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The Role of Epidemic Resistance Plasmids and International High-Risk Clones in the Spread of Multidrug-Resistant *Enterobacteriaceae*

Amy J. Mathers,[a] Gisele Peirano,[b,c] Johann D. D. Pitout[b,c,d,e]

University of Virginia, Charlottesville, Virginia, USA[a]; Division of Microbiology, Calgary Laboratory Services,[b] and Departments of Pathology and Laboratory Medicine[c] and Microbiology, Immunology and Infectious Diseases,[d] University of Calgary, Calgary, Alberta, Canada; Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa[e]

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Published 29 April 2015

Citation Mathers AJ, Peirano G, Pitout JDD. 29 April 2015. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. Clin Microbiol Rev doi:10.1128/CMR.00116-14.

Address correspondence to Johann D. D. Pitout, johann.pitout@cls.ab.ca.

doi:10.1128/CMR.00116-14

SUMMARY

Escherichia coli sequence type 131 (ST131) and *Klebsiella pneumoniae* ST258 emerged in the 2000s as important human pathogens, have spread extensively throughout the world, and are responsible for the rapid increase in antimicrobial resistance among *E. coli* and *K. pneumoniae* strains, respectively. *E. coli* ST131 causes extraintestinal infections and is often fluoroquinolone resistant and associated with extended-spectrum β-lactamase production, especially CTX-M-15. *K. pneumoniae* ST258 causes urinary and respiratory tract infections and is associated with carbapenemases, most often KPC-2 and KPC-3. The most prevalent lineage within ST131 is named *fimH30* because it contains the H30 variant of the type 1 fimbrial adhesin gene, and recent molecular studies have demonstrated that this lineage emerged in the early 2000s and was then followed by the rapid expansion of its sublineages H30-R and H30-Rx. *K. pneumoniae* ST258 comprises 2 distinct lineages, namely clade I and clade II. Moreover, it seems that ST258 is a hybrid clone that was created by a large recombination event between ST11 and ST442. Epidemic plasmids with $bla_{\text{CTX-M}}$ and bla_{KPC} belonging to incompatibility group F have contributed significantly to the success of these clones. *E. coli* ST131 and *K. pneumoniae* ST258 are the quintessential examples of international multidrug-resistant high-risk clones.

INTRODUCTION

The *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella pneumoniae*, are common causes of nosocomial and community infections among humans (1). One of the most urgent areas of antimicrobial drug resistance is the rapid evolution of fluoroquinolone, cephalosporin, and carbapenem resistance among *Enterobacteriaceae*, which has spread globally during the last decade. Recently, the World Health Organization (WHO) released a report entitled *Antimicrobial Resistance*: *Global Report on Surveillance 2014* (2). This report focused on antibiotic resistance among bacteria responsible for common, serious infections, including bloodstream-associated infections (BSIs), urinary tract infections (UTIs), and intra-abdominal infections (IAIs). It states that resistance to certain antibiotics among frequently isolated bacteria is rife in certain parts of the globe.

For *E. coli*, this report states the following: "Resistance to one of the most widely used antibacterial medicines (i.e., the fluoroquinolones) for the treatment of UTIs caused by *E. coli* is very widespread. In the 1980s, when these drugs were first introduced, resistance was virtually zero. Today, there are countries in many parts of the world where this treatment is now ineffective in more than half of patients" (2). Specifically, for *K. pneumoniae*, the WHO report states the following: "Resistance to the last resort agents (i.e., carbapenem antibiotics) for the treatment of life-threatening infections caused by a common intestinal bacteria, *K. pneumoniae* has spread to all regions of the world. *K. pneumoniae* is a major cause of hospital-acquired infections such as pneumonia, BSIs, infections in newborns and intensive care unit patients. In some countries, because of resistance, carbapenem antibiotics would not work in more than half of people treated for *K. pneumoniae* infections" (2).

The global dissemination of drug-resistant organisms is troublesome for medical practitioners because it decreases the available options for appropriate treatment. This contributes to increased patient mortality and morbidity (3). Therefore, it is not surprising that the global spread of drug resistance was recently recognized as a major threat to human health (4). Antimicrobial resistance endangers some of the accomplishments of modern medicine; e.g., in a world without antibiotics, it would be difficult to provide effective chemotherapy to cancer patients, since most will die due to opportunistic multidrug-resistant (MDR) infections.

This pandemic comes at a time when Moore's law has enabled the analysis of large amounts of sequencing data, enabling molecular epidemiologists to study antimicrobial resistance (5). The analysis of such data will enable bioinformaticians to determine the global dissemination of clones and plasmids responsible for antimicrobial resistance in ways that were not feasible 10 years ago. Hopefully, such new insights can yield urgently needed clues on how to prioritize tactics for limiting the global spread of multidrug resistant *Enterobacteriaceae*.

"Eminent or successful" bacterial clones are a powerful source for the propagation of antimicrobial-resistant genetic components (i.e., genes, integrons, transposons, and plasmids) (6). They are able to provide stable platforms for the maintenance and propagation of genes responsible for antimicrobial resistance and have played an essential role in the recent global emergence of multidrug resistance among Gram-negative organisms, especially the *Enterobacteriaceae*.

Plasmids are extrachromosomal elements of circular DNA present in bacteria, which replicate independently of the host genome. The horizontal transfer of plasmids containing resistance genes is an essential mechanism for the dispersion of antimicrobial resistance (7). This free movement of plasmid-borne genes responsible for drug resistance has also been central to the recent and rapid global increase in antimicrobial resistance (8). Antimicrobial resistance plasmids can broadly be divided into 2 main groups, namely, the narrow-host-range group, which most often belongs to incompatibility group F (IncF), and the broad-host-range group, which belongs to the IncA/C, IncL/M, and IncN. Broad-host-range plasmids can easily be transferred between dif-

ferent species, while narrow-host-range plasmids tend to be restricted to species (9). Epidemic resistance plasmids belonging to IncF with divergent replicon types (e.g., FIA, FIB, and FII) have the ability to acquire resistance genes and then rapidly disseminate among *Enterobacteriaceae*, especially among certain clones within species (9).

The focus of molecular epidemiologists when investigating antimicrobial-resistant bacteria has been on analyzing chromosomal DNA, because the initial spread of successful clones did not necessarily require an in-depth investigation of the role that plasmids played in the dissemination of drug resistance genes. Molecular and bioinformatics tools for analyzing large amounts of plasmid DNA combined with refined techniques for large-scale chromosomal analysis will likely provide a better understanding of the ceaseless movement of drug-resistant genes throughout the microbial world (5).

The pandemics caused by multidrug-resistant *E. coli* and *K. pneumoniae* (including fluoroquinolone-, cephalosporin-, and carbapenem-resistant isolates) are due mostly to the global dissemination of certain high-risk clones, namely, *E. coli* sequence type 131 (ST131) and *K. pneumoniae* ST258. There is a strong relationship of *E. coli* ST131 and *K. pneumoniae* ST258 with IncF epidemic plasmids containing FIA and FII replicon types. The reasons for the particular success of these high-risk clones and their association with certain epidemic resistance plasmids are uncertain. However, their ability to spread swiftly is beyond dispute.

This review provides insights into how antibiotic resistance evolves and spreads in bacterial populations and attempts to highlight recent information about the continuous interplay between bacterial clones and antimicrobial resistance plasmids. This article expands the knowledge regarding the role, the importance, and the interdependence of high-risk clones and epidemic plasmids in global spread among multidrug-resistant *Enterobacteriaceae*. It also provides an overview on rapid laboratory methods that will aid clinicians and infection control measures in combating the spread of high-risk clones.

EXPANDED-SPECTRUM β-LACTAMASES

Overview

Antibiotic therapy with cephalosporins that contain the oxyimino side chain (i.e., cefotaxime, ceftazidime, ceftriaxone, and cefepime) is considered one of the choice treatments for serious infections due to *Klebsiella* spp. and *E. coli*. Moreover, carbapenem-resistant *E. coli* and *Klebsiella* spp. are troublesome for medical physicians, since these drugs are often the last efficient treatment left for serious infections (1).

β-Lactamases are the most common cause of resistance to various β-lactam agents. Several different schemes have traditionally been used for the classification of β-lactamases. These enzyme can be divided into either classes A, B, C, and D (referred to as the molecular or Ambler classification based on amino acid sequences) or groups 1, 2, and 3 (referred to as the functional or Bush-Jacoby classification based on substrate and inhibitor profiles). Class A, C, and D β-lactamases are serine enzymes, while class B β-lactamases require divalent zinc ions for their activity (10). Group 1 is referred to as serine cephalosporinases, and group 2 is referred to as serine β-lactamases, which includes penicillinases and broad-spectrum enzymes, while group 3 is also called metallo-β-lactamases (MBLs) (10).

For the sake of simplicity, we use the term "expanded-spectrum" β-lactamases in this review article to stipulate those enzymes with activity against the cephalosporins with oxyimino side chains and/or the carbapenems. These enzymes consist of the class C plasmid-mediated or imported AmpC β-lactamases (e.g., CMY types), class A extended-spectrum β-lactamases (ESBLs) (e.g., CTX-M, SHV, and TEM types), and carbapenemases (e.g., KPC types [class A]; MBLs, e.g., VIM, IPM, and NDM types [class B]; and the oxacillinases, e.g., OXA-48-like enzymes [class D]). The production of expanded-spectrum β-lactamases causes nonsusceptibility to various β-lactam agents, and *Enterobacteriaceae* with these enzymes are often coresistant to a variety of other classes of antibiotics. The most common global type of ESBL is the CTX-M β-lactamases, while the NDM, OXA-48, and KPC β-lactamases are the most frequent carbapenemases among nosocomial and community isolates of *Enterobacteriaceae* (11). Infections with ESBL- and carbapenemase-producing *Enterobacteriaceae* in the developed world are sometimes associated with travel to a region of endemicity (12). The features of the expanded-spectrum β-lactamases are outlined in Table 1.

CTX-M β-Lactamases

ESBLs provide resistance to most of the β-lactam drugs and are inhibited by certain β-lactamase inhibitors (13) (see Table 1 for a summary on which *Enterobacteriaceae* produce these enzymes and the classification, spectrum of activity, inhibition properties, types, regions of endemicity, and molecular epidemiology of ESBLs). The SHV or TEM types of ESBLs were common during the 1980s and 1990s (13), while the CTX-M types became prominent since 2000 (14). Currently, the CTX-M enzymes are present in various types of bacteria from all continents, being especially frequent in *E. coli* (15).

The CTX-M types belong to the molecular class A or functional group 2be β-lactamases and include at least six lineages (i.e., CTX-M-1-like, CTX-M-2-like, CTX-M-8-like, CTX-M-9-like, CTX-M-25-like, and KLUC-like) that differ from each other by ≥10% amino acid homology (14). The association of plasmids carrying $bla_{\text{CTX-M}}$ that belong to incompatibility groups IncF, IncN, and IncK with certain insertion sequences (ISs) (e.g., IS*Ecp1* or IS*CR1*) is able to capture and mobilize $bla_{\text{CTX-M}}$ genes effectively among members of the *Enterobacteriaceae*. IS elements can also act as strong promoters for the high-level expression of $bla_{\text{CTX-M}}$ (16) (Table 1). CTX-M-15 is the most universal type of ESBL among *E. coli* isolates and has been associated with the presence of a clone named ST131 (17). It seems that this clone or sequence type accounted for the global distribution and increase in the prevalence of *E. coli* with $bla_{\text{CTX-M-15}}$ during the early to mid-2000s (more details are provided in the high-risk clone section, below).

E. coli strains with CTX-M enzymes are responsible for nosocomial and community UTIs, BSIs, and IAIs (14), and risk factors include the preceding use of antibiotics and visiting certain regions of endemicity (18). The worldwide dissemination of *E. coli* with CTX-M enzymes has been very efficient and involved health care settings, community, livestock, companion animals, wildlife, and the environment (19). Studies have also shown high transmission rates of *E. coli* with $bla_{\text{CTX-M}}$ genes within households (20).

The CTX-M pandemic significantly contributed to the rapid global increase in the rate of cephalosporin resistance among *Enterobacteriaceae* with subsequent increased usage of the carbapenems for the medication of infections due to these MDR bacteria.

TABLE 1 Characteristics of *Enterobacteriaceae* that produce expanded-spectrum β-lactamases

Enzyme	*Enterobacteriaceae* member	Class	Example(s)	Spectrum of activity	Agent inhibited	Area(s) of endemicity	Molecular epidemiology
Extended-spectrum β-lactamases	*E. coli* *K. pneumoniae* Others (rare)	A	CTX-M-14, -15, others TEM, SHV	Penicillins Cephalosporins Monobactams	Clavulanic acid Tazobactam Sulbactam	Worldwide	ST131 IncF plasmids IS*Ecp1*
Plasmid-mediated or imported AmpC β-lactamases	*K. pneumoniae* *E. coli* *Salmonella* spp. Others (rare)	C	CMY, FOX, ACT, MOX, DHA	Penicillins Cephalosporins (not cefepime) Cephamycins Monobactams	Cloxacillin Boronic acid Avibactam	Worldwide	IncA/C, other plasmids
Metallo-β-lactamases	*K. pneumoniae*	B	NDM-1	Penicillins	Metal chelators, e.g., EDTA, dipicolinic acid	Japan (IMP), Taiwan (IMP), Indian subcontinent (NDM), Balkan states (NDM)	IncA/C, IncN, other plasmids
	E. coli Others (rare)		IMP, VIM	Cephalosporins Cephamycins Carbapenems		Greece (VIM)	Class I integrons
KPC carbapenemases	*K. pneumoniae* *Enterobacter* spp. *E. coli* Others	A	KPC-2, -3 Others	Penicillins Cephalosporins Cephamycins Carbapenems	Clavulanic acid (weak) Tazobactam (weak) Boronic acid Avibactam	USA, Greece, Italy, Israel, China, Brazil, Colombia, Argentina	Tn*4401* IncFII plasmids CC258
OXA β-lactamases	*K. pneumoniae* *E. coli* Others (rare)	D	OXA-48, OXA-163 OXA-181 OXA-204 OXA-232	Penicillins Temocillin β-Lactamase inhibitor combinations Carbapenems	NaCl	Turkey North Africa (Morocco, Tunisia)	Tn*1999* IncL/M plasmids

Unfortunately, CTX-M-producing *E. coli* isolates are often coresistant to various antibiotic classes, which include co-trimoxazole, the aminoglycosides, and the fluoroquinolones (15). This has important clinical implications because some of these drugs (e.g., co-trimoxazole) are popular oral treatment options for community-acquired uncomplicated lower UTIs. Fortunately, fosfomycin, amdinocillin, and nitrofurantoin retain sufficient activity against a high percentage of *E. coli* isolates with $bla_{\text{CTX-M}}$ genes (16).

CTX-M-producing *E. coli* is an important component among global multidrug-resistant bacteria and should be regarded as a major target for surveillance, infection control, and fundamental investigations in the field of antimicrobial drug resistance (21).

AmpC β-Lactamases or Cephalosporinases

Enterobacteriaceae with AmpC β-lactamases are important causes of cephalosporin and cephamycin resistance (see Table 1 for a summary on which *Enterobacteriaceae* produce imported or plasmid-mediated class C cephalosporinases and the classification, spectrum of activity, inhibition properties, types, regions of endemicity, and molecular epidemiology of plasmid-mediated AmpC β-lactamases). Since high-risk clones and epidemic plasmids do not play important roles in the global dissemination of AmpC enzymes, we refer the reader to some excellent review articles on this topic (22–24).

KPC β-Lactamases

The most clinically significant class A carbapenemases are the KPC (i.e., *Klebsiella pneumoniae* carbapenemase) types (25) (see Table 1 for a summary on which *Enterobacteriaceae* produce these enzymes and the classification, spectrum of activity, inhibition properties, types, regions of endemicity, and molecular epidemiology of KPCs). KPC-2 and -3 are the most prevalent isoenzymes among KPCs, and bacteria with these β-lactamases are nonsusceptible to a variety of β-lactam drugs, including the majority of β-lactamase inhibitor combinations (26). KPC enzymes are especially prevalent in *Klebsiella* spp. and are found to a lesser extent in *Enterobacter* spp. (25).

Several hospital outbreaks, most often due to *K. pneumoniae* with $bla_{\text{KPC-2}}$ and $bla_{\text{KPC-3}}$, have been reported in North America (especially the United States), South America (Colombia and Argentina), Europe (Greece, Italy, and Poland), Asia (China), and the Middle East (Israel) (26–28). Regions where KPC-producing bacteria are endemic are shown in Table 1 (28). *K. pneumoniae* ST258 isolates with $bla_{\text{KPC-2}}$ and $bla_{\text{KPC-3}}$ have significantly contributed to the worldwide distribution of this resistance trait (more details are provided in the section on high-risk clones, below) (28).

Plasmids with bla_{KPC} in association with the mobile element Tn*4401* are responsible for the effective spread of these genes among different types of *Enterobacteriaceae* (29) and, with other antibiotic resistance determinants on the same plasmid, provide an easy mechanism for carbapenemase genes to effectively spread as hitchhiker genes, even in the absence of carbapenem selection (30). *Enterobacteriaceae* with bla_{KPC} are often multidrug resistant to classes such as the aminoglycosides, fluoroquinolones, and co-trimoxazole (31).

NDM β-Lactamases

The MBL designated NDM was described for *K. pneumoniae* and *E. coli* from Sweden during the late 2000s. This patient was previously admitted to a New Delhi hospital in India (32) (see Table 1 for a summary of which *Enterobacteriaceae* produce these enzymes and the classification, spectrum of activity, inhibition properties, types, regions of endemicity, and molecular epidemiology of NDMs). Patients visiting certain high-risk regions and then returning to their respective home countries with NDM-producing bacteria have been described, and these enzymes are some of the most common carbapenemases identified in countries such as Canada, the United Kingdom, and France (11). Since high-risk clones and epidemic plasmids do not seem to play important roles in the global dissemination of NDMs, we refer the reader to some excellent review articles and recent reports on this topic (11, 33–36).

OXA-48-Like β-Lactamases

The molecular class D β-lactamases are commonly referred to as OXAs and comprise >400 enzymes, with some variants that possess carbapenemase activity (also referred to as carbapenem-hydrolyzing class D β-lactamases [CHDLs]) (37) (see Table 1 for a summary of which *Enterobacteriaceae* produce these enzymes and the classification, spectrum of activity, inhibition properties, types, regions of endemicity, and molecular epidemiology of CHDLs in *Enterobacteriaceae*). CHDLs are common in *Acinetobacter* spp., but the OXA-48-like types of CHDLs are most often encountered among the *Enterobacteriaceae* (37, 38). OXA-163 is different from other OXA-48-like enzymes in that this enzyme has the ability to hydrolyze the oxyimino cephalosporins. *Enterobacteriaceae* with OXA-163 are common in certain South American countries, especially Argentina (39). Since high-risk clones and epidemic plasmids do not seem to play an important role in the global dissemination of OXA-48 derivatives, we refer the reader to some excellent review articles on this topic (37, 38).

INTERNATIONAL MULTIDRUG-RESISTANT HIGH-RISK CLONAL LINEAGES

Various definitions have been used to describe the criteria for resistance among MDR *Enterobacteriaceae*. We use the recent definition of MDR *Enterobacteriaceae* that was adopted by the European Center for Disease Prevention and Control (40). This definition states that a MDR *Enterobacteriaceae* isolate is nonsusceptible to at least 1 drug in >3 antimicrobial categories, including aminoglycosides, cephalosporins (divided into 3 groups), cephamycins, antipseudomonal penicillins with β-lactamase inhibitors, penicillins, penicillins with β-lactamase inhibitors, monobactams, carbapenems, folate pathway inhibitors, glycylcyclines, fluoroquinolones, phenicols, phosphonic acids, polymyxins, and tetracyclines.

A bacterial clone refers to the progeny of one bacterial cell through asexual reproduction, implying that the same clonal lineage consists of very closely related isolates that have recently diverged from a common ancestor (41). However, bacterial genomes are plastic and are subjected to genome rearrangements (i.e., deletions and insertion sequences, etc.) and, to various extents, to localized recombinational events. Thus, bacterial isolates assigned to the same clone may not be identical, as recent descendants of the same common ancestor may differ somewhat in genotype (42). Therefore, the strict definition of a clone tends to be loosened slightly in bacteriology, and clones are defined as isolates

that are indistinguishable or highly similar to each other, as identified by using a particular molecular typing procedure.

It is important to take into account that the identification of clones depends very much on the molecular typing technique used. The most common molecular techniques currently used by molecular epidemiologists to determine if isolates are clonally related are multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), PCR typing (such as enterobacterial repetitive intergenic consensus [ERIC] or randomly amplified polymorphic DNA [RAPD] methods), and multilocus variable-number tandem-repeat analysis (MLVA) (43). PFGE is a technique with some of the highest levels of discrimination for typing of bacteria, and in the mid-1990s, Tenover and colleagues provided guidelines to define clones or clusters (i.e., possibly related with 4 to 6 band differences) (44). MLST and MLVA are less discriminatory than PFGE, and clones identified by these techniques often have different pulsotypes; e.g., an international collection of *K. pneumoniae* ST258 strains (identified by using MLST) consisted of 4 different pulsotypes that showed a distinctive geographical distribution (45).

In this article, the term clone refers to any bacterium propagated from a single colony isolated at a specific time and place showing common phylogenetic origins. This implies that such isolates have similar traits, as determined by methods (e.g., biochemical and molecular typing methods) indicating that they belong to the same group or lineage and possess a common ancestor. This implies that whole-genome sequencing will provide all of the information necessary to identify clones (43). The term clone has also become useful in molecular epidemiology, particularly in the study of possible relationships between isolates from different geographical areas. It has become well recognized that not all isolates of pathogenic species are necessarily equal and that in a typical pathogenic species, a small number of clones, clusters, or lineages are greatly overrepresented among those isolates recovered from particular types of infection (46).

Short of whole-genome sequencing, MLST has often been used to type several members of the *Enterobacteriaceae*, especially *E. coli* and *K. pneumoniae*. This genotyping method assigns all isolates that are related to each other to a certain sequence type or strain type using a numerical system (e.g., ST1 and ST2, etc.). Two standardized MLST schemes are widely used to type *E. coli* (e.g., Achtman and Pasteur schemes), while the Pasteur MLST scheme is often utilized for *K. pneumoniae* (for more details on MLST, including the Achtman and Pasteur MLST schemes, see the section on laboratory methods, below).

As described previously, an eminent or successful bacterial clone is a powerful source for the propagation of genetic antimicrobial-resistant components (i.e., genes, integrons, transposons, and plasmids) (6). Drug-resistant determinants are provided to the offspring in a vertical fashion, and such eminent or high-risk clones increase the prevalence of antibiotic resistance by their enhanced ability to survive and reproduce efficiently. Moreover, due to the ability of high-risk clones to survive for long periods of time, they also play import roles in the horizontal transfer of drug resistance determinants to other bacteria, acting as efficient donors and recipients.

International multidrug-resistant high-risk clones have a global distribution and can remain viable for prolonged time periods in diverse areas (47). High-risk clones have acquired certain adaptive traits that increase their pathogenicity and survival skills, which is accompanied by the acquisition of antibiotic resistance determinants. These clones have the tenacity and flexibility to accumulate and then provide resistance and virulence genes to other isolates. High-risk clones have contributed to the spread of global multidrug resistance through the transmission of different types of genetic platforms, including plasmids, and resistance genes among Gram-negative bacteria (6). Examples of some multidrug-resistant high-risk clones among *Enterobacteriaceae* are *E. coli* ST38, ST69, ST131, ST155, ST393, ST405, and ST648 and *K. pneumoniae* ST14, ST37, ST147, and ST258 (47).

High-risk clones most likely possess some types of biological factors that lead to increased "fitness," providing these strains with a Darwinian edge over other isolates of the same species (46). Such advantages will provide them with the abilities to outcontest other bacteria and become the principal part of the bacterial populace in that area. This will provide these clones with increased opportunities to spread as well as time to acquire antimicrobial drug resistance determinants from other bacteria. The spread of multidrug-resistant high-risk clones is especially facilitated by the selective pressures of antimicrobial drugs present in health care settings and used during food animal husbandry.

To qualify as an international multidrug-resistant high-risk clone, clones must possess the following characteristics (47): (i) a global distribution, (ii) an association with various antimicrobial resistance determinants, (iii) the ability to colonize and persist in hosts for long time intervals (>6 months), (iv) the ability for effective transmission among hosts, (v) enhanced pathogenicity and fitness, and (vi) the ability to cause severe and/or recurrent infections.

ESCHERICHIA COLI SEQUENCE TYPE 131

Initial Studies Pertaining to *E. coli* ST131

Extraintestinal pathogenic *E. coli* (ExPEC) is an important cause of nosocomial and community infections in humans (especially UTIs, BSIs, and IAIs) (48). Resistance to certain antimicrobial classes (especially the fluoroquinolones and cephalosporins) among ExPEC isolates was rare before 2000 but has increased exponentially since the mid- to late 2000s (49).

During the mid-2000s, molecular typing of *E. coli* with $bla_{\text{CTX-M-15}}$ using PFGE in the United Kingdom and Canada identified different pulsotypes among these isolates. These pulsotypes were related but showed <80% similarity and did not fulfill the Tenover criteria for relatedness (44). The pulsotypes from the United Kingdom were named clones A to E, while the Canadian pulsotypes were named clones 15A and 15AR (related to A) (50, 51). In 2008, investigators from Spain and France collected *E. coli* with $bla_{\text{CTX-M-15}}$ from different countries, including Spain, France, Canada (including isolates from PFGE clusters 15A and 15AR), Portugal, Switzerland, Lebanon, India, Kuwait, and South Korea (52, 53). Both groups of investigators performed MLST on this collection, and ST131 was present in all the countries that provided isolates for the studies (52, 53). Clusters A to E from the United Kingdom were later identified as also belonging to ST131 (54). All isolates of multidrug-resistant *E. coli* ST131 typed as belonging to serotype O25b:H4 belonged to phylogenetic group B2 and harbored IncF types of plasmids containing $bla_{\text{CTX-M-15}}$ (52, 53). The results of these two initial studies suggested that ST131 had appeared simultaneously in the community setting and seemingly unrelatedly in separate areas of the globe without any obvi-

ous link between the patients. The results suggested that the appearance of ST131 was due to a contaminated common source (e.g., water or food) and/or was being introduced into different regions via travelers returning to their respective home countries.

Subsequently, ST131 with CTX-M-15 was described in the United Kingdom (54), the rest of Canada (55), Italy (56), Turkey (57), Croatia (58), Japan (59), the United States (60), South Africa (61), Brazil (62), and Norway (63). Healthy Parisians in France were also found to be rectally colonized with *E. coli* ST131 without CTX-M β-lactamases (64). Interestingly, ST131 showed a high prevalence among fluoroquinolone-resistant ESBL-negative *E. coli* urinary isolates obtained from Canada (65). It quickly became apparent that *E. coli* ST131 strains with $bla_{\text{CTX-M-15}}$ were present among global isolates recovered from the community setting (66); hospitals (67); long-term-care settings, including nursing homes (68); and, interestingly, companion animals (69).

Molecular epidemiologists were intrigued with this unexpected global appearance of *E. coli* ST131 mostly in the community setting, apparently in the same time frame but without any obvious connections between these patients. The first studies to provide insight into this fascinating issue occurred in Calgary, Canada, and Auckland, New Zealand. The Auckland study reported a series of patients that presented to a local health care facility with community-onset UTIs due to CTX-M-15-producing *E. coli*. This was the first encounter of those authors with *E. coli* with $bla_{\text{CTX-M-15}}$, and of special interest was that all the patients had recently visited India as tourists or emigrated from India (70). The population-based surveillance study from Calgary showed that recent travel (i.e., within the previous 3 months) to the Indian subcontinent, the African continent, and the Middle East was linked with notable high risks for developing community-onset UTIs (including upper UTIs) with CTX-M-producing *E. coli* (71). A follow-up study by the same investigators in Calgary showed that these UTIs in returning travelers were due mostly to ST131 strains with $bla_{\text{CTX-M-15}}$ (72).

A retrospective molecular epidemiology investigation from Calgary, Canada, during 2000 to 2007 highlighted that *E. coli* ST131 with $bla_{\text{CTX-M-15}}$ had appeared during the mid-2000s as an important etiology of community-acquired health care-associated BSIs, especially during the closing period of that study (66). Community-onset cases were categorized into community-acquired or health care-associated infections (73). Health care-associated infections included patients attending community clinics, patients from long-term-care facilities, or patients with infections that occurred within the first 48 h of admittance to an acute-care facility. *E. coli* ST131 was more likely to be multidrug resistant, to possess the *aac(6′)-Ib-cr* gene, and to trigger community-onset BSIs, especially those secondary to upper UTIs.

It seems that the unexpected and sudden global appearance of *E. coli* with $bla_{\text{CTX-M-15}}$ in the community was related to the emergence of ST131, which was then followed by the subsequent expansion of this multidrug-resistant clone. Global surveillance studies have shown that >50% of *E. coli* isolates from community specimens obtained on the Indian subcontinent contain ESBLs (74). Therefore, it is possible that the rectal colonization of travelers returning from certain regions of endemicity could possibly have been critical for the early global spread of ST131 (12). However, it seems that international travel is not essential for the current *E. coli* ST131 global pandemic: a follow-up study during 2011 from the Calgary region of Canada showed that international travelers to regions of endemicity such as India had rectal colonization rates for *E. coli* ST131 similar to those of nontravelers (75).

The sudden worldwide appearance of *E. coli* with CTX-M-15 was most likely due to the acquisition of certain IncF epidemic plasmids harboring $bla_{\text{CTX-M-15}}$ by a high-risk clone such as ST131. The combination of drug-resistant epidemic plasmids harboring multiple antibiotic resistance determinants with the increased fitness of the high-risk clone due to several virulence factors enabled ST131 to move effortlessly between the community, different hospitals, and long-term-care facilities. The horizontal transfer of plasmids with $bla_{\text{CTX-M-15}}$ between *E. coli* ST131 and non-ST131 isolates was also described in certain areas such as France, Spain, and Portugal (76).

Investigators from the United States and Canada characterized ~200 ExPEC isolates with various levels of co-trimoxazole and fluoroquinolone resistance. These urine isolates originated from Manitoba (Canada) and were acquired during the early 2000s (65). The overall prevalence rate of ST131 was 23%, and of special interest was that 99% of isolates of this clone were fluoroquinolone resistant, but only 2% were resistant to the cephalosporins (65). Results from this study provided some evidence that *E. coli* ST131 was initially a fluoroquinolone-resistant clone and later acquired plasmids harboring $bla_{\text{CTX-M}}$ genes.

One of the first *in vitro* studies that investigated *E. coli* ST131 for the presence of different virulence factors related to ExPEC was performed by Johnson and colleagues (77). Those researchers investigated 127 ExPEC isolates obtained in the United States during 2007 from the SENTRY and MYSTIC surveillance programs. The overall prevalence rate of ST131 was 17%, and this clone was specifically associated with multidrug resistance and had a significantly higher overall virulence score than did non-ST131 *E. coli* isolates. It is interesting to note that the presence of some virulence factors, such as the uropathogenic specific protein (*usp*), outer membrane protein (*ompT*), secreted autotransporter toxin (*sat*), aerobactin receptor (*iutA*), and pathogenicity island marker (*malX*), corresponded specifically to ST131. The results of that study demonstrated that ST131 showed a typical virulence profile (compared to those of other ExPEC isolates), and it seemed that multidrug resistance in association with some virulence factors may be essential for the ecological triumph of this clone. Subsequent studies from different investigators have shown similar virulence profiles among ST131 isolates (19). Of special interest is the low prevalence of classical ExPEC-associated virulence factors (i.e., *pap* [P fimbriae], *cnf1* [cytotoxic necrotizing factor], and *hlyD* [alpha-hemolysin]) among isolates that belong to ST131. The precise roles of these virulence factor genes remain to be elucidated; however, it is possible that certain recognized virulence factors possibly play a part in the fitness and adaption of ST131 (78). Virulence factor genes such as *sat*, *iutA*, *malX*, *usp*, *iha*, *hra*, and *ompT* might increase the ability of *E. coli* ST131 to efficiently colonize human tissues rather than being responsible for virulence and causing infection (79).

As described above, PFGE analysis showed that *E. coli* ST131 is not a single entity and can be separated into various pulsotypes. Molecular surveillance has demonstrated that ST131 isolates with similar PFGE clusters are present in different regions or countries (53, 76, 80). A study that investigated a global collection of ST131 isolates (isolates were obtained from 9 countries in North America, South America, Europe, the Middle East, Asia, and Oceania) identified 15 distinct pulsotypes; nearly half of the ST131 isolates

belonged to four distinctive clusters showing a global distribution (81). However, ST131 isolates with less related pulsotypes can be present within the same location (80, 81). Johnson and colleagues genotyped >500 global ST131 ExPEC isolates obtained from the late 1960s until 2010 from multiple materials, including materials from humans, animals, and the environment, by PFGE (82). This study proved that ST131 is highly divergent when PFGE is used to analyze this clone. Those researchers also showed that a certain number of closely related pulsotypes preside internationally, and these so-called "high-frequency" clusters appeared only during the last 10 years or so (82). A retrospective molecular surveillance study from Calgary, Canada, from 2000 until 2010 identified a prominent closely related pulsotype of ST131 causing a significant increase in the number of ESBL-producing *E. coli* strains isolated from blood cultures (83). This cluster became especially dominant during 2009 and 2010.

Although ST131 first came to the attention of the medical community because of its particular association with ESBL-producing *E. coli*, especially ExPEC with $bla_{\text{CTX-M-15}}$, it became apparent during the late 2000s that most isolates of this clone were initially ESBL negative and resistant to the fluoroquinolones (19). Global surveillance studies have shown that ST131 was a fluoroquinolone-resistant clone during the early 2000s and became strongly associated with $bla_{\text{CTX-M-15}}$ toward the end of the 2000s (84).

Recent Developments Pertaining to *E. coli* ST131

The presence of ST131 among human clinical *E. coli* isolates varies by geographic region and host population. Recent surveillance studies have shown that the overall prevalence ranges from ~10% to nearly 30% of all *E. coli* clinical isolates (84).

Epidemiology and clinical issues. Like other ExPEC isolates, ST131 causes a variety of extraintestinal infections, including BSIs, pneumonia, UTIs, IAIs, and wound infections. ST131 is strongly associated with community-onset infections, especially in patients with regular contact with health care settings. This was initially illustrated by infections due to ESBL-producing isolates (66, 83) and more recently by a population-based cohort study from the United States (85). Interestingly, *E. coli* ST131 has not been responsible for large nosocomial outbreaks in intensive care or high-care units.

Infections with ST131 are most common among the elderly, and this sequence type has a high prevalence among residents of nursing homes and long-term-care facilities (85). ST131 isolates have also been detected in nonhuman sources such as companion animals, other animals, food sources, and the environment (19). However, the prevalence of ST131 is substantially higher among humans than in animals, produce, or the environment (86). It seems that the ST131 pandemic is primarily a human-based phenomenon and that this clone has somehow successfully adapted to human hosts. However, global studies regarding the prevalence of ST131 among nonhuman hosts are lacking.

There is a clear association between previous antibiotic consumption and colonization followed by infection due to ESBL-producing *E. coli*, including ST131 isolates. Antimicrobial agents such as the fluoroquinolones and cephalosporins have most often been implicated in selection for colonization and subsequent infections due to *E. coli* ST131 (85). The high prevalence of ST131 in community environments is associated with the extensive use of antimicrobial agents (i.e., the outpatient setting of health care settings, nursing homes, and long-term-care centers). This indirectly supports the notion that antimicrobial usage played a role in the selection of ST131. However, the underlying basis for why *E. coli* ST131 has not been implicated in nosocomial outbreaks remains an enigma.

Population structure. The most prevalent lineage within ST131 is named *fimH30* because it contains the H30 variant of the type 1 fimbrial adhesin gene *fimH* (87). The *fimH30* lineage was first identified among ST131 isolates obtained from different geographical regions and sources by means of subtyping of >1,000 historical and recent ExPEC isolates (both ST131 and non-ST131) using a combination of typing strategies, including sequencing of the *fimH*, *gyrA*, and *parC* genes; MLST; and PFGE (87). Johnson and colleagues observed that the *fimH30* ST131 lineage consisted of nearly 70% of recent fluoroquinolone-resistant *E. coli* isolates, while it remained infrequent (i.e., <1%) among fluoroquinolone-susceptible ST131 isolates. The ST131 *fimH30* lineage first appeared in the early 2000s, became prominent in the mid-2000s, and then expanded rapidly during the late 2000s. The majority of strains of the *fimH30* lineage have very close genetic similarity, suggesting that they originated from a single *fimH30*-carrying ancestor (88). It seems that the dramatic global emergence of fluoroquinolone-resistant ExPEC ST131has been driven by the clonal expansion and dissemination of the *fimH30* lineage. Additional support for a single ancestor was provided by the linkage of the ST131 *fimH30* lineage with a specific *gyrA* and *parC* allele combination, despite proof for the widespread generation of different *gyrA* and *parC* allele combinations among non-H30 lineages (87). A PCR-based assay that detects *fimH30*-specific single-nucleotide polymorphisms (SNPs) is available to rapidly and cost-effectively detect the H30 lineage (89).

Price and colleagues, using whole-genome sequencing combined with phylogenetic SNP analysis, recently identified two important sublineages within the ST131 *fimH30* lineage, called H30-R and H30-Rx, because of their extensive antimicrobial resistance profiles (88). Those authors initially analyzed just over 500 ST131 isolates collected between 1967 and 2011 from different geographic regions by using PFGE and found that the *fimH30* lineage showed significantly different pulsotypes irrespective of whether the isolates were susceptible or resistant to a fluoroquinolone or contained $bla_{\text{CTX-M}}$. These findings suggested that fluoroquinolone-resistant mutations and CTX-M genes were horizontally acquired over a long period of time. However, when 105 of the isolates with significantly different pulsotypes underwent next-generation sequencing (NGS) combined with SNP analysis to reconstruct the phylogeny of ST131, the fluoroquinolone-resistant *fimH30* lineage and strains carrying *fimH30* with $bla_{\text{CTX-M-15}}$ formed a tight cluster irrespective of whether their pulsotypes were different. Moreover, when those authors performed additional sequencing with increased coverage, they found that within the fluoroquinolone-resistant *fimH30* lineage, isolates with $bla_{\text{CTX-M-15}}$ formed a distinct cluster, named the H30-Rx sublineage, that was separated from ESBL-negative fluoroquinolone-resistant *fimH30* isolates by 3 core genome SNPs (88).

The phylogenetic analysis undertaken by Price and colleagues indicated that the ST131 *fimH30* lineage comprised a series of nested sublineages that were most likely derived from a single common fluoroquinolone-susceptible *fimH30* ancestor (84). These investigators named these sublineages as follows: the H30-R sublineage or subclone, which shows fluoroquinolone resistance without $bla_{\text{CTX-M-15}}$, and the H30-Rx sublineage or subclone,

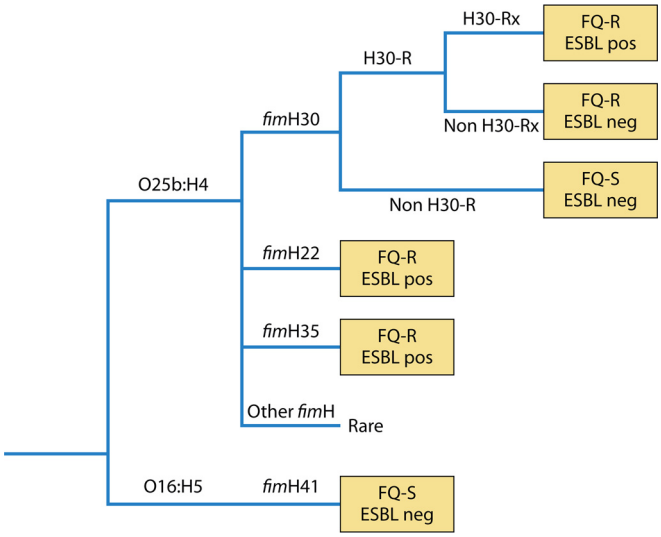

FIG 1 Population structure of the *Escherichia coli* ST131 *fimH30* lineage, H30 sublineages, and other ST131-associated lineages. FQ-R, fluoroquinolone resistant; FQ-S, fluoroquinolone sensitive.

which shows fluoroquinolone resistance with $bla_{\text{CTX-M-15}}$ (88). This clonal structure results in a succession of antimicrobial resistance among the *fimH30*-associated lineages, from the most susceptible H30 lineage (fluoroquinolone susceptible and CTX-M negative), to the more resistant H30-R sublineage (fluoroquinolone resistant and CTX-M negative), to the most extensively resistant H30-Rx sublineage (fluoroquinolone resistant and CTX-M positive). Whole-genome sequencing combined with SNP analysis indicated that the clonal expansion of the ST131 *fimH30* lineage is the most dominant and important vehicle for the increasing global prevalence of fluoroquinolone resistance and $bla_{\text{CTX-M-15}}$ among ExPEC strains. These results were later independently confirmed by Petty and colleagues (90). The population structure of the *E. coli* ST131 *fimH30* lineage, H30 sublineages, and other ST131-associated lineages is illustrated in Fig. 1.

The prevalences and distributions of the *fimH30* lineage and the H30-R and H30-Rx ST131 sublineages were initially described for populations within the United States. A study of *E. coli* clinical isolates from various U.S. veterans centers obtained during 2011 showed that the prevalence of ST131 was only 7% among fluoroquinolone-susceptible ExPEC isolates, 78% among fluoroquinolone-resistant ExPEC isolates, and 64% among ESBL-producing *E. coli* isolates; the ST131 *fimH30* lineage accounted for 12.5% of fluoroquinolone-susceptible ST131 isolates, which increased to 95% of fluoroquinolone-resistant and 98% of ESBL-producing ST131 isolates (89). Similarly, in a case-control study conducted in the region of Chicago, IL, >50% of ESBL-producing ExPEC isolates were identified as ST131; 98% were further characterized as *fimH30*, while 92% were characterized as H30-Rx (91). These studies supported the notion that there is a strong alliance linking the H30-Rx sublineage with $bla_{\text{CTX-M-15}}$. However, not all ST131 isolates with CTX-M-15 are members of the H30-Rx sublineage.

In a population-based study of consecutively collected ExPEC isolates in Minnesota, the prevalence of the ST131 *fimH30* lineage was high among elderly patients (i.e., >60 years of age), while infections due to non-H30 ST131 isolates were more common among younger patients (i.e., <2 years of age) (92). In a different multicenter U.S. study that analyzed >1,600 ExPEC isolates, clonotype CH40-30 (which corresponded to the *fimH30* lineage of ST131) was the most prevalent clonotype among all ExPEC isolates and was statistically associated with recurrent or persistent UTIs and the presence of sepsis (93). The next-generation sequencing study by Price and colleagues described above also observed that sepsis was significantly associated with infections due to the H30-Rx sublineage (88).

The first study outside the United States to determine the prevalence and distribution of the ST131 *fimH30* lineage and its respective sublineages was a retrospective population-based surveillance study investigating BSIs due to fluoroquinolone-resistant ExPEC in Calgary, Canada (2000 to 2010) (94). This study demonstrated that the deluge of the H30-Rx sublineage toward the latter part of the study period was responsible for the significant increase in the prevalence of ST131 and fluoroquinolone-resistant ExPEC (94) (Fig. 2). This study recognized the relationship of H30-Rx with primary sepsis and BSIs due to prostate biopsies. This sublineage was also more likely to contain *aac(6′)-lb-cr*.

O16:H5 H41 and other ST131 lineages. As described above, several investigators noticed during the late 2000s that *E. coli* ST131 strains with ESBLs exhibited different pulsotypes (52, 55, 60, 61, 66). Interestingly, some ST131 isolates belonged to a well-defined cluster that was separated from the more prominent O25b:H4 clade and tested negative by Clermont ST131 PCR (170) (for more details on Clermont PCR, see the section on laboratory methods, below). This cluster contained $bla_{\text{CTX-M-14}}$ and was susceptible to the fluoroquinolones, and with additional serotype and *fimH* allele characterization, it tested positive for O16:H5 and belonged to the *fimH41* lineage (81). Of interest, the O16 and O25b serotypes within ST131 were identified as representing different STs by using the Pasteur Institute MLST scheme, but isolates with these different serotypes were recognized as the same ST by the Achtman MLST scheme (84).

The O16:H5 ST131 *fimH41* lineage had distinct combinations of *gyrA* and *parC* alleles, and surveillance data showed that this lineage comprised 1 to 5% of *E. coli* ST131 isolates (95). The *fimH41* lineage is associated with resistance to trimethoprim-sulfamethoxazole and gentamicin (compared to the *fimH30* ST131 lineage), while ESBLs and fluoroquinolone resistance are rarely detected with this lineage. O16:H5 ST131 *fimH41* isolates with $bla_{\text{OXA-48}}$ in the United Arab Emirates and Morocco have recently been described (96). A rapid PCR screening test was developed to detect the *fimH41* lineage and combined the O16 *rfb* gene with the ST131-specific *mdh* and *gyrB* alleles (95) (for more details on this PCR, see the section on laboratory methods, below).

Two additional ST131 lineages with the O25b:H4 serotype but showing significant different pulsotypes from the main *fimH30* cluster were recently reported (81). These isolates belonged to the *fimH22* and *fimH35* lineages and were also associated with $bla_{\text{CTX-M-15}}$, fluoroquinolone resistance, and the presence of *aac(6′)-lb-cr*. O25b:H4 ST131 *fimH22* isolates with bla_{KPC} from Argentina and Colombia were recently described (96). The population structure of the *E. coli* ST131 *fimH30*, *fimH41*, *fimH22*, and *fimH35* lineages as well as the H30-R and H30-Rx sublineages is illustrated in Fig. 1.

Virulence. A Spanish study proposed a new classification system for ST131, where it is classified into four virulence-associated groups (i.e., groups A, B, C, and D) (also named virotypes) (97). Those investigators performed *in vitro* virulence characterization

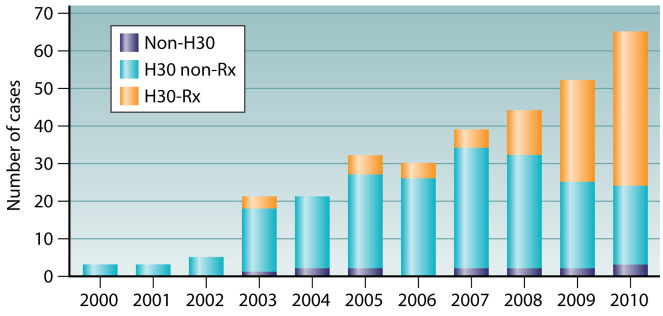

FIG 2 Numbers of isolates of *Escherichia coli* ST131 and the H30-R and H30-Rx sublineages isolated from blood in the Calgary Region from 2000 to 2010. (Adapted from reference 94.)

on a large global collection of ST131 isolates and identified four virulence factors that showed a distinctive distribution among the different virotypes. These virulence factors include *afa* (GenBank accession number FM955459) (Afa/Dr adhesion), *iroN* (catecholate siderophore receptor), *ibeA* (invasion of brain endothelium), and *sat* (secreted autotransporter toxin) (97). Virotypes A and B were related to antibiotic nonsusceptibility, $bla_{\text{CTX-M-15}}$, and *aac(6′)-lb-cr*. Virotype C was present in several countries and was linked to invasive infections compared to other virotypes such as virotypes A and B (97).

A U.S. study described the ExPEC-associated virulence profiles of ST131 and its sublineages (H30-R and H30-Rx) among a large *E. coli* collection from the Midwest near the Great Lakes region (91). Those researchers identified distinctive virulence profiles among these sublineages, with the H30-Rx sublineage having the highest aggregative virulence score (91). Colpan and colleagues showed that the fluoroquinolone-susceptible ST131 *fimH30* lineage had a significantly different virulence profile from that of non-ST131 fluoroquinolone-susceptible isolates. Moreover, as this lineage gained resistance to the fluoroquinolones and acquired ESBLs, isolates tended to progressively accumulate additional virulence factors, especially among the ST131 *fimH30* lineage that acquired CTX-M-15 (89).

***E. coli* ST131 and carbapenemases.** The development of resistance to the carbapenems in community pathogens such as *E. coli* is worrisome to the medical fraternity (49). NDM and OXA-48 are the most prevalent carbapenemases present in nosocomial and community isolates of *E. coli*, while the VIM, IPM, and KPC β-lactamases are not yet commonly encountered in this species (11). Infections with carbapenemase-producing *E. coli* often occur in patients who had recently visited certain regions of endemicity, such as the Indian subcontinent for NDMs and North Africa or Turkey for OXA-48 (12).

Due to the revolutionary worldwide triumph of ST131, carbapenemases among isolates of this clone have been carefully monitored by molecular epidemiologists. The carbapenemase gene bla_{NDM} was first identified in ST131 isolates during 2010 from patients in Chicago and Paris (98, 99). Both patients had previously visited the Indian subcontinent. This was followed by case reports of ST131 with bla_{VIM} from Italy (100); ST131 with bla_{KPC} from Ireland (101), France (102), the United States (103), Italy (104), Taiwan (105), and China (106); ST131 with $bla_{\text{OXA-48}}$ from the United Kingdom (107), Ireland (108), Algeria (109), and Spain (110); and ST131 with bla_{IMP} from Taiwan (111). The largest cluster of ST131 with carbapenemases was recently described in Pittsburgh, PA (112). The authors of that study characterized 20 isolates of KPC-producing *E. coli*: 60% belonged to the ST131 *fimH30* lineage, while the bla_{KPC} plasmid belonged to the IncF type with FII_{k} replicons.

A recent study by the SMART and AstraZeneca global surveillance programs showed that 35% of 116 carbapenemase-producing *E. coli* isolates belonged to ST131, which was associated with fluoroquinolone resistance, the presence of bla_{KPC}, the *fimH30* lineage, and virotype C (96). Genes for ESBLs such as $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$ were also present in some of the ST131 isolates with carbapenemases. ST131 was also isolated from Argentina, China, Colombia, Ecuador, India, Italy, Jordan, Morocco, Panama, Philippines, Puerto Rico, Thailand, Turkey, the United Arab Emirates, the United States, and Vietnam. *E. coli* ST131 with carbapenemases poses a significant new public health risk due to its worldwide distribution and relationship with the dominant *fimH30* lineage.

Does ST131 Qualify as an International Multidrug-Resistant High-Risk Clone?

The pandemic emergence of *E. coli* ST131, and specifically its H30-R and H30-Rx sublineages, occurred over <10 years. It is a remarkable antimicrobial resistance success story rivaling the global pandemics caused by clones within methicillin-resistant *Staphylococcus aureus* (i.e., ST5, ST8, and ST36) and *Streptococcus pneumoniae* (i.e., ST236 and ST320).

Global distribution and prevalence. *E. coli* ST131 was initially described among isolates with $bla_{\text{CTX-M-15}}$ from the following countries: Canada, France, Switzerland, Portugal, Spain, Kuwait, Lebanon, India, and South Korea (52, 53). Subsequently, ST131 was identified among ESBL, non-ESBL, fluoroquinolone-resistant, and fluoroquinolone-susceptible *E. coli* isolates from all corners of the world (19). If investigators decide to determine the presence of ST131 among *E. coli* isolates collected from human sources, they will most likely detect ST131 among their collection. ST131 is still overrepresented among antimicrobial-resistant ExPEC isolates; recent global surveillance showed that ST131 consistently accounts for ~60% to 80% of fluoroquinolone-resistant isolates and 50 to 60% of ESBL-producing isolates but only 0% to 7% of fluoroquinolone-susceptible isolates (84). *E. coli* ST131 has a true global distribution and is present among ExPEC isolates on all continents, except possibly Antarctica.

Association with antimicrobial resistance mechanisms. *E. coli* ST131 was initially described in ExPEC isolates with CTX-M-15 and today is known to also be associated with fluoroquinolone resistance (83, 89, 91). Population genetics indicated that fluoroquinolone resistance in the ST131 *fimH30* lineage is due mostly to *gyrA1AB* and *parC1aAB* mutations in gyrase and topoisomerase IV, respectively (87). The *gyrA1AB* and *parC1aAB* mutations are present in 71% of fluoroquinolone-resistant and in 62% of ESBL-positive *E. coli* isolates from various Veterans Administration hospitals in the United States (89). ST131 is also associated with the presence of the plasmid-mediated quinolone resistance determinant *aac(6′)-lb-cr* (17). This resistance determinant causes decreased susceptibility to the fluoroquinolones ciprofloxacin and norfloxacin as well as resistance to the aminoglycosides tobramycin and amikacin.

Various expanded-spectrum β-lactamases (e.g., CTX-Ms, CMYs, SHV ESBLs, and TEM ESBLs) have been detected in ST131

isolates (19), with CTX-M-15 in combination with TEM-1 and OXA-30 being by far the most common. The presence of carbapenemases is still rare among ST131 isolates, although some enzymes have been reported in different parts of the world (19).

Other resistance determinants that have been characterized in ST131 isolates include *mph*(A) (responsible for resistance to the macrolides), *catB4* (responsible for resistance to chloramphenicol), *tetA* (responsible for tetracycline resistance), *dfrA7* (responsible for trimethoprim resistance), *aadA5* (responsible for streptomycin resistance), and *sul1* (responsible for sulfonamide resistance). Therefore, isolates belonging to ST131 are most often nonsusceptible to the cephalosporins, monobactams, fluoroquinolones, trimethoprim-sulfamethoxazole, and the aminoglycosides (19).

Ability to colonize human hosts. Because intestinal colonization with ExPEC is believed to be a prerequisite for extraintestinal infection, it is possible that the enhanced ability to colonize the intestinal tract is partly responsible for the widespread dissemination of ST131. Several studies have shown that asymptomatic individuals can be rectally colonized with *E. coli* ST131 (19). In a mouse model of intestinal colonization, an ST131 isolate surpassed other commensal *E. coli* isolates mixed in a 1:1 ratio and administered enterally into streptomycin-pretreated mice. Although that study was limited by its evaluation of only a single ST131 isolate, it provided evidence that some ST131 isolates have the ability to efficiently colonize the intestine, bladder, and kidney of mice (113). It is unclear whether the prevalence or duration of intestinal colonization in humans is different for ST131 compared to other *E. coli* isolates. It is also unclear if the dominant *fimH30* ST131 lineage, including the sublineages (i.e., H30-R and H30-Rx), has intestinal colonization abilities different from those of other ST131 lineages (e.g., *fimH41*, *fimH35*, and *fimH22*).

The prevalence of *E. coli* ST131 among rectal isolates of *E. coli* varied considerably with the population studied, geographical regions, host characteristics, the presence of resistance mechanisms, as well as the time frame when the study was conducted: rectal colonization rates of ST131 that varied from 0 to 44% have been reported (19).

Effective transmission among hosts. The transmission of ST131 between different household family members (father to daughter, daughter to mother, and sister to sister) and companion animals (dogs and cats in particular) has previously been documented (19, 84). A study from Switzerland showed that ST131 isolates were more likely to be transmitted between members of the same family than within patients in the hospital environment (20). A French day care center recently reported that 7 children were rectally colonized with ST131 that produced CTX-M-15, suggesting that this clone was effectively transmitted within the day care setting (114).

It seems that *E. coli* ST131 can effectively be transmitted between members of the same family, and this might have played an important role in the emergence and dissemination of this sequence type within the community setting. However, whether ST131 is more efficiently transmitted than other *E. coli* isolates is uncertain and deserves additional studies.

Enhanced pathogenicity and fitness. In several molecular epidemiological studies, ST131 isolates, compared to non-ST131 ExPEC isolates, consistently had a large number of ExPEC-associated virulence factor genes and had significantly high aggregate virulence scores (77, 115). Moreover, within ST131, the *fimH30* lineage and specifically the H30-Rx sublineage had characteristic virulence profiles with higher virulence scores than those of non-H30 ST131 *E. coli* isolates, most likely playing an important role in the overall virulence and fitness of this sublineage (91).

In vitro studies that investigated the maximal growth rate and the ability of ST131 to produce biofilms showed that this clone had a high metabolic potential, most likely by increasing the fitness and ability of ST131 to establish intestinal colonization for long periods of time (116). *In vivo* studies that investigated the virulence potential of ST131 in animal models indicated that this sequence type kills mice (117) but is less virulent than non-ST131 *E. coli* in the *Caenorhabditis elegans* and zebra fish embryo models (118).

Cause of severe and/or recurrent infections. It is unclear if ST131 isolates cause more severe infections than other ExPEC *E. coli* isolates, but clinical epidemiological data suggest that ST131 isolates are more likely to cause upper UTIs than lower UTIs (84). In a series of studies from Australia, ST131 accounted for 30% of pyelonephritis isolates among ExPEC isolates from women, versus only 13% of cystitis isolates and 4% of fecal isolates. Similar prevalence trends were seen among men and children (116). A study from the United Kingdom showed similar results, where the prevalence of ST131 was 21% among bacteremia isolates, compared to only 7% among urinary isolates (119).

Whether ST131 is associated with worse clinical outcomes than other ExPEC isolates is unclear. Some studies suggest that ST131 is more likely to cause persistent or recurrent UTIs (85), while other investigators have found no significant difference in outcomes of infections with ST131 versus other ExPEC isolates (120). When investigators adjusted for host factors, no differences in cure or mortality were found between patients with infections due to ST131 and those with infections due to other ExPEC isolates (93). Of special interest, isolates of the H30-Rx sublineage of ST131 have demonstrated statistical and epidemiological associations with sepsis (88).

In summary, *E. coli* ST131 isolates clearly have all of the essential characteristics that define a high-risk clone (Table 2). In fact, this sequence type might be the quintessential example of an international multidrug-resistant high-risk clone.

KLEBSIELLA PNEUMONIAE SEQUENCE TYPE 258

Initial Studies Pertaining to *K. pneumoniae* ST258

The rate at which carbapenem resistance in *K. pneumoniae* has disseminated globally and the consequences thereof have raised cause for alarm among the medical community at large. To date, bla_{KPC} has been found in >100 different STs, but this pandemic is driven primarily by the spread of KPC-producing *K. pneumoniae* isolates that are members of clonal complex 258 (CC258) (121). CC258 (the founder member is ST292) consists of one predominant ST, namely, ST258, and, to a lesser extent, ST11, ST340, and ST512, which are single-locus variants (SLVs) of ST258 (30, 121). *K. pneumoniae* ST258 is a prototype of a high-risk clone, and recent information about the epidemiology, genetic rearrangement, and evolution of this successful clone has provided insights into the global spread of antimicrobial drug resistance.

K. pneumoniae with bla_{KPC} was first identified in a non-ST258 isolate during 1996 in the Southern United States (122). During the late 1990s to early 2000s, there were sporadic reports of *K. pneumoniae* with bla_{KPC} from the Northeastern United States;

TABLE 2 Characteristics of *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST258 that define them as high-risk clones

Characteristic	Description	
	Escherichia coli ST131	*Klebsiella pneumoniae* ST258
Global distribution	Endemic to all continents except Antarctica	ST258 is endemic to the USA, Israel, Greece, Italy, Poland, and Colombia; ST11 has been reported in China and Brazil; ST512 has been reported in Israel, Italy, and Colombia; ST340 has been reported in Brazil and Greece
Association with various antimicrobial resistance determinants	Various but associated with fluoroquinolone resistance and CTX-M-15 (CTX-M-14 to a lesser extent)	Various but associated with KPC-2 and KPC-3
Ability to colonize and persist in hosts for long periods of time	Rectal colonization for up to 6 mo	Rectal colonization for up to 12 mo
Effective transmission among hosts	Transmission among family members	Successful nosocomial transmission for months after introduction
Enhanced pathogenicity and fitness	Higher aggregate ExPEC-associated virulence scores; high metabolic potential and biofilm production	Unclear
Causes severe and/or recurrent infections	More likely to cause upper UTIs and recurrent UTIs, and the H30-Rx sublineage is associated with sepsis	Mortality rates are higher than with non-ST258 *K. pneumoniae* (most likely due to the patient's underlying conditions)

however, large outbreaks due to related isolates were not described (123). In 2009, the U.S. Centers for Disease and Prevention in collaboration with investigators from Israel performed MLST on *K. pneumoniae* with bla_{KPC}, and they identified ST258 among isolates from the New York area collected during 2005 (124). As time progressed, ST258 was detected in geographically diverse regions of the United States, and in 2009, it became apparent that ST258 was the predominant clone in this country, being responsible for 70% of *K. pneumoniae* isolates with bla_{KPC} obtained from different parts of the country (125). During the mid-2000s, Israel experienced several nosocomial outbreaks of infections due to *K. pneumoniae* with bla_{KPC} that were caused by a clone (identified by PFGE) named clone Q (124). Interestingly, clone Q has a pulsotype similar to that of ST258, present in the United States. This was followed by global reports of ST258 among *K. pneumoniae* isolates with bla_{KPC} from countries such as Greece (126), Norway, Sweden (127), Italy (128), Poland (129), Canada (130), Brazil (131), and South Korea (132), suggesting that this ST had characteristics of international multidrug-resistant high-risk clones. Recent reports from Israel (133) and Italy (134) demonstrated the endemicity and persistence of CC258 over time while remaining the predominant clone among *K. pneumoniae* isolates with bla_{KPC}. Interestingly, Israel has seen an overall dramatic decrease in the incidence of KPCs among *K. pneumoniae* isolates, but ST258 still remains the most predominant clone (133).

The other SLVs from CC258 have the following global distribution: ST11 is the major ST among *K. pneumoniae* isolates with bla_{KPC} from Asia (especially China) (135) and has also been described in Latin America (especially Brazil) (121). Other STs that belong to CC258 have been reported in Colombia (i.e., ST512), Italy (i.e., ST512), Israel (i.e., ST512), Brazil (i.e., ST340), and Greece (i.e., ST340) (121).

Recent Developments Pertaining to *K. pneumoniae* ST258

Population structure. The diversity in *K. pneumoniae* genomes is due primarily to mobile genes that move frequently by horizontal transfer between bacteria, including plasmids, phages, integrative conjugative elements (ICEs), and insertion elements (IEs) (30). DeLeo and colleagues recently performed whole-genome sequencing on two *K. pneumoniae* ST258 urinary isolates from New Jersey and then did supplementary sequencing on a different global collection of just over 80 CC258 clinical isolates (136). A phylogenetic single-nucleotide polymorphism (SNP) analysis of the core genomes of these isolates showed that *K. pneumoniae* ST258 isolates belonged to two well-defined lineages, named clade I and clade II. Clade I was associated with KPC-2, and clade II was associated with KPC-3. The genetic divergence between these two clades occurred in a 215-kb area that included the genetic material used for capsule polysaccharide biosynthesis (*cps*), an important virulence factor for *K. pneumoniae*.

That same group then compared the genetic structures of the *cps* regions and dispersal of SNPs in the core genomes of ST258 clades I and II with other *K. pneumoniae* sequence types (i.e., ST11, ST442, and ST42) (137). Kreiswirth and colleagues found a 1.1-Mbp area in ST258 clade II that is identical to that of ST442, while the remaining part of the ST258 genome was homologous to that of ST11. This indicated that ST258 clade II is a hybrid or crossbreed clone that was created by a large recombination event between ST11 and ST442. Those investigators then identified the same *cps* regions in ST42 and ST258 clade I. The similarity of the areas surrounding the *cps* regions from ST42, ST258 clade I, and ST258 clade II indicated that ST258 clade I evolved from ST258 clade II due to the replacement of the *cps* region from ST42. Recent developments regarding the population structure of *K. pneumoniae* ST258 are illustrated in Fig. 3.

The integrative conjugative element ICEKp258.2 contains gene clusters for a type IV pilus (i.e., *pilV*) and a type III restriction-modification system. A type IV pilus increases the exchange of plasmid DNA between bacteria and facilitates the attachment of bacteria to objects. The type IV pilus may therefore facilitate the movement of resistance genes (137). A type III restriction-modification system could also be responsible for "host specificity," i.e., preventing the exchange of certain mobile elements, including

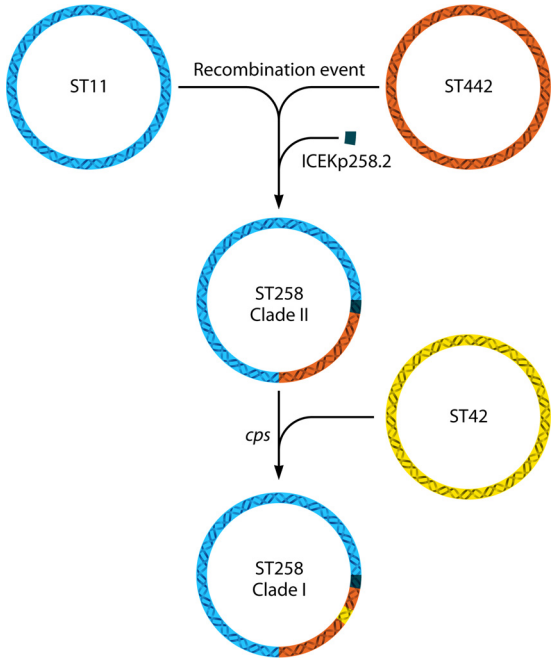

FIG 3 Population structure of *Klebsiella pneumoniae* ST258. ICE, integrative conjugative element; *cps*, capsule polysaccharide biosynthesis gene region.

plasmids, among bacteria (137). A study from Israel investigated the specific association of ICEKp258.2 with *K. pneumoniae* ST258 and other sequence types by searching for the presence of *pilV* and showed that this gene cluster was present only among ST258 isolates but was absent in non-ST258 *K. pneumoniae* isolates (including SLVs of ST258 such as ST11) (138). Their results shed some light on the different behaviors of ST258 and its close relative ST11: ST258 (which contains ICEKp258.2) is specifically associated with KPC and narrow-host-range IncF plasmids (30), while ST11 (which lacks ICEKp258.2) is associated with various carbapenemases (KPC, VIM, IMP, NDM, and OXA-48) on a broad range of plasmids (e.g., IncF, IncA/C, InL/M, IncN, and nontypeable plasmids) (139–141). Therefore, the restriction of plasmids and specific mobile elements by a type III restriction-modification system on ICEKp258.2 may explain some of the differences observed between these SLVs (i.e., ST258 and ST11). Moreover, *pilV* may also in part explain the high transmissibility and survival abilities of ST258 on living and nonliving surfaces. It is possible that ICEKp258.2 contributes significantly to the ecological success of *K. pneumoniae* ST258 (137).

Association with antimicrobial resistance mechanisms. High-risk clones act as stable hosts that harbor resistance genes, transposons, integrons, and plasmids, which allow such elements to spread with such clones (6). One of the most consistent hallmarks of *K. pneumoniae* ST258 is its association with multidrug resistance determinants (30). The majority of antimicrobial-resistant determinants present in ST258 are plasmid mediated, and this clone often contained more than one plasmid, each with multiple individual resistant determinants (136). A recent study that used next-generation sequencing with long reads examined the contents on plasmids in a single ST258 isolate. Those investigators identified four plasmids containing 24 different resistance genes (142). Several investigators have shown that *K. pneumoniae* ST258 isolates have multiple antimicrobial resistance determinants responsible for aminoglycoside resistance [i.e., *aac(6′)-Ib*, *aadA2*, and *aph(3′)-Ia*], β-lactam resistance (i.e., bla_{KPC}s, $bla_{\text{OXA-9}}$, $bla_{\text{SHV-11}}$, and $bla_{\text{TEM-1}}$), fluoroquinolone resistance (i.e., *oqxA* and *oqxB*), macrolide-lincosamide-streptogramin B resistance (i.e., *mphA*), chloramphenicol resistance (i.e., *catA1*), trimethoprim resistance (i.e., *dfrA12*), and sulfonamide resistance (i.e., *sul1*) (142, 143). ST258 isolates can also decrease the number of porin channels, which leads to nonsusceptibility to additional classes of antimicrobial drugs, leaving clinicians with very limited treatment options (144).

The emergence of colistin resistance among ST258 isolates is a worrisome finding since this antimicrobial agent has remained one of the last-line salvation therapies for infections due to *K. pneumoniae* isolates with bla_{KPC} (144). Colistin resistance among ST258 isolates has been documented in several diverse geographical regions, which likely indicates that colistin-resistant ST258 mutants can be selected over time after the introduction of this agent for the treatment of infections due to *K. pneumoniae* ST258 (144–146).

Ability to colonize human hosts. A recent study from Germany investigated the rectal colonization of patients following an outbreak due to *K. pneumoniae* ST258 and showed that 25% of patients remained colonized for up to 1 year after the outbreak was contained. Some patients were persistently colonized even beyond this time (147). A study from Greece found that 73% of the patients were rectally colonized with *K. pneumoniae* ST258 with bla_{KPC} on average within 9 days of exposure to this clone (148). Similar data have been reported from Italy, which showed the effective colonization of neonatal patients by *K. pneumoniae* ST258 (149) as well as long-term acute-care populations in the United States (150). However, in contrast to *E. coli* populations, ST258 isolates have remained present largely among nosocomial patients with health care associations and are not readily identified among community dwellers.

Transmission within the health care setting. A detailed analysis of a recent intensive care unit (ICU) outbreak due to *K. pneumoniae* ST258 in the United States indicated that this clone can be very successfully transmitted between hosts for a long period of time (i.e., months) after the identification of the initial index patient (151). In Italy, the successful spread of ST258 occurred rapidly within the hospital setting (128). The most notable and pronounced outbreak due to ST258 was experienced in Israel after the introduction of this clone during the early to mid-2000s (124). There were sharp increases in the numbers of cases and outbreaks in Israel during 2006 to 2007 in acute-care hospitals, which were driven primarily by ST258. After the nationwide implementation of infection control guidelines during the late 2000s to limit the spread of *K. pneumoniae* with bla_{KPC}, there has been a steep decline in the incidence of infections due to ST258 throughout Israel (133).

Enhanced pathogenicity and fitness. Capsular polysaccharide is a recognized virulence factor enabling *K. pneumoniae* to evade phagocytosis (152). Recent papers have highlighted the likely importance of unique capsular polysaccharides within ST258 and their probable importance in promoting the global success of this clone (136). Characterization of the genome regions of different clades of ST258 (i.e., clade I and clade II) showed that that capsule polysaccharide biosynthesis regions *cps-1* and *cps-2* (also referred to as $cps_{207\text{-}2}$ and $cps_{\text{BO-4}}$) are significantly different between clades

TABLE 3 Laboratory methods for detection of *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST258

Method	Characteristic(s) of detection of:	
	Escherichia coli ST131	*Klebsiella pneumoniae* ST258
NGS	High-resolution, accurate, and reproducible; not yet routine	High-resolution, accurate, and reproducible; not yet routine
MLST	Gold standard; expensive and time-consuming; 2 schemes (Achtman and Pasteur)	Gold standard; expensive and time-consuming; Pasteur scheme
PFGE	Used during the late 2000s; poor method since ST131 consists of different pulsotypes	Used during the late 2000s; poor method since ST258 consists of different pulsotypes
Repetitive-sequence-based PCR typing	Standardized fingerprinting kit; rapid and expensive	Standardized fingerprinting kit; rapid and expensive
MLVA	Rapid, cost-effective, and comparable to MLST	Not yet described
PCR	Several techniques; rapid and inexpensive for screening a large no. of isolates	Several techniques; rapid and inexpensive for screening a large no. of isolates; multiplex for clades I and II
MALDI-TOF MS	Rapid and inexpensive; not yet routine	Not yet described

I and II and are likely involved in the global distribution of these clades (137, 153). This *cps* region of diversification could be advantageous for *K. pneumoniae* ST258 clades I and II, which manages to change the polysaccharide structure as a mechanism to evade host defenses. Capsule switching is used by certain bacteria to escape the host immune response (154). It is possible that the DNA interchange that occurs up- and downstream of the *cps* regions may be an important method used by *K. pneumoniae* to quickly expand and change over time (154). It would be interesting to determine what selection process is responsible for capsule switching among *K. pneumoniae* ST258 clades I and II.

As described above, *pilV* is present on ICEKp258.2 and may in part be responsible for the high transmissibility and survival abilities of *K. pneumoniae* ST258. This element is present in both clades of ST258, but it seems to have been acquired after the large recombination event between ST11 and ST442 to create ST258 clade II. It seems that the genetic material for KPC and the ICEKp258.2 element were independently acquired at two distinct points in time (136). A type IV secretion system might have been beneficial to *K. pneumoniae* ST258 by increasing the fitness and survival abilities of this clone (137).

It is unclear if ST258 isolates are more virulent than other *K. pneumoniae* isolates. ST258 lacks well-characterized *K. pneumoniae* virulence factors, including the K1, K2, and K5 capsular antigen genes; the aerobactin genes; and the regulator of mucoid phenotype gene, *rmpA* (156). A recent study demonstrated that ST258 is nonvirulent in animal models, is highly susceptible to serum killing, and can rapidly undergo phagocytosis (156). A different group showed that not all ST258 isolates produce the same results in a mouse lethality model, but similar results were obtained in a moth (*Galleria mellonella*) virulence model (157). There has been evidence of virulence in a nematode model with *Caenorhabditis elegans* killing, and this effect was not diminished when the isolates were cured of bla_{KPC} plasmids (158). This study suggested that the bla_{KPC} plasmids contained additional factors that could result in persistence but not necessarily enhanced virulence. There was also some strain-to-strain variability in the nematode model.

In summary, *K. pneumoniae* ST258 clearly has all of the essential characteristics that define a high-risk clone (Table 2).

LABORATORY METHODS FOR DETECTION OF *E. COLI* ST131 AND *K. PNEUMONIAE* ST258

Due to the unprecedented success of *E. coli* ST131 and *K. pneumoniae* ST258, several investigators have designed rapid methods for the detection of these sequence types to aid molecular epidemiologists and surveillance studies (Table 3). Such techniques have facilitated the enhanced detection of these sequence types as part of surveillance programs. These techniques include PCR, PFGE, DiversiLab repetitive PCR typing, abbreviated MLVA, and, more recently, MALDI-TOF mass spectrometry.

Next-generation sequencing (NGS) is a promising new technology with substantial potential for clinical microbiology (159). This technique uses PCR to amplify individual DNA molecules that are immobilized on a solid surface, enabling molecules to be sequenced in parallel, leading to decreased costs and rapid turnaround (160). NGS provides high-resolution DNA data in a rapid fashion, which can be used for accurate typing of medically important organisms. Several *E. coli* ST131 and *K. pneumoniae* ST258 isolates have undergone NGS (88), and it is likely that this technique, combined with more user-friendly and rapid bioinformatics, will most likely become the gold standard for the identification of these sequence types in the near future.

Multilocus Sequence Typing

MLST is a sequence-based molecular typing method that determines the nucleotide sequences of some (i.e., 6 to 10) conserved housekeeping genes (161). This makes it suitable for continuous surveillance and is excellent for collating data obtained from several separate sources via Web-based databases. MLST is often used to examine the evolutionary relationships among bacteria, which implies some form of common ancestry among isolates with the same ST. Relatedness between isolates determined by using MLST is based on polymorphisms within conserved housekeeping genes. MLST determines the population structures of different species and has led to the identification of certain global antimicrobial-resistant STs or clones (161). MLST does not have the necessary discrimination to detect genetic changes among isolates involved in outbreak situations (43).

MLST is perfect for tracing multidrug-resistant clones or STs across the globe (161). Unfortunately, MLST is expensive, since it often uses Sanger sequencing, but this technique remains the

benchmark for the identification of *E. coli* ST131 and *K. pneumoniae* ST258.

As described above, there are 2 MLST schemes that are often used to identify *E. coli* ST131. The Achtman scheme (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) uses the gene sequences of seven housekeeping genes: *adk* (adenylate kinase), *fumC* (fumarate hydratase), *gyrB* (DNA gyrase), *icd* (isocitrate/isopropylmalate dehydrogenase), *mdh* (malate dehydrogenase), *purA* (adenylosuccinate dehydrogenase), and *recA* (ATP/GTP binding motif). The Pasteur Institute *E. coli* scheme (http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html) uses eight housekeeping genes: *dinB* (DNA polymerase), *icdA* (isocitrate dehydrogenase), *pabB* (*p*-aminobenzoate synthase), *polB* (polymerase II [Pol II]), *putP* (proline permease), *trpA* (tryptophan synthase subunit A), *trpB* (tryptophan synthase subunit B), and *uidA* (beta-glucuronidase). The scheme from the Pasteur institute tends to identify different sequence types among ST131 isolates (as identified by the Achtman scheme). Most investigators use the Achtman MLST scheme to routinely identify ST131.

The Pasteur Institute is the most popular MLST scheme used to identify *K. pneumoniae* ST258 (162). (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). This scheme uses the following seven housekeeping genes: *rpoB* (beta-subunit of RNA polymerase), *gapA* (glyceraldehyde-3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphorine E), *infB* (translation initiation factor 2), and *tonB* (periplasmic energy transducer).

Pulsed-Field Gel Electrophoresis

PFGE is the benchmark for molecular typing of medically important bacteria during outbreak investigations (43). PFGE involves the cutting of bacterial DNA into fragments of various sizes, followed by separation into fingerprints by gel electrophoresis, and this technique is often used to track clones or clusters during outbreak investigations (163). Unfortunately, PFGE is arduous, and it takes days to obtain results, while personnel need to be technically skilled in this method to obtain reliable and reproducible results. Additionally, comparison of various pulsotypes created in different locations remains a problem.

PFGE was extensively used during the late 2000s to recognize isolates that belonged to *E. coli* ST131 (66) and *K. pneumoniae* ST258 (125). However, PFGE is not a very good method to identify both sequence types because they consist of different pulsotypes that display high levels of intralineage genetic variations (45, 81). Both high-risk clones often display <80% similarity and often do not satisfy the criteria for relatedness outlined previously by Tenover et al. (44).

Repetitive-Sequence-Based PCR Typing

Three separate studies, from Canada, the United Kingdom, and the United States, evaluated the ability of the DiversiLab fingerprinting kit, a type of repetitive-element PCR, to identify *E. coli* ST131 (164, 165) and *K. pneumoniae* ST258 (166). The DiversiLab system allowed the discrimination of ST131 isolates from isolates of other *E. coli* sequence types and of ST258 isolates from isolates of other *K. pneumoniae* sequence types. The DiversiLab system offers kit-based standardized typing results in a rapid fashion but is unfortunately rather expensive, and this prohibited its widespread utilization and the implementation of this technique for the recognition of *E. coli* ST131 and *K. pneumoniae* ST258.

Multilocus Variable-Number Tandem-Repeat Analysis

An abbreviated version of MLVA for the detection of *E. coli* ST131 that uses a benchtop capillary electrophoresis instrument was developed by Nielsen et al. (167). The exclusion of two loci affected the discriminatory power of the abbreviated MLVA, making it less discriminatory than the original assay. However, this approach can be used as a high-throughput assay and provided rapid typing results for *E. coli* ST131 that were comparable to those provided by MLST and PFGE.

PCR

PCR-based techniques offer rapid and inexpensive methodologies (compared to MLST and PFGE) to identify *E. coli* ST131 and *K. pneumoniae* ST258. These techniques are the most popular approaches used to screen for ST131 and ST258 among large numbers of *E. coli* and *K. pneumoniae* isolates. The PCR screening methods are based on the recognition of different SNPs within the O serogroup and housekeeping genes specific for *E. coli* ST131 (19) and *K. pneumoniae* ST258 (168).

Several PCR screening methods are widely available to determine whether an *E. coli* isolate belongs to ST131. These techniques include a PCR for SNPs within the 5′ portion of the *rfb* locus specific for O25b (169) and SNPs within the *pabB* gene (170). Johnson and colleagues designed a sequencing method based on SNPs within the *mdh* and *gyrB* genes (65). A novel and rapid technique based on sequencing of the *fumC* and *fimH* loci (called CH typing or clonotyping) identified the *fimH30* ST131 lineage as clonotype CH40-30 (171). Blanco and colleagues designed a triplex PCR that was based on the detection of the O25b *rfb* allele, $bla_{\text{CTX-M-15}}$, and the *afa/draBC* virulence factor (172). A recent PCR screening test combined the O16 *rfb* variant with the ST131-specific alleles of *mdh* and *gyrB* that can distinguish between the two serogroups of ST131, namely, O25b (*fimH30*) and O16 (*fimH41*) (95). PCR methods are also available for the identification of the *fimH30* lineage and the H30-Rx sublineage of ST131 (87, 91, 95). The identification of the H30-Rx sublineage requires an additional sequencing step to be performed. Note that PCR-based screening of *E. coli* ST131 may infrequently identify isolates that belong to clonal complex 131 (which varies from ST131 by one or two alleles) as belonging to ST131 and very rarely can misidentify non-ST131 *E. coli* isolates as belonging to ST131.

The multiplex real-time PCR for the detection of *K. pneumoniae* ST258 described by Chen and colleagues targets 2 SNPs in the *tonB* allele that are unique to ST258 and related STs (168). This PCR also included primers against the *gapA* allele (present in all *K. pneumoniae* isolates), which serves as an internal control. The regular multiplex PCR method described by Adler and colleagues detects the presence or absence of 2 genes, namely *pilv-l* and *prp* (138). Whole-genome sequencing has shown that the *pilv-l* allele is specific for ST258 and related STs. Both studies included ST258 and ST512 as positive controls in their evaluation. Chen and colleagues also developed a multiplex PCR for the identification of different lineages within ST258, i.e., clade I and clade II (173).

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

MALDI-TOF MS is a high-throughput methodology based on the identification of the mass-to-charge ratios of peptides and small proteins, most of which are ribosomal (174). It has been increasingly adopted by clinical microbiology laboratories all over the

world for quick and reliable microbial identification to the species level based on the comparison of mass spectral fingerprints (obtained from single colonies or crude extracts) with those in previously established reference databases. The use of MALDI-TOF MS for bacterial typing is based on the principle that sequence variations within a given taxon or subspecies will be translated into the corresponding protein or peptide sequences and supported by a similar clustering obtained from either mass spectral data or genomic sequences, highlighting the usefulness of peak patterns as taxonomic markers (175). The advantages of simplicity, speed, and low cost associated with MALDI-TOF MS and its potential application on a large-scale basis might be useful to enable timely and appropriate diagnosis, infection control, and individual patient management decisions.

Japanese and Portuguese investigators evaluated MALDI-TOF MS for the identification of *E. coli* ST131. The study from Japan used Flexanalysis software to successfully distinguish ST131 (O25b and O16 serotypes) from other sequence types with a sensitivity of 97.0% and a specificity of 91.5% (176). The study from Portugal showed that MALDI-TOF MS fingerprinting analysis was able to discriminate between ST131, ST69, ST405, and ST39 isolates and between phylogenetic group B2 ST131 isolates and other phylogenetic group B2 isolates that did not belong to ST131 (177). MALDI-TOF MS is a promising tool for the detection of *E. coli* ST131 that might be available for routine use in the near future.

To our knowledge, studies that have evaluated MALDI-TOF MS for the detection of *K. pneumoniae* ST258 have not yet been reported.

ROLE OF PLASMIDS IN HIGH-RISK *E. COLI* ST131 AND *K. PNEUMONIAE* ST258 CLONES

Overview

Since plasmids are often maintained and exchanged independently of the bacterial host genome, they undergo evolutionary changes that are unrelated to the host. To understand the interdependence of the acquisition of plasmids and their subsequent dissemination in high-risk clones, a brief description of the classification of and similarities and differences among plasmids is required.

Plasmids consist of extrachromosomal DNA and require independent mechanisms for maintenance and coinheritance into daughter cells (9). Large portions of plasmid DNA show high degrees of plasticity, consisting of several types of mobile elements, such as insertion elements and transposons. This plasticity has challenged studies investigating the evolutionary history and relatedness of different plasmids, even when investigators used the latest sequencing technologies. However, DNAs that are required for the replication of plasmids are conserved and can therefore be used as regions for the comparison and classification of different plasmids obtained over different time periods, from different geographical areas, and from different bacteria. This is referred to as the "incompatibility group typing" scheme, which is based on the characterization of unique or conserved replication areas on different plasmids and is used primarily to demonstrate the relatedness and behavior of different plasmid groups (178, 179). It is important to remember that not all plasmids can be typed with this technique; plasmids that do not show results with incompatibility typing are often referred to as nontypeable. Plasmids that belong to different incompatibility groups exhibit variable behavior characteristics (e.g., narrow-bacterial-host-range plasmids versus broad-host-range plasmids and high-copy-number plasmids versus low-copy-number plasmids).

The classification scheme of broad-host-range versus narrow-host-range *Proteobacteria* plasmids is based on the diversity of the bacterial hosts into which plasmids can be transferred and then successfully maintained in a sustainable manner (180). The narrow-host-range group of plasmids most often belongs to incompatibility group F (IncF), which contains different replicons (e.g., FIA, FIB, and FII), while the broad-host-range group includes the IncA/C and IncN types (among others). Broad-host-range plasmids can easily be transferred between different species, while narrow-host-range plasmids tend to be restricted to certain species or even clones within species. This concept is important when evaluating plasmids in high-risk clones, as there is a predominance of narrow-range plasmids (specifically IncF with certain β-lactamases [e.g., $bla_{\text{CTX-M-15}}$, $bla_{\text{KPC-2}}$, and $bla_{\text{KPC-3}}$]) in these clones. These plasmids have recently been termed "epidemic resistance plasmids" due to their propensity to acquire resistance genes and rapid dissemination among the *Enterobacteriaceae* (9). IncF plasmids use postsegregational killing and addiction systems to ensure their propagation among high-risk clones. IncF plasmids are often present in *E. coli*, and up to 70% of plasmids characterized in human and avian *E. coli* isolates belonged to IncF with different replicons (181).

Plasmids have coevolved with bacteria, providing them with rapid ways to evolve and adapt through the accumulation of point mutations, deletions, and insertions. Plasmids can provide intact functional genes to various bacteria that will assist them in adapting to harsh conditions and unsuitable environments (182). Plasmids often contain various combinations of virulence, fitness, and antimicrobial resistance genes and have contributed significantly to the success of antimicrobial-resistant bacteria (183).

Once a resistance plasmid has been transferred to and has replicated in a new bacterial host, the presence of antimicrobial agents will create artificial selective pressure that will select for a bacterial population with such resistance plasmids. However, in the absence of antimicrobial agents, the resistance plasmid may be retained only transiently by the bacterium if it is unstable within the host (184). The long-term stability and persistence of resistance plasmids in various bacterial populations during the absence of antibiotic selection pressures has received surprisingly little attention. Very little is known about the impact of plasmid stability on the long-term survival and host range of resistance plasmids, especially in the absence of selection pressure created by antimicrobial agents.

As described above, there is a predominance of narrow-range plasmids (i.e., IncF) among high-risk clones such as *E. coli* ST131 and *K. pneumoniae* ST258, and it seems that such clones with IncF plasmids provide a stable environment for antimicrobial resistance genes (185). Antimicrobial resistance determinants on epidemic plasmids provide a selective advantage to high-risk clones and are likely central to their success (186, 187). IncF plasmids are diverse, are complex, tend to have a narrow host range, and are present in low copy numbers (188). The concept of incompatibility for this group is somewhat of a misnomer, with several descriptions of multiple replicons being present in the same plasmid with one replicon remaining silent while another replicon is acting as the area of replication (188).

Plasmids Associated with *E. coli* ST131

The initial description of *E. coli* ST131 in 2008 by Coque and colleagues included the characterization of eight plasmids harboring $bla_{CTX\text{-}M\text{-}15}$ in this ST (52). The sizes of the plasmids varied between 100 kb and 150 kb, with 3 different restriction fragment length polymorphism patterns, and they all belonged to IncF with FII and FIA replicons.

The first plasmids from ST131 to be entirely sequenced were obtained in the United Kingdom and included IncF plasmids (i.e., pEK499 and pEK516) and IncI1 plasmid pEK204 (189). Plasmid pEK499 turned out to be a fusion of FII and FIA replicons and contained the following genes for antimicrobial determinants: $bla_{TEM\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, *aac(6′)-Ib-cr*, *mph*(A), *catB4*, *tetA*, *dfrA7*, *aadA5*, and *sul1*. pEK499 also harbored the following postsegregational killing systems that are responsible for stable plasmid inheritance during vertical spread: the *hok-mok* killing protein and modulator system, the *pemI-pemK* toxin-antitoxin system, the *vagC-vagD* virulence-associated genes, and the *ccdA-ccdB* toxin-antitoxin system. These postsegregational killing systems could be responsible for the persistence of pEK499-like plasmids among ST131 isolates, which can occur even in the absence of antibiotic selection pressure. IncF plasmid pEK516 contains the FII replicon with the following antimicrobial resistance genes: $bla_{TEM\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, *aac(6′)-Ib-cr*, *aac(3)-II*, *catB4*, and *tetA*. Plasmids pEK516 and pEK499 display 75% DNA sequence similarity, despite pEK516 being substantially smaller than pEK499. Plasmid pEK516 also carries the *pemI-pemK* and *hok-mok* postsegregational killing systems as well as the type I partitioning locus *parM* and *stbB*. Plasmid pEK204 belongs to a different incompatibility group, IncI1 (i.e., a broad-host-range type of plasmid) and contains $bla_{CTX\text{-}M\text{-}3}$ and $bla_{TEM\text{-}1}$ accompanied by an IS*Ecp1*-like mobile element upstream of $bla_{CTX\text{-}M\text{-}3}$. These initial plasmid studies were then followed by several global molecular epidemiology studies that characterized plasmids associated with CTX-M enzymes among ST131 isolates. The majority of the plasmids associated with $bla_{CTX\text{-}M\text{-}15}$ belonged to IncF and contained the fused FIA-FII or FII replicons (19). IncF plasmids present in *E. coli* ST131 can also encode β-lactamases other than CTX-M-15, such as CTX-M-14, SHV-2, and SHV-12, while $bla_{CTX\text{-}M\text{-}15}$ was also identified on plasmids that belong to other incompatibility groups, such as IncI1, IncN, and IncA/C, as well as being on *pir*-type plasmids (19).

The narrow-host-range IncF and broad-host-range plasmids (i.e., IncN, IncN2, IncI1, IncHI2, IncL/M, IncA/C, IncK, IncX4, and IncU) as well as the rolling circle replication (RCR) plasmid families have been associated with CTX-M enzymes, but the IncF plasmids are by far the most common Inc type detected in *E. coli* ST131 isolates harboring $bla_{CTX\text{-}M}$ (190). $bla_{CTX\text{-}M\text{-}15}$ has been found mainly on IncF plasmids with fused FIA-FII or FII replicons in ST131, whereas Inc plasmids with different replicons (i.e., FIB) have been identified in non-ST131 ExPEC (191). Some IncF plasmids with $bla_{CTX\text{-}M\text{-}15}$ also carry multiple virulence determinants (e.g., *iutA ompT*, *hlyF*, *iss*, and *iroN*) (192). IncF plasmids provide various antimicrobial resistance determinants and virulence factors that confer additional selective and significant advantages for the ST131 host. IncF plasmids also use postsegregational killing and addiction systems to ensure their propagation and maintenance within *E. coli* ST131 and have played an essential role in the dissemination of $bla_{CTX\text{-}M\text{-}15}$ among isolates of this high-risk clone, even in the absence of artificial selection pressure created by antimicrobial agents. The introduction of such IncF plasmids created the H30-Rx sublineage, and in some areas, this sublineage is more prevalent than the H30-R sublineage (Fig. 2) (94). Banerjee and colleagues have shown that isolates of the H30-Rx sublineage with $bla_{CTX\text{-}M\text{-}15}$-containing plasmids had significantly higher virulence scores than did isolates of the H30-R sublineage and non-H30 ST131 isolates, implying greater virulence potential created by the presence of such IncF plasmids and possibly providing an explanation for the high prevalence of this sublineage (91). A different study showed that as ST131 gained resistance to the fluoroquinolones and acquired ESBLs, isolates (especially those that had acquired $bla_{CTX\text{-}M\text{-}15}$) tended to accumulate additional virulence factors, thus implying that the virulence factors on plasmids contribute to the success of the H30-Rx sublineage (89).

Plasmids Associated with *K. pneumoniae* ST258

Several different KPC-containing plasmids have been identified in ST258. They belong to IncF (with FII_{k1}, FII_{k2}, and FIA replicons), IncI2, IncX, IncA/C, IncR, and ColE1, and these plasmids often contain various genes encoding nonsusceptibility to different antimicrobial drugs (30). However, the most predominant bla_{KPC}-carrying plasmid type associated with *K. pneumoniae* ST258 is IncF with FII_k replicons (185). The first bla_{KPC}-carrying plasmid identified in ST258 (named clone Q at that time) was obtained in 2006 from Israel and was named pKpQIL (193). This plasmid is a 113-kb IncF plasmid with an FII_{k2} replicon containing Tn*4401a*, with a backbone very similar to that of plasmid pKPN4, first characterized in 1994 in a non-KPC antimicrobial-resistant *K. pneumoniae* isolate obtained in Massachusetts (193).

A retrospective plasmid analysis of *K. pneumoniae* strains with bla_{KPC} from the New York and New Jersey areas isolated during the early 2000s showed that ST258 contained $bla_{KPC\text{-}2}$ and $bla_{KPC\text{-}3}$ on pKpQIL-like plasmids, which were nearly identical to the Israeli plasmid pKpQIL described in 2006 (194). pKpQIL-like plasmids from the New York and New Jersey isolates were associated mostly with $bla_{KPC\text{-}2}$ and to a lesser extent with $bla_{KPC\text{-}3}$, whereas the pKpQIL plasmids from Israel were associated mainly with $bla_{KPC\text{-}3}$ (194, 195). This suggests that ST258 isolates with $bla_{KPC\text{-}3}$ on pKpQIL plasmids were introduced into Israel from the United States during the mid-2000s (a founder effect), followed by clonal expansion in Israel. The $IncFII_k$ plasmids are also the most common plasmids identified among ST258 isolates with bla_{KPC} from several different geographically diverse areas, including Canada, Poland, the United States, Israel, Brazil, Italy, and Norway (131, 185, 196). There also appears to be an association between different plasmid Inc groups and ST258 clades I and II. The $bla_{KPC\text{-}3}$-associated IncI2 plasmids and $bla_{KPC\text{-}3}$-associated IncFIA plasmids were found exclusively in clade II, while the pKpQIL-associated $IncFII_{k2}$ plasmids with $bla_{KPC\text{-}2}$ were detected in both clades I and II (136). pKpQIL plasmids were not restricted to only ST258 and were present in 33% of non-ST258 *K. pneumoniae* isolates in the New York area (194).

The complete sequencing of plasmids associated with ST258 from large collections reveals that they are evolving over time through large genetic rearrangements (185, 197, 198). This process is creating hybrid plasmids, as was previously described in Italy, with ST258 isolates containing two different IncF plasmids, namely, pKpQIL-IT and pKPN-IT, as well as a ColE-like plasmid with $bla_{KPC\text{-}2}$ (199). Both pKpQIL-IT and pKPN-IT have a very high degree of homology to the historic plasmids pKPN4 and

TABLE 4 Summary of *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST258

Characteristic	Description[a]	
	Escherichia coli ST131	*Klebsiella pneumoniae* ST258
Clonal complex	CC131 (with ST131 being dominant)	CC258 (with ST258 being dominant; also includes ST11, ST340, ST437, and ST512)
Multidrug-resistant high-risk clone	Yes	Yes
Region(s) of endemicity	Global	USA, Greece, Italy, Israel, Columbia
Resistance determinants	Various but associated with fluoroquinolone resistance and CTX-M-15 (CTX-M-14 to a lesser extent)	Various but associated with KPC-2 and KPC-3
Mobile element	IS*Ecp1*	Tn*4401*
Plasmids	Various incompatibility groups, but IncF with virulence factors is the most common	Various incompatibility groups, but $IncFII_k$ is the most common
Clades/lineages	O25 *fimH30* lineage that consists of the H30-R and H30-Rx sublineages; O16 *fimH41* lineage	Clade I associated with KPC-2; clade II associated with KPC-3
Population biology	H30-R sublineage is associated with fluoroquinolone resistance, and H30-Rx is associated with CTX-M-15	Clade II is a hybrid of ST11 and ST442; clade I is a hybrid of clade II and ST42
Methods of acquisition	Community-onset health care associated, LTCFs	Nosocomial, LTCFs
Types of infection	UTIs, IAIs, BSIs	UTIs, RTIs, BSIs
Mortality	Unclear if the rate is higher than that with non-ST131 *E. coli*	Higher rate than that with non-ST258 *K. pneumoniae* (most likely due to the patient's underlying conditions)

[a] UTIs, urinary tract infections; IAIs, intra-abdominals infections; BSIs, bloodstream-associated infections; RTIs, respiratory tract infections; LTCFs long-term-care facilities.

pKPN3 from a non-KPC-producing *K. pneumoniae* strain isolated in 1994 (199). This suggests that certain ancestral plasmids are particularly well suited to *Enterobacteriaceae* such as *K. pneumoniae* and are good candidates for sustaining the presence of bla_{KPC} through multiple independent insertion and transposition events. This is further supported by a recent study from South Korea that demonstrated that ancestral plasmids were present among ST258 isolates from various geographical regions and were obtained as early as 2002 (143).

Logic might dictate that when a single bla_{KPC} plasmid enters ST258 isolates at a single time point, it will develop diversity over time. However, it appears that *K. pneumoniae* ST258 isolates acquired various types of bla_{KPC} plasmids at multiple time points and in different geographical locations around the globe, which was then followed by the notable local spread of ST258 isolates with the specific KPC plasmid (30).

It seems that the presence of plasmids with bla_{KPC} is central to the success of ST258. The loss of pKpQIL by ST258 isolates has limited the ability of these isolates to successfully disseminate compared to other *K. pneumoniae* isolates without bla_{KPC} and ST258 isolates with pKpQIL (187). This suggests that bla_{KPC} in combination with other virulence or persistence factors on pKpQiL-like plasmids promoted the fitness and survival of ST258 isolates. This is further supported by the epidemiological observation that non-ST258 *K. pneumoniae* isolates with bla_{KPC} did not demonstrate the same global success as did ST258 isolates with bla_{KPC}. It appears that the successful global dissemination and survival of *K. pneumoniae* ST258 are dependent in part on the combination of bla_{KPC} on IncF plasmids with factors inherently present on the chromosome of this high-risk clone (186).

IncI2 with bla_{KPC-3} can also successfully pair with ST258 and was recently detected in 23% of ST258 isolates from the New York and New Jersey areas (200). Interestingly, this IncI2 plasmid also contained type IV pili, which may contribute to the successful dissemination of *K. pneumoniae* ST258.

Broad-Host-Range Plasmids

Broad-host-range plasmids (i.e., IncA/C, IncL/M, and IncN) are rare in *E. coli* ST131 and *K. pneumoniae* ST258 isolates (19, 30). This is a curious finding, and it seems that at this time, broad-range plasmids do not play a meaningful role in the success and global dominance of *K. pneumoniae* ST258 and *E. coli* ST131.

CONCLUSION

There is no doubt that the high-risk *E. coli* ST131 and *K. pneumoniae* ST258 clones are important human pathogens, have spread extensively throughout the world, and are responsible for the sudden increase in multidrug resistance among *E. coli* (17) and *K. pneumoniae* (121) isolates, respectively. ST131 is responsible for extraintestinal infections (especially UTIs), is most often fluoroquinolone resistant, and can also be linked with the production of CTX-M-15 (19), while ST258 is known to cause UTIs, respiratory tract infections, and BSIs and is associated with carbapenemase production, most often KPC-2 and KPC-3 (201) (Table 4).

Biological evolution is defined as "descent with modification within a defined population," with the sole purpose of adaptation to environmental changes (202). This definition encompasses microevolution (i.e., changes in gene frequency within a population from one generation to the next) and macroevolution (i.e., the descent of different species from a common ancestor over many generations). Evolution provides the means for members of a population to adapt, survive, and reproduce in an effective manner. For evolution to progress in an orderly and efficient fashion, a selection process for desirable traits is required. Natural selection is due to the interaction of a population with the environment, while artificial selection (also referred to as selective breeding) occurs due to human intervention. Antimicrobial therapy with the subsequent selection of antimicrobial-resistant mutations is considered to be one of the most efficient artificial selection pressures introduced by humans (203).

E. coli ST131 has long been "flying under the antimicrobial

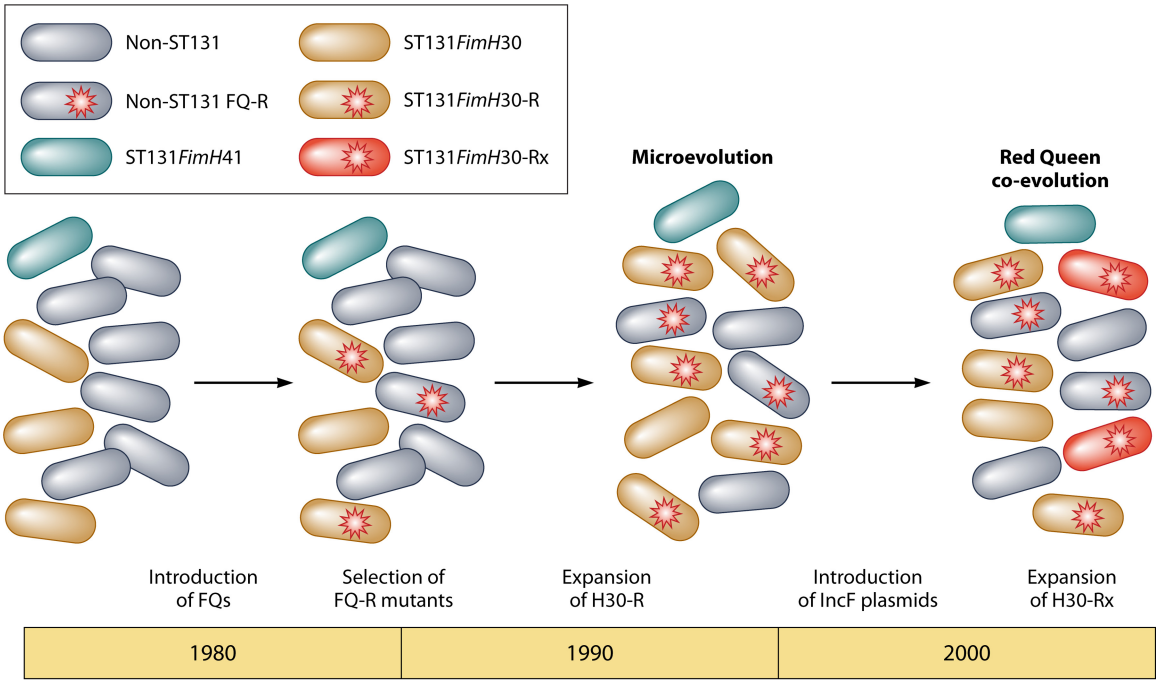

FIG 4 Change in the population structure of *Escherichia coli* due to selection pressures created by the fluoroquinolones (FQ) and the oxyimino cephalosporins FQ-R, fluoroquinolone resistant.

resistance radar" and therefore did not generate the same awareness as other multidrug-resistant bacteria. Retrospective molecular epidemiology surveys have demonstrated that ST131 was rare among *E. coli* isolates from the 1990s and early 2000s (84, 94). However, during the mid- to late 2000s, the numbers of fluoroquinolone-resistant ST131 isolates increased substantially, which was followed by an explosive increase in the number of isolates with $bla_{\text{CTX-M-15}}$ toward the end of the 2000s (19). The reasons for this sudden increase are not clear or well understood. Recent studies utilizing advanced technologies such as whole-genome sequencing combined with phylogenetic analysis have demonstrated that a lineage within ST131, named *fimH30*, emerged during the early 2000s. This was then followed by the rapid expansion of its sublineages H30-R and H30-Rx, which were responsible for the global emergence of fluoroquinolone resistance and CTX-M-producing ExPEC isolates. The widespread use of antimicrobials possibly selected for ST131, resulting in the emergence of ST131. The strong association between ST131 and fluoroquinolone resistance suggested that the use of fluoroquinolones has contributed to the expansion of the H30-R ST131 sublineage. Likewise, the use of oxyimino cephalosporins may have driven the expansion of the H30-Rx sublineage, which is strongly associated with CTX-M-15. The selection pressures created by the widespread use of the fluoroquinolones and the oxyimino cephalosporins dramatically changed the population structure of *E. coli*, especially among ExPEC isolates, and created one of the most prevalent and extensive global antimicrobial-resistant *E. coli* sublineages known to the medical community (84). This is an excellent example of microevolution in action due to the artificial selection pressure created by the use of antimicrobial agents (Fig. 4).

The prevalences of certain virulence genes such as *sat*, *iutA*, *malX*, *usp*, *iha*, *hra*, and *ompT* are consistently high among ST131 isolates (compared to non-ST131 ExPEC isolates), and these genes might increase the versatility and competitiveness of ST131 and the capacity of ST131 to effectively infect the human body (Fig. 5). The exact role of these virulence genes remains to be explained; however, it is possible that a combination of factors contributes to the fitness of ST131 rather than being directly involved in the pathogenesis of infection (79). *In vivo* studies suggest that fluoroquinolone-resistant *E. coli* isolates undergo compensatory mutations and become as fit as fluoroquinolone-susceptible isolates (46).

This brings up some intriguing issues. Is *E. coli* ST131 inherently more fit than other ExPEC clones and therefore better able to survive in certain environments? Is this "fitness" due to certain virulence factors (e.g., *sat*, *iutA*, *malX*, *usp*, *iha*, *hra*, and *ompT*) that provided ST131 with opportunities to be exposed to and acquire certain narrow-host-range IncF plasmids? Did the *gyrA-parC* compensatory mutations (i.e., *gyrA1AB* and *parC1aAB*), selected by fluoroquinolones, reduce the fitness costs associated with IncF plasmids? Is it perhaps possible that the mutations accommodating IncF plasmids increased the fitness of ST131 isolates, enabling them, with the aid of certain virulence factors and antimicrobial resistance determinants on the CTX-M-15 plasmids, to outcompete other ExPEC clones? Colpan and colleagues have shown that fluoroquinolone-susceptible *E. coli* ST131 isolates have different virulence factor profiles than those of fluoroquinolone-susceptible non-ST131 isolates, and as ST131 isolates gained resistance to the fluoroquinolones and acquired ESBLs (especially CTX-M-15), they tended to accumulate additional virulence factors (89). Furthermore, *in vitro* studies have shown that ST131 has high metabolic potential and an enhanced ability to produce biofilms (19). These studies suggest that the inherent

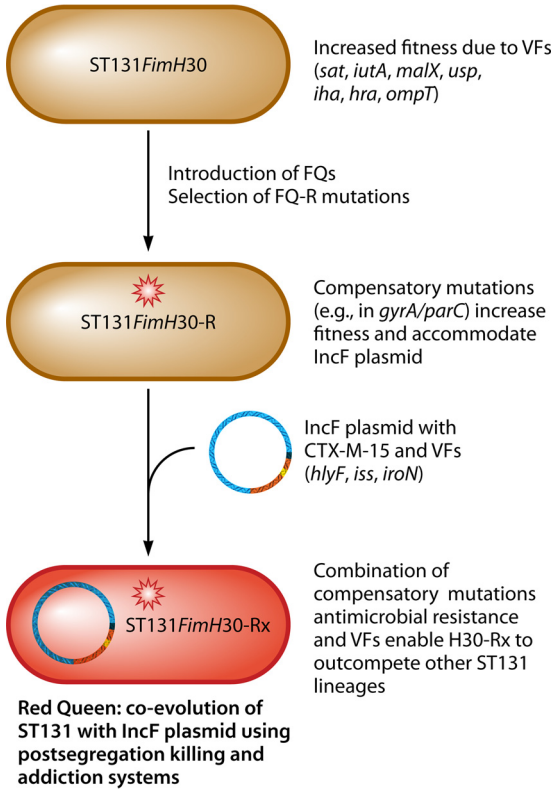

FIG 5 Possible factors that make the *Escherichia coli* ST131 *fimH30* lineage such a successful high-risk clone. VFs, virulence factors; FQ-R, fluoroquinolone resistant.

combination of different virulence factors, biofilm production, and metabolic potential provides ST131 isolates with the means to be "more fit" than other ExPEC clones. Moreover, it seems that ST131 (especially the *fimH30* lineage) has the ability to counteract the fitness cost associated with antimicrobial resistance and also tends to accumulate virulence factors, as this clone/lineage is becoming progressively more antimicrobial resistant (89). With its combination of multidrug resistance and ecological success, the ST131 *fimH30* lineage counters the hypothesis that increased antimicrobial resistance entails a significant fitness cost. *E. coli* ST131 is most likely the best example of an international multiresistant high-risk clone (Fig. 5).

The role of IncF plasmids with $bla_{\text{CTX-M-15}}$ in the success of ST131 is another intriguing issue. IncF plasmids provide various antimicrobial resistance determinants, but some plasmids also encode multiple virulence factors that confer additional selective and significant advantages for the ST131 host (192). IncF plasmids use postsegregational killing and addiction systems to ensure their propagation among high-risk clones and have played an essential role in the dispersion of $bla_{\text{CTX-M-15}}$ among *E. coli* ST131 isolates by creating the H30-Rx sublineage. A population-based molecular surveillance study (2000 to 2010) from Calgary showed that the H30-Rx sublineage with $bla_{\text{CTX-M-15}}$ became the most dominant sublineage toward the later part of the study period (94) (Fig. 2). It is therefore possible that the coevolution of certain sublineages within high-risk clones (i.e., H30-Rx) and epidemic plasmids (e.g., IncF) provided rapid and continual adaptation for certain sublineages, giving them the additional ability to outcompete other sublineages without such epidemic plasmids (Fig. 5). This is consistent with both the macro- and microevolutionary versions of the Red Queen hypothesis, as demonstrated recently by using the microscopic roundworm *Caenorhabditis elegans* as a host and the bacterium *Serratia marcescens* as a parasite to create a host-parasite coevolutionary system (204) (Fig. 4 and 5).

Recent molecular studies have shown that *K. pneumoniae* ST258 consists of 2 distinct lineages, namely, clade I and clade II (136). Clade I was specifically associated with $bla_{\text{KPC-2}}$, and clade II was associated with $bla_{\text{KPC-3}}$ (Table 4). The genetic differentiation of the two clades occurred in a 215-kb region that included genes responsible for capsule polysaccharide biosynthesis, indicating that these 2 clades had distinct evolutionary pathways. Additional investigation showed that ST258 clade II is a cross-hybrid clone that was created by a large recombination event between ST11 and ST442 (137). Moreover, it seems that ST258 clade I was created by the *cps* replacement in ST258 clade II that originated from ST42 (Fig. 3). The integrative conjugative element ICEKp258.2 contains gene clusters for a type IV pilus (i.e., *pilV*) and a type III restriction-modification system. *pilV* on ICEKp258.2 may in part be responsible for the high transmissibility and durability of ST258 on foreign surfaces, and it seems that this integrative conjugative element contributes significantly to the epidemiological success of *K. pneumoniae* ST258 (137).

The use of antimicrobial agents will continue to create selection pressure that gives high-risk clones the opportunity to become effective intestinal colonizers and provides opportunities for them to cause infections. To prescribe more effective empirical antimicrobial therapy, clinicians will need to have an increased awareness of *E. coli* ST131 and *K. pneumoniae* ST258, more specifically the prevalence and extensive antimicrobial resistance capabilities of these high-risk clones, including which patients are at risk for infection. *E. coli* ST131 and *K. pneumoniae* ST258 cause infections similar to those caused by non-ST131 *E. coli* and non-ST258 *K. pneumoniae* isolates, respectively, and clinical risk factors most likely will be insufficient to reliably identify patients with an increased risk of infections by these clones. Therefore, the medical community needs cost-effective diagnostic tests that have the ability to rapidly detect *E. coli* ST131 and *K. pneumoniae* ST258. PCR- and sequence-based approaches have been developed and reliably detect both clones in a matter of hours (Table 3), but unfortunately, due to cost and personnel issues, these methods are difficult to implement routinely in a diagnostic laboratory. Such approaches might provide timely and appropriate antimicrobial therapy that can help to improve clinical outcomes with infections due to *E. coli* ST131 and *K. pneumoniae* ST258.

E. coli ST131, *K. pneumoniae* ST258, and their lineages/clades are now major threats to public health due to their worldwide distribution and association with multidrug resistance. The recent emergence of *E. coli* ST131 with carbapenemases poses a significant additional public health menace due to its relationship with the very dominant *fimH30* lineage.

Future studies should be undertaken to determine if antibiotic stewardship and which infection control bundles will decrease colonization and infections by *E. coli* ST131 and *K. pneumoniae* ST258. Additionally, vaccines that target *E. coli* ST131 serotypes O25b and O16 and those that target the *cps* region of *K. pneumoniae* ST258 clades I and II should also be developed. This might reduce the reservoir of asymptomatic individuals colonized with

these high-risk clones and will reduce the spread of and infection due to *E. coli* ST131 and *K. pneumoniae* ST258.

ACKNOWLEDGMENTS

This work was supported in part by a research grant from Calgary Laboratory Services (grant number 10006465).

J.D.D.P. previously received research funds from Merck and AstraZeneca. A.J.M. has received research funds from Rempex Pharmaceuticals. G.P. has nothing to declare.

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Amy J. Mathers, M.D., is an Assistant Professor of Medicine and Pathology at the University of Virginia in the Division of Infectious Diseases and International Health, where she serves as Clinical Director of Antimicrobial Stewardship and Assistant Director of Clinical Microbiology. She received her bachelor's of science in Molecular Biology at Humboldt State University in California. She completed her medical degree at Loyola Stritch School of Medicine in Chicago, IL. During Internal Medicine Residency at the Maine Medical Center in Portland, ME, she investigated the strain diversity of *Borrelia burgdorferi*. After completing her Infectious Diseases fellowship at the University of Virginia, she transitioned to faculty in 2009. Focusing on the urgent clinical problem of carbapenem resistance in *Enterobacteriaceae*, she has been evaluating detection methods and molecular transmission of carbapenemase genes for the last 8 years. Using next-generation sequencing, she seeks to better understand the origin, transmission, and sustainability of genes of antimicrobial resistance.

Gisele Peirano, Ph.D., is a Research Associate in the Microbiology section of Calgary Laboratory Services in Calgary, Alberta, Canada. She graduated from Rio de Janeiro State University (1997) and received her M.Sc. (2000) and Ph.D. (2005) in Bacteriology from the Oswaldo Cruz Institute, Rio de Janeiro, Brazil. She was awarded a Foreign Affairs, Trade and Development Canada postdoctoral fellowship at the Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada. Her main research interest is resistance to antimicrobial agents among Gram-negative bacteria, especially the molecular epidemiology of newer β-lactamases such as extended-spectrum β-lactamases and carbapenemases. She has published over 40 papers in this field.

Johann D. D. Pitout, M.D., is a Medical Microbiologist and Professor at the University of Calgary and Calgary Laboratory Services. His main research interests are resistance to antimicrobial agents among Gram-negative bacteria, especially the laboratory detection, characterization, and molecular epidemiology of bacteria with newer β-lactamases such as AmpC, extended-spectrum β-lactamases, and carbapenemases. He has also been involved in population-based surveillance studies and studies of the role of high-risk clones among bacteria producing these newer types of β-lactamases. He has published over 120 papers in this field.