Pandemic Bacteremic *Escherichia Coli* Strains: Evolution and Emergence of Drug-Resistant Pathogens

Yael Yair and Uri Gophna

Contents

Abstract In recent years, there have been several pandemics of *E. coli* strains which are highly virulent and antibiotics resistant. Here, we discuss one recent pandemic strain, ST131. These *E. coli* strains are members of the virulence-associated phylogenetic group B2 and exhibit extraintestinal virulence factors, including various adhesins, toxins, siderophores, and protectins. This group often also harbors a diverse range of antimicrobial resistance types and mechanisms and may have particular metabolic capacities that enable it to colonize many individuals asymptomatically, while out competing other *E. coli* strains. Here, we discuss this clonal group in the context of other pathogenic *E. coli* and focus on its specific characteristics in terms of resistance, virulence, and metabolism.

Y. Yair (✉) · U. Gophna
Department of Molecular Microbiology and Biotechnology,
Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel
e-mail: yael.yairhelmann@gmail.com

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1 Introduction: Pathogenic *Escherichia Coli* in Human Health and Disease

Since its discovery in 1885, *Escherichia coli* has been one of the best studied Gram-negative bacteria, and commonly used as a "workhorse" for molecular biology and biochemistry. *E. coli* strains of biological significance to humans are generally categorized as commensal strains, typically found in a healthy human gut microbiome (Eckburg 2005) and pathogenic strains. In humans, pathogenic *E. coli* strains are responsible for two types of infections: gastrointestinal infections and extraintestinal infections that include urinary tract infections (UTIs), bloodstream infections, and neonatal meningitis (Russo and Johnson 2000). The intestinal infections can be complicated by extraintestinal syndromes, such as in the case of Shiga toxin-producing intestinal strains that can cause hemolytic uremic syndrome (Rasko et al. 2011). Among the strains causing intestinal infections, there are six defined categories (also referred to as "pathotypes") of pathogenic strains of *E. coli*: enterotoxigenic (ETEC), Shiga toxin-producing/enterohemorrhagic (STEC/EHEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent (DAEC) (Russo and Johnson 2000). The most common extraintestinal *E. coli* (ExPEC) infections occur at multiple anatomical sites such as the urinary tract (Kaper et al. 2004), the respiratory tract, the cerebral spinal fluid, meningitis (mostly in neonatal), and peritoneum (spontaneous bacterial 58 peritonitis) (Soriano et al. 1995; Russo 2003). The majority of UTIs in young healthy women are caused by ExPEC strains (85–95%), and along with Group B Streptococcus, ExPEC is considered to be a leading cause of neonatal meningitis worldwide (Russo 2003; Poolman and Wacker 2015).

ExPEC strains can often be found in a normal healthy human gut, without causing any clinical symptoms (Eckburg 2005). Thus, they can be asymptomatically carried by many, and later be transmitted via contact, infecting the urinary tract or blood, often of the same individual that has been hosting them for years. When bacteria are present in the blood in large numbers, this can lead to other infections and sometimes trigger a serious body-wide inflammatory response called sepsis, which can be life-threatening, since it may progress to one or more organ failure, that often leads to death (Nguyen et al. 2006). Once ExPEC strains enter the bloodstream, for example, as a result of advanced UTI or transrectal ultrasound-guided (TRUS) biopsy, they can cause bacteremia that can lead to sepsis (coliseptisemia) (Johnson and Russo 2002). In fact, although TRUS prostate biopsy is generally considered to be a relatively safe medical procedure, severe sepsis has been described in 0.1–3.5% of cases after TRUS biopsy, with ExPEC being the most common cause (Williamson 2012; Lange et al. 2009).

In the past decade, there has been a rapid increase in the rates of hospitalization and mortality due to ExPEC infections, mainly because of the spread of antibiotic resistance among clinical isolates (De Kraker et al. 2013). In 2010, the estimated economic burden of UTI-associated hospitalization in the USA alone was estimated

to be around 2.3 billion dollars and between 85 and 95% of the cases were *E. coli* related (Poolman and Wacker 2016). *E. coli* is also one of the most common causes of community-acquired bacteremia and sepsis (De Kraker et al. 2013). In seniors, *E. coli* is the most common source of community-acquired bacteremia (Jackson et al. 2005). In the USA, at 2001, it was estimated that about 40,000 deaths per year are caused by *E. coli*-associated sepsis (Russo 2003), and it is the most common bacterial species associated with septicemia (Elixhauser et al. 2006), with cost of nearly $15.4 billion in aggregate hospital costs. In Europe, similar trends have been observed with an increase of reported *E. coli* bacteremia cases from 20,151 reports in 2002 to 32,194 reports in 2008. Thus, ExPEC strains have a great impact on public health and represent an increasing economic burden on society.

2 *E. coli* Sequence Type 131: A Worldwide Pandemic Clone

In 2008, reports of a previously unknown *E. coli* clonal group emerged from three different continents, noticed by two research groups who were studying CTX extended-spectrum-β-lactamase (ESBL)-producing *E. coli* (Nicolas-Chanoine et al.

Fig. 1 Reported cases of ST131 worldwide, as of 2017 (Nicolas-Chanoine et al. 2014; Vignoli 2016; Chattaway et al. 2016; Eibach et al. 2016; Begum and Shamsuzzaman 2016; Ouedraogo et al. 2016; Yahiaoui et al. 2015; Ebrahimi et al. 2016; Hristea et al. 2015; Markovska et al. 2017; Rogers et al. 2011; Severin et al. 2010; Peirano et al. 2014). **Africa**: Algeria, Burkina Faso, Cameroon, Central African Republic, Egypt, Guinea-Bissau, Ghana, Kenya, Madagascar, Morocco, Nigeria, South Africa, Tanzania, and Tunisia. **Europe**: Belgium, Bulgaria, Croatia, Denmark, France, Germany, Hungary, Italy, Netherland, Norway, Portugal, Romania, Spain, Sweden, Switzerland, the Czech Republic, and the United Kingdom. **Asia**: Bangladesh, Cambodia, China, India, Israel, Jordan, Japan, Kuwait, Lebanon, the Philippines, South Korea, People's Democratic Republic of Laos, Pakistan, Russia, Turkey, Thailand, and United Arab Emirates. **North America**: Canada, the United States. **South America**: Argentina, Brazil, Colombia, Ecuador, Mexico, Panama, Puerto Rico, and Uruguay. **Oceania**: Australia and New Zealand

2008; Coque 2008). This clonal group, which later became known as sequence type 131 (ST131), caught the attention of the clinical research community due to several unique characteristics: increased occurrence of resistance to antimicrobial agents, enhanced virulence, and fast spread. The two initial studies showed that ST131 had emerged predominantly in the community, and was simultaneously identified in different parts of the world spanning three continents. Since then, ST131 has spread globally (Fig. 1).

The serotype most associated with ST131, O25:H4, was also identified among both intestinal and extraintestinal adherent-invasive *E. coli* (AIEC) strains (Martinez-Medina et al. 2009).

3 ST131—Characterization of a Novel Pandemic Lineage

The first step in identifying the mysterious clonal group was to examine basic characteristics—phylogenetic group, serotype, O antigen, and virulence genes, as well as multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE). Most ExPEC strains belong to group B2, while a small fraction belongs to group D and most commensal strains have been shown to belong to group A (Clermont et al. 2000). Phylogenetic analysis of the ST131 clone revealed that like other ExPEC strains it belonged to phylogenetic group B2 (Nicolas-Chanoine et al. 2008; Coque 2008; Clermont et al. 2008). The lineage was identified as belonging to ST131 (Nicolas-Chanoine et al. 2008; Coque 2008; Wirth et al. 2006) based on MLST, perhaps the most widely accepted bacterial typing method today. As for the three surface antigens O, H, and K (the specific part of the LPS, the flagella, and the capsule, respectively) (Ørskov and Ørskov 1984), most ST131 isolates exhibit serotype O25b:H4, except for a small subset of strains that exhibit serotype O16:H5 (Nicolas-Chanoine et al. 2008; Johnson et al. 2014), and some isolates that cannot yet be typed for either O (Dahbi et al. 2013) or H antigens (Suzuki et al. 2009).

Although all ST131 are coherent and homogenous when examined according to their MLST-determining genes, there is significant within-lineage genetic variations as shown by the PFGE method which is based on the specific digestion (using rare-cutting restriction enzymes) of DNA into fragments of varying sizes, followed by the separation of these DNA fragments by gel electrophoresis using a periodically changing electric field. In contrast to its otherwise clonal character ST131 presents highly variable PFGE profiles, and different pulsotypes can be observed depending on geographic location, time periods, and ecological niches (Johnson et al. 2012). Pulsotype comparison of 579 ST131 isolates resolved 170 distinct pulsotypes (Johnson et al. 2012), with a small number of dominant pulsotypes, including one pulsotype (968) that accounts for 24% of the general ST131 population. In summary, the ST131 lineage, while monolithic in terms of the sequence of housekeeping genes, is highly variable in terms of its genome content, and often contains different “flexible genome” elements, such as plasmids and prophages.

4 What Are the Reasons for the Worldwide Rapid Dissemination of ST131?

The initial consensus hypothesis for explaining the rapid worldwide dissemination of ST131 was that these bacteria are more virulent, combining the B2 group background with additional novel virulence traits (Banerjee and Johnson 2014; Johnson et al. 2010). Typical virulence-associated genes in ExPEC are adhesin-encoding genes (such as *papAH*, *papC*, *papEF*, *papG*, *sfa/focDE*), toxin-encoding genes (*such as hlyA*, *hlyF*, *cnf1*), siderophore-related genes (*iroN*, *fyuA*, *ireA* and *iutA*), protectin/invasin-encoding genes (*kpsM II*, *kpsMT III*, *iss,* etc.), pathogenicity island markers, and miscellaneous genes (*cvaC*, *usp*, *ompT*, *clbB*, etc.). These genes contribute to the pathogenic potential and are seldom found in non-pathogenic strains (Johnson and Stell 2000).

Indeed, 12 virulence genes were found to be significantly more prevalent among ST131 than among non-ST131 isolates: *iha* and *fimH* (adhesin–siderophore receptor and type I fimbriae, respectively), *sat* (a secreted toxin), *astA* (an enteroaggregative *E. coli*-associated toxin), *fyuA* and *iutA* (yersiniabactin and aerobactin receptors, respectively), *kpsM II-K2* and *kpsM II-K5* (group II capsular polysaccharide synthesis), *usp* (uropathogenic-specific protein), *traT* (surface exclusion, serum resistance-associated), *ompT* (outer membrane protease), and *malX* (a pathogenicity island marker). The overall virulence scores were significantly higher for ST131 isolates compared to most of the non-ST131 isolates (Johnson et al. 2010). Similarly, in ESBL-producing isolates, 11 virulence genes (*papG III*, *afaFM955459*, *cnf1*, sat, hlyA, kpsM II-K2, *kpsM II-K5*, *traT*, *ibeA*, *malX,* and *usp*) were significantly associated with ST131, whereas only *papG II* and *tsh* were significantly associated with non-ST131 strains (Coelho et al. 2011). Analysis of 130 clinical ST131 isolates revealed four distinct virotypes of ST131 (labeled arbitrarily as A, B, C, and D) (Blanco et al. 2013), with an additional virotype added later on (virotype E (Dahbi et al. 2013)), based on the presence or absence of four distinctive virulence genes, including *afa* FM955459 (specific for an ST131 clone encoding an *Afa/Dr* adhesin), *iroN*, *ibeA* (invasion of brain endothelium), and *sat*. The patterns were as follows (Table.1):

Table 1 Distribution of *afa* FM955459, *iroN*, *ibeA*, and *sat* genes among the different main virotypes of ST131

Virotype/ gene	*Afa* FM955459	*IroN*	*IbeA*	*Sat*
A	+	−	−	∓
B	−	+	−	∓
C	−	−	−	+
D	−	∓	+	∓
E	−	−	−	

Table 2 Summary of selected studied pointing the major finding regarding ST131 virulence phenotype and genotypes in experimental, in vivo models, and epidemiological surveys

Year	Geographic location	Method	Non-ST131	ST131	Main findings	Reference
2011	Spain	Genotypic characterization (virulence factor genes profile analyses)	64	30	ST131 isolates have higher virulence scores than non-ST131 isolates	Coelho et al. (2011)
2012	USA and Canada	Animal model (mouse subcutaneous sepsis model)	34	27	ST131 isolates are not more virulent than other ExPEC isolates	Johnson et al. (2012)
2012	France and Spain	Animal models (*Caenorhabditis elegans* and zebrafish embryos)	5	3	No advantage detected for the ST131 isolates	Lavigne et al. (2012)
2017	Spain	Epidemiologic survey	56	33	ST131 isolates are more drug resistance but not more clinically virulent and no significant differences in risk factors or prognosis	Morales-Barroso (2017)
2010	USA	Genotypic characterization (virulence factor gene profile analyses)	73	54	ST131 isolates have higher virulence scores compared to non-ST131 isolates	Johnson et al. (2010)
2014	Spain	Mouse subcutaneous sepsis model	2	23	Broad virulence diversity among ST131. All ST131 isolates exhibited some lethality rate for mice	Mora et al. (2014)
2008	France, Tunis and Central African Republic	Mouse lethality assay, adhesion on human cells, biofilm production, and genomic characterization	4	4	ST131 isolates yielded 100% lethality rate for mice	Clermont et al. (2008)
2012	Portugal, Spain, United Kingdom, USA, France, Norway, Czech Republic, South Korea, Switzerland, and Croatia	Adherence in vitro to abiotic surfaces	1	32	None of the ST13 isolates was able to form biofilm in the tested conditions	Novais et al. (2012)

Virotype D isolates exhibited significantly higher virulence scores than did those of other virotypes and was significantly associated with younger patients and community acquisition (Blanco et al. 2013). In contrast, virotype B was associated with older patients and a lower likelihood of symptomatic UTI, but a higher likelihood of respiratory tract infection, while virotype C was significantly associated with a generally higher likelihood of symptomatic infections. Overall, ST131 isolates of the major serotype O25b:H4 exhibited higher virulence scores than ST131 isolates of the rarer serotype O16:H5 (Dahbi et al. 2013).

In contrast to molecular epidemiology, experimental studies in animal models do not clearly support the hypothesis that ST131 is more virulent than other *E. coli* strains. When ST131 isolates were compared to non-ST131 ExPEC isolates in a mouse subcutaneous sepsis model, no significant advantage for the ST131 was observed in terms of lethality and clinical illness (Johnson et al. 2012). These findings were also supported by studies in *Caenorhabditis elegans* and zebrafish embryos (Lavigne et al. 2012). Some evidence that ST131 is not necessarily hyper-virulent comes from a recent case–control study from 2017 that showed that the non-ESBL-producing ST131 strains did not cause a worse clinical outcome in human bacteremia (in terms of mortality, severe sepsis, hospitalization time, etc.) than non-ST131 isolates (Morales-Barroso 2017). Taken together, the studies contradict the assumption that the rapid emergence and global dominance of ST131 are due to enhanced virulence.

The findings concerning the virulence of ST131 in comparison to non-ST131 strains are summarized in Table 2.

From these experiments, it appears that ST131 strains are not significantly more virulent in vivo than the non-ST131 strains. Yet, it is highly likely that there are other factors, such as enhanced metabolic capacities, and capacity for asymptomatic carriage (discussed below), that have contributed to the success of ST131 as a global pathogen. Indeed, there is evidence that ST131 isolates have higher metabolic potential compared to non-ST131 isolates (Vimont et al. 2012; Gibreel 2012), in terms of catabolic enzyme repertoire but further study is required, such as in vivo colonization studies that involve competition between ST131 isolates and other strains, or ST131 mutant that lack these enzymatic functions.

5 Carriage of ST131 in the Community

A key factor that probably contributes to the global dissemination of ST131 is its carriage among healthy individuals. ST131 lineage is strongly associated with community-onset infections, and carriage rates in healthy subjects can range from 7% in independent healthy Parisians (Leflon-Guibout et al. 2008) up to over 35% in long-term care facilities (LTCF) for the elderly in Italy (Giufrè 2017). ST131 is associated with older age, intensive antibiotic treatment, and high prevalence among residents in nursing homes and LTCF (Banerjee et al. 2013), which may represent the largest human reservoir for ST131. Taken together with the potential

of ST131 for increased virulence, these findings indicate the opportunistic nature of this lineage. Indeed, several case reports of transmission of ST131 within household, resulting in severe or fatal extraintestinal infections (Morales-Barroso 2017; Mora et al. 2014), demonstrate its potential for causing deadly opportunistic disease. Since, like other ExPEC lineages, ST131 has the ability to colonize healthy individuals without causing any symptoms, health authorities should consider future measures in order to prevent its dissemination, or in the very least take steps to prevent infections in high-carriage communities.

6 ST131 in Companion and Non-companion Animals—Additional Natural Reservoirs

One of the intriguing aspects of the ST131 clonal group is its natural reservoirs. ST131 is found among drug-resistant *E. coli* isolates in companion and non-companion animals (Rogers et al. 2011)—dogs, cats (Ewers et al. 2010; Pomba et al. 2009; Johnson et al. 2009), poultry (Mora et al. 2010; Cortes et al. 2010), horses (Ewers et al. 2010), and pigs. In non-companion animals, ST131 was found in glaucous-winged gulls (Hernandez et al. 2010), seagulls (Simoes et al. 2010), and rats (Guenther et al. 2010). A European collection of 177 ESBL-producing *E. coli* isolates collected from eight countries, mainly obtained from companion animals with various clinical manifestations, revealed that 5.6% of the isolates were ST131 O25b (Ewers et al. 2010). Many clinical ST131 isolates from companion animals were found to have high resemblance to human clinical ST131 isolates based on their virulence genotype, resistance characteristics, and PFGE profiles. These findings suggest either recent or ongoing zoonotic transmission between humans and animals (Rogers et al. 2011), and have been corroborated by more recent genomic analysis showing that most, though not all, ST131 strains frequently cross-host species boundaries (McNally et al. 2016). A possible reason for the relative lack of ST131 case reports in animals might be the veterinary sector's relatively limited microbiological diagnosis and reporting systems, probably resulting in many unreported cases. Reports of ST131 carriage among non-companion animals are even more rare, since such animals are little studied. Although according to most data, the ST131 pandemic appears mostly a human-based phenomenon, the risk of inter-species transmission of these multi-resistant strains between humans and animals should be seriously considered (Table 3).

Table 3 A summary of all reported ST131 cases in companion and non-companion animals

Geographic location	Year	Total number of isolates	% of ST131	ST131 isolates	Total number of isolates	Species	Reference
Portugal	2007–2008	139	9%	4	Feces	Seagull (*L. fuscus, L. cachinnans*)	Simoes et al. (2010)
Spain	2004–2006	61	1.6%	1	Chronic cystitis	Dog	Pomba et al. (2009)
USA	2008	5	20%	1	Urine, feces	Dog and cats	Johnson et al. (2009)
Germany	2010	211	0.47%	1	Feces	Brown rat (*Rattus norvegicus*)	Guenther et al. (2010)
Spain	2009–2010	100	8%	8	Retail chicken	Poultry	Mora et al. (2010)
Spain	2007–2009	463	1.5%	7	Clinical	Chickens	
Spain, France, Belgium	1991–2001	1601	0.18%	3	Clinical	Turkeys, chicken	
Spain	2003	57	1.8%	1	Feces	Chickens	
Spain	2003	59	1.6%	1	floor	Poultry	Ortes et al. (2010)
Russia	2010	145	0.68%	1	Fecal or cloacal	Mainly Glaucous-winged gull (*L. glaucescens*), Tufted Puffin (*F. cirrhata*) and Black-headed gull (*L. ridibundus*)	Hernandez et al. (2010)

(continued)

Table 3 (continued)

Geographic location	Year	Total number of isolates	% of ST131	ST131 isolates	Total number of isolates	Species	Reference
Germany, Italy, the Netherlands, France, Spain, Denmark, Austria, Luxembourg	2008–2009	84	10.7%	9	Clinical	Dogs	Ewers et al. (2010)
		50	2%	1	Clinical	Horse	
		7	0%	0	Clinical	Cattle	
		2				Guinea pigs	
		1				Pygmy rabbit	
		1				Pig	
		1				American Kestrel	
		31				Cats	
Australia	2007–2009	120	6.6%	8	Clinical	Dogs	Platell et al. (2011)
		5	20%	1	Clinical	Cat	
Australia	2009	232	2.1%	5	Feces	Dogs	Guo et al. (2013)

7 Drug Resistance Among the ST131 Clonal Group

Extended-spectrum β-lactamase (ESBL)-producing bacteria are resistant to most beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams. These enzymes cleave the amide bond in the β-lactam ring, and thus inactivate those antibiotics. The rapid dissemination of antibiotic resistance among bacteria is an alarming trend and considered to be one of the world's main health threats (Bonnet 2003). In recent years, CTX-M enzymes have become the predominant ESBLs encountered in the clinic. These enzymes have originated from *Kluyvera* spp. (Pitout et al. 2005) and are generally plasmid-associated. *Klebsiella pneumoniae* and *E. coli* are the major ESBL-producing organisms isolated worldwide. The ST131 clonal group initially caught the attention of researches because of its CTX-M-15 ESBL (Nicolas-Chanoine et al. 2008). Since then, ST131 isolates carrying CTX-M enzymes were reported in many countries worldwide (Rogers et al. 2011). In Canada, a multi-center study that included 209 clinical isolates revealed that 46% of ESBL isolates belonged to the ST131 clonal group, with the vast majority (91%) of these strains producing CTX-M-15 (Peirano et al. 2010).

It was previously reported that CTX-M-producing *E. coli* isolates often carry resistance to additional antibiotic classes, which can include co-trimoxazole, aminoglycosides, and fluoroquinolones (Pitout and Laupland 2008). Indeed, fluoroquinolone resistance is one of the most frequently reported resistances among ST131 strains, including ESBL-producing ones. There are several mechanisms that can lead to fluoroquinolone resistance in ST131, and they provide varying levels of resistance. High-level fluoroquinolone resistance in *E. coli* was reported to be caused by chromosomal mutations of genes coding the fluoroquinolone targets, which are *gyrA*, *gyrB*, *parC,* and *parE* (Rogers et al. 2011). The aminoglycoside-modifying enzyme AAC(6′)-Ib-cr also contributes to quinolone resistance via acetylation of selected fluoroquinolones. Low-level resistance can also be conferred by the presence of plasmid-mediated quinolone resistance genes, including *qnrA*, *qnrS,* and *qnrB*. Population analysis performed on historical and recent ST131 isolates found that fluoroquinolone resistance in the ST131 fimH30 sub-lineage is mostly due to *gyrA1AB* and *parC1aAB* mutations in genes encoding gyrase and topoisomerase IV, respectively (98% of FQ-R isolates) (Johnson et al. 2013).

Resistance to the carbapenemases among *E. coli* is yet another alarming trend worldwide (Nordmann and Poirel 2014). Three major carbapenemases have been reported: KPC (*Klebsiella pneumoniae* carbapenemases), NDM (New Delhi metallo-β-lactamase), and OXA-48 (for "oxacillinases"). An extended analysis of 116 carbapenemase-producing *E. coli* isolates found that 35% of the isolates belonged to ST131. 58% of ST 131 isolates were positive to the bla_{KPC}, 32% for $bla_{OXA\text{-}48\text{-}like}$, 7% for $bla_{NDM\text{-}1}$, and 2% for $bla_{IMP\text{-}14}$ (Peirano et al. 2014).

It is obvious that antimicrobial resistance is widespread among the ST131 clonal group, and it is safe to assume that this feature has a strong impact on the spread of ST131 in the community, helping this lineage to replace other, antibiotic-sensitive

strains in a world where antibiotic exposure is common. Thus, their antibiotic resistance is making ST131 more abundant as well as harder to eliminate (Banerjee and Johnson 2014).

8 "A Shark Among Sharks"—The H30 and H30-Rx Sub-clones of ST131

In order to better understand the genetic structure of the ST131 clone, an analysis of 350 historical and more recent ST131 isolates and over 700 non-ST131 *E. coli* isolates was performed in 2010, which utilized a variety of typing strategies, such as sequencing of selected genes (*fimH*, *gyrA,* and *parC*), MLST, and PFGE. This analysis identified 185 unique PFGE types and 7 distinct *fimH*-based putative clonal lineages of ST131: H15, H22, H27, H30, H35, H41 and H94, with H30 being the dominant allele (Johnson et al. 2013). The high genetic similarity of most H30 isolates to one another suggested that they originate from a single fimH30-carrying ancestor. Moreover, this H30 ST131 sub-clone was closely associated with fluoroquinolone resistance (FQ-R) and with ESBL production mediated by CTX-M-15. Since this lineage was rare among fluoroquinolone-susceptible ST131 isolates (<1%), it was suggested that FQ-R is associated almost exclusively with the ST131 H30 sub-clone, which originated from a single strain about 14 years ago (Johnson et al. 2013; Banerjee et al. 2013). The H30 ST131 clone was found to be associated with persistent infections, subsequent hospital admissions, and subsequent new infections (Johnson et al. 2016). A recent study from 2017 suggests that H30 isolates tend to be less frequently nosocomially acquired, and more frequently affect patients aged >65 years. Moreover, H30 isolates were also found to be more frequently resistant to ciprofloxacin and less frequently resistant to trimethoprim/sulfamethoxazole (Morales-Barroso 2017). In addition, the H30 lineage was found to be associated with virotype C (see above) and CTX-M-14 (Peirano et al. 2014).

An important sub-lineage within H30 is the single, highly virulent sub-clone, H30-Rx. This clone was identified by whole-genome single-nucleotide polymorphism (SNP) analysis performed on 105 ST131 isolates cultured from humans and animals between 1967 and 2011 (Price 2013). High-resolution phylogenetic analysis enabled the identification of a single-ancestral sub-clone within H30-R, the fluoroquinolone-resistant H30 sub-clone. Because of its more extensive resistance characteristics, this CTX-M-15-associated sub-clone was designated H30-Rx. Assessment of the demographic, geographic, and clinical prevalence of H30-Rx revealed that the relative prevalence of H30-Rx was highest among German Hospital isolates (where it even exceeded the prevalence of other H30-R isolates), intermediate among US-based hospital isolates, and lowest among the US outpatient isolates (Price 2013). In addition, a more recent study that analyzed a global collection of ESBL-producing *E. coli* isolates found that the majority of ST131 (92%) isolates belonged to the H30 lineage, and 82% H30 isolates belonged to the H30-Rx sub-lineage. The H30-Rx lineage was recovered from all 9 countries

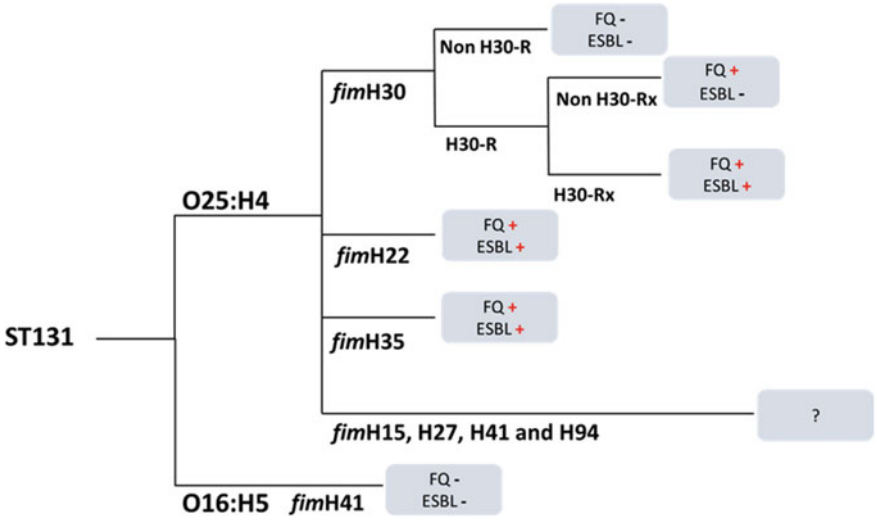

Fig. 2 General structure of the population of *E. coli* ST131. Presented are the two main serotypes (O25b:H4 and O16:H5) with lineages and sub-lineages producing or not producing ESBL enzymes and being resistant or sensitive against fluoroquinolones (FQ). Adapted from Schaufler (2017), Mathers et al. (2015)

examined in that study (spanning all five continents) and also showed strong association with drug resistance (having the $bla_{CTX\text{-}M\text{-}15}$, and *aac(6′)-lb-cr* genes) and virotype A (Peirano et al. 2014). Moreover, H30-Rx isolates were found to have higher resistance scores compared to non-H30-Rx ST131 isolates and were associated specifically with CTX-M-15. Three virulence genes (*iha*, *sat*, and *iutA*) were more prevalent among H30 than non-H30 ST131 isolates. Thus, the H30 and H30-Rx sub-clones are considered to be more drug-resistant and have virulence profiles that are distinct from those of non-H30 ST131 (Banerjee et al. 2013). Several studies were performed in order to determine the prevalence and distribution of ST131 sub-lineages worldwide. For instance, a population-based study performed in Minnesota, USA, revealed that 88% of all ST131 infections were due to the H30 sub-lineage. The H30 sub-lineage was most common among adults over 50 years old and its prevalence was positively correlated with age. However, among children under the age of 10, both H30 and non-H30 ST131 isolates were highly prevalent, suggesting that both the old and the young are more vulnerable to ST131 and its sub-lineage (Banerjee et al. 2013). Another population-based study from Canada revealed that 46% of FQ-R *E. coli* isolates were ST131, and 96% of these belonged to the H30 sub-lineage, with 32% belonging to the H30-Rx sub-lineage. The study identified the association of H30-Rx sub-lineage with the clinical features of primary sepsis, upper UTIs, and complication of prostate biopsies. Predictably, the H30-Rx sub-lineage was also associated with multidrug resistance, and with the presence of $bla_{CTX\text{-}M\text{-}15}$ and *aac(6′)-lb-cr* resistance genes (Peirano and Pitout 2014) (Fig. 2).

Taken together, its high prevalence in the young and old, its pathogenicity, and drug resistance make the H30 sub-lineage potentially the most important *E. coli* from a public health perspective.

9 Conclusions

The global spread of ST131 is probably a combination of different factors. Unlike epidemic strains that cause outbreaks that are eventually contained by the medical community, pandemic lineages such as ST131 require a constant reservoir to maintain their extended footprint. The high asymptomatic carriage rates of ST131 provide this reservoir within the human population, but how did they replace other *E. coli* lineages and have become so commonly carried remains unclear. While ST131 is not more infective than other *E. coli* strains, they appear to be stable for longer periods within their hosts (Giufrè 2017), implying that they are fitter either due to resistance to antibiotics, to which carriers are often exposed, especially in LTCF, to increased metabolic potential, or both. A key question is how these strains interact in the colon with many other intestinal bacteria, and also additional *E. coli* strains. Residents of LTCF have been shown to have a microbiota that is less diverse than community-dwelling elderly subjects (Claesson et al. 2012), and one may speculate that such microbiota may be more conductive to colonization with ST131. Furthermore, this less diverse microbiota was associated with higher levels of inflammatory markers raising the question of whether ST131 strains contribute more to chronic inflammation than other *E. coli* lineages.

It remains to be determined whether more responsible antibiotics usage world-wide will lead to a decrease in ST131 carriage or whether specific anti-ST131 measures such as vaccination or phage therapy (Pouillot et al. 2012; Green 2017) will have to be undertaken in order to reduce the burden of this lineage to healthcare systems and to human health.

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