestinal E. coli pathotypes of animal origin.

Virulence and antibiotic resistance in E. coli of animal origin. Antimicrobial resistance in E. coli isolates may vary according to the animal host it was isolated from. For instance, chicken and pork E. coli isolates may exhibit a higher degree of resistance and more virulence genes than beef isolates.85,86 Research has also showed that there is a potential human origin to antibiotic resistance in bacteria from domestic/companion animals,86,87 as E. coli isolated from domestic/companion animals tend to present the same resistance and virulence of E. coli isolated from humans.

In fact, the presence of the human-associated ST131 in domestic/companion animals is documented,86,87 presenting similar resistance phenotypes and VFs. Furthermore, for other serogroups, like O6, the hypothesis of zoonotic potential between animal/human seems valid.88,89

Among E. coli pathotypes that may be ingested by humans, EHEC is a group that is increasingly isolated worldwide.90,91 EHEC includes known virulent strains, such as E. coli O157:H7, which due to its virulence potential and low infectious dose makes it one of the most threatening serotypes. The pathogenicity of E. coli O157:H7 mainly results from the production of shiga toxins (stx1, stx2 and its variants), intimin (eaeA), and enterohemolysin A (hlyA). Antimicrobial resistance in these strains is a matter of increasing concern, and the association with some virulence traits in the same bacteria remains unclear. Together, current data from Asia, Europe and North America indicate that drug-resistant E. coli O157 may indeed co-express stx1, eaeA and hlyA, but little is known of the genetic mechanisms that may facilitate the concurrent acquisition and transmission of these traits.92,93 In feedlot beef cattle, studies have also reported E. coli O157:H7 resistance to antibiotics used in feed, but also to ciprofloxacin used in human medicine.94 These studies, and others,95–97 indicate that the use of quinolones may trigger the development of resistance in E. coli O157 in food-producing animals, regardless of geographical location or animal species.

A relationship was evaluated between imported feed contaminated with E. coli O157:H7 and sporadic cases of hemolytic-uremic syndrome (HUS) in Northern Italy in 2002.98 Although, HUS causing strains were multi-resistant to antibiotics and...
expressed more frequently the stx1 and stx2 genes, strains isolated from imported calves from France and Spain were more susceptible to antibiotics, and presented more frequently the iucD gene, which suggested independent circulation. Also food-borne, the recent 2011 HUS outbreak in Germany was caused by another STEC (Shiga toxin E. coli) pathotype, E. coli O104:H4. Therefore, contaminated meat as well as vegetables, expressed more frequently the stx1 and stx2 genes, strains isolated from imported calves from France and Spain were more susceptible to antibiotics, and presented more frequently the iucD gene, which suggested independent circulation. Also food-borne, the recent 2011 HUS outbreak in Germany was caused by another STEC (Shiga toxin E. coli) pathotype, E. coli O104:H4. Therefore, contaminated meat as well as vegetables, and goats. In Jordan, ampicillin and tetracycline are used in selection of non-pathogenic intestinal flora of the calves, lambs with increased use of antibiotics. This in turn may have led to the resistance in non-STEC and non-NTEC isolates, in association periods, it cannot be verified whether a decrease of resistance among domestic/companion animals, STEC have been less detected, but normally associated with infected human owners. Nonetheless, the simple presence of STEC strains among these types of animals should be investigated when owners are infected.

**Controversies in non-E. coli O157 pathotypes.** Contradictory data have been published on the association of virulence and antibiotic resistance in non-O157 pathotypes. For example, STEC, also called verotoxicogenic E. coli (VTgEC), isolated from diarrheic dairy calves, may exhibit very low degrees of multidrug resistance. Similarly, necrotrogenic E. coli (NTEC) also with low multidrug resistance, may concurrently express fimbriate F41, F17, F5 and aerogenes. Conversely, the highest level of multidrug resistance was detected among non-Ehec, non-toxigenic, aerogenes-negative strains suggesting that low frequency of virulence factors may be associated with a high multiple resistance to antibiotics. However, other reports have documented that STEC strains from calves may be significantly more resistant to multiple antimicrobial agents than NTEC, non-STEC, and non-NTEC strains. As the latter studies were performed at different periods, it cannot be verified whether the decrease in resistance occurred in STEC strains and conversely was an increase in resistance in non-STEC and non-NTEC isolates, in association with increased use of antibiotics. This in turn may have led to the selection of non-pathogenic intestinal flora of the calves, lambs and goats. In Jordan, ampicillin and tetracycline are used in livestock without control. Furthermore, feed-derived resistance to ampicillin and tetracycline in STEC of sheep and goats may be co-expressed with high degrees of resistance to chloramphenicol, nitrofurantoin, nalidixic acid, ciprofloxacin, sulmethamoxazole-trimethoprim, and interestingly also to gentamicin, not used in such agricultural settings. This further highlights the importance of cross-selection for resistance mechanisms.

E. coli isolated from food may contain a number of VFs, including intimin (eaeA), shiga toxin (stx), aerobactin (iucD), EAggEC heat-stable enterotoxin (espF), and cytolethality-associated plasm (pap). Studies have observed that strains that were resistant to amoxicillin/clavulanic acid, cephalosporin and amikacin did not contain these virulence factors, whereas that do were susceptible to the antibiotics. Curiously, the strains with eae plus iucD presented higher frequency of resistance to multiple antibiotics, while strains harboring only iucD gene expressed lower levels of resistance. Similarly, E. coli strains with the pap gene were more resistant to several antibiotics than those lacking the pap gene. The mechanisms regulating these phenomena, which may be serotype-dependent, remain obscure.

Multidrug resistant E. coli O26 strains may contain a class 1 integron, a genetic mobile element often associated with spread of antimicrobial resistance. The presence of class 1 integrons among highly virulent strains increases the risk of dissemination of antimicrobial resistance. Indeed, the association between ESBL and class 1 integrons has been well established.

High degrees of resistance have been observed among some STEC strains isolated from animal wastewater, and in these strains, the strains most susceptible to the majority of antibiotics were also those that carried more than one stx gene. It was postulated that this observation may reflect the presence of more than one bacteriophage in the genome of the strains, and the fact that some antibiotics induce the lytic cycle of these phages. In turn, in the presence of certain antibiotics, the lytic cycle of the bacteriophages may be induced more readily than if only one phage was present. Conversely, other studies found that some chicken strains may exhibit high resistance and low virulence due to the predominance of non-B2 groups with low expression of PAs.

In E. coli isolates from diarrheic and non-diarrheic pigs, virulent EPEC and ETEC exhibited more frequently a relatively high level of resistance to gentamicin, neomycin, and sulphamethoxazole-trimethoprim. Of course, research is needed to determine whether virulence genes detected in pathogenic isolates may be linked, at some level, to genes coding for antibiotic resistance. However, some E. coli strains encoding transport and aerobactin VFs, do not show any type of inverted relationship between VFs and resistance.

Among the non-O157 serogroups, several serogroups (O1, O2, O65 and O78) are normally associated with avian pathogenic E. coli (APEC). APEC strains can present high levels of resistance to tetracycline, sulphamethoxazole and fluoroquinolones. Resistance to several antibiotics has been related with the presence of APEC IncI or IncH12 plasmids, pAPEC-02-R and pAPEC-O1-R, respectively, also responsible for the horizontal transmission of VFs.

Together, these data reflect the controversial nature of the topic. Importantly, the studies also illustrate that observations available to date are mostly correlative in nature. Unequivocal evidence for cause-to-effect relationships, and for co-transmission mechanisms of resistance and virulence, is sorely needed. Regardless, animal and environment strains represent a pool of putatively enteropathogenic bacteria that can act as reservoirs for antibiotic resistance genes. Hence, the possibility exists that combinations of VFs and resistance determinants may occur over time, especially in E. coli, since its genome has such a high degree of plasticity.

**Concluding Remarks**

E. coli is a highly adaptable microorganism that has evolved sophisticated means of antibiotic resistance. Antimicrobial resistance represents a clear threat to public health worldwide. Many antimicrobial genes are inserted in conjugative plasmids
that may also carry virulence factors determinants, and might be selected by antibiotic selected pressure. However, the genetic association between resistance and virulence traits is poorly understood. Despite controversial findings, evidence indicates that there is, at least in certain strains, a correlation between virulence and antimicrobial resistance, and often these are clonal. Such clones have a high capacity of acquiring antimicrobial resistance, including FQ-resistance, and of expressing VFs. However, evidence also suggests reverse relationships between the expression of VFs and quinolone resistance have been documented in uropathogenic E. coli isolates. In these cases, research needs to determine whether chromosomal mutations might influence the transcriptional events that modulate expression of specific VFs. Alternatively, or as a coinciding consequence, the loss of VFs might increase bacterial fitness in such strains. Regardless, successful virulent and resistant clones are being disseminated worldwide, as illustrated by the UPEC-associated pandemic clone E. coli ST131, or strains of EHEC, such as E. coli O157, and the recent O104. Future research needs to characterize the genetic mechanisms linking these two phenotypes in other bacterial lineages with shared genetic backgrounds. Studies need to investigate the interplay of resistance and virulence at genetic levels, and concurrently identify the expression of these traits, in order to help us improve our management of infectious diseases.

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