Epidemiologic Analysis of a Worldwide Collection of *Escherichia coli* ST131 Using the 1928D Core Genome Multilocus Sequence Typing Reveals Country Specific and Globally Disseminated Clades

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Introduction

- Extraintestinal lineage of pathogenic *Escherichia coli* belonging to multilocus sequence type (ST) 131 has emerged as an international multidrug-resistant (MDR) high-risk clone
- Increasing antimicrobial resistance among *E. coli* isolates worldwide can be associated with the expansion of ST131 isolates that harbor virulence factors and cause more severe infections when compared to other antimicrobial-resistant *E. coli* isolates
- Analyzing core genomic sequencing data using >2,500 genes instead of multilocus sequence typing (MLST) that evaluates only 7 genes enables further discrimination of this global clone
- We evaluated the epidemiology of a global ST131 *E. coli* collection and unrelated STs using the core genome MLST (cgMLST) and resistance gene profiles generated by the bioinformatics tool 1928D to better understand the evolution and dissemination of ST131 isolates and compared them to a small set of non-ST131 *E. coli*

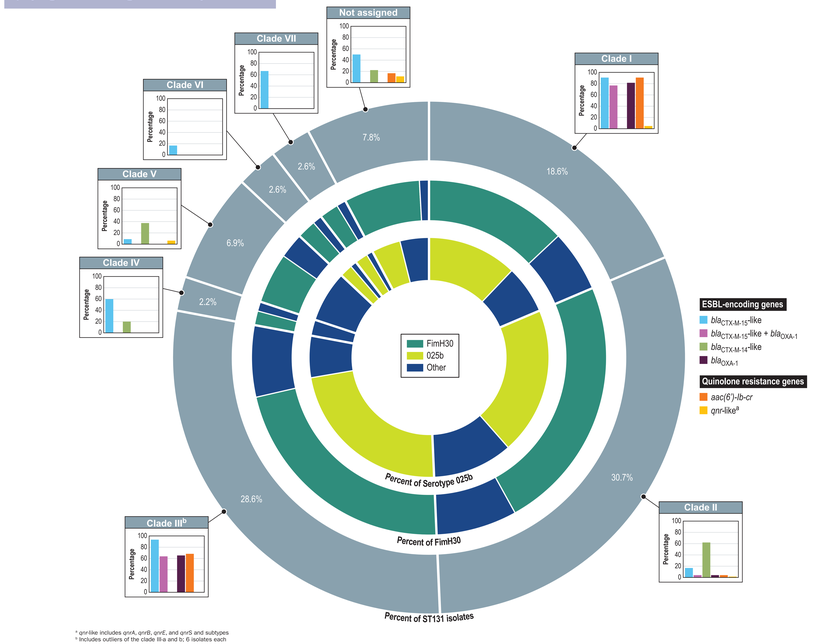
Materials and Methods

- A total of 259 *E. coli* clinical isolates belonging to ST131 (n=206), ST131-single loci variants (SLV; n= 25), and non-ST131 (n=28) collected from 27 countries during 2016-2018 were selected for analysis
- All isolates were subjected to whole genome sequencing on MiSeq (Illumina, San Diego, CA, USA)
  - High-quality input DNA was extracted and purified using the KingFisher Cell and Tissue DNA kit (Thermo Scientific, Waltham, Massachusetts, USA) in a robotic workstation KingFisher™ Flex Magnetic Particle Processor (Thermo Scientific)
  - DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina) and sequenced with a target depth of coverage >70X
  - Each raw data set was quality assured, error corrected, and assembled using SPAdes v. 3.11.0
  - An *in-silico* PCR with zero mismatches as described in the literature was used to identify serotype 025b, and Fimtyper (https://cge.cbs.dtu.dk/services/FimTyper/) was used to identify isolates belonging to FimH30 type
- Raw reads (FASTQ files) were uploaded onto the 1928 Diagnostics *E. coli* platform (1928D v. 2019-07.5) for analysis
  - MLST, cgMLST, and resistance gene prediction outputs were generated
  - Custom analysis was performed comparing >2,500 genes to determine allele differences and core genome MLST analysis among isolates
  - Nodes on the phylogenetic tree with distances of ≤50 alleles were selected for designating clades, between 51 and 60 were considered outliers of the clade, and >60 were considered distinct clades
- Allelic distance among the major clades assigned was analyzed to identify alleles that distinguish these clades to add further granularity

Results

- Among 231 ST131 and SLV *E. coli* isolates, 7 clades were identified applying cgMLST allele distance of ≤50 as a cutoff (Table 1, Figure 1)
  - The majority of ST131 isolates were categorized into clades I, II, or III (77.9%; 180/231)
  - Isolates within the 3 major clades were observed on multiple continents (Table 1)
  - A total of 18 isolates were not assigned to clades since their allelic distance from ST131 clades was >60
- The most allelic distance between the 7 ST131 clades was 216, while unrelated STs showed variable allelic distance among isolates within that ST
  - Isolates belonging to ST1193 were closely related genetically and displayed an allelic distance of 30 despite originating from different continents (countries of Australia, United States, and Brazil)
  - Other STs showed more variability among isolates (ST167, allelic distance 552; ST38, allelic distance 150; and ST69, allelic distance 179)
  - >2,000 differences were observed among ST131 and unrelated types
- Clades I, II, and III were very similar and only showed <60 allelic distance
  - Based on >95% concordance, 11 alleles differentiated clades II and III from clade I, while 6 alleles separated clades I and III from clade II (Table 2)
  - Outliers of clade III were not included in this analysis
- Isolates in clades I to IV were ciprofloxacin resistant (MIC, ≥4 mg/L) and the majority carried an extended-spectrum β-lactamase gene (164/185, 91.3%; Figure 1)
  - Isolates in clades I and III predominantly carried $bla_{CTX\text{-}M\text{-}15}$ (39/43 and 62/66), $bla_{OXA\text{-}1}$ (35/43 and 43/66), and *aac(6')-Ib-cr* (39/43, 45/66)
  - The majority of isolates in clade II carried $bla_{CTX\text{-}M\text{-}14}$-like (44/71) and less frequently carried $bla_{CTX\text{-}M\text{-}15}$-like (11/71) and/or *aac(6')Ib-cr* (3/71)
- Other resistance genes prevalent among 231 ST131 isolates were *dfrA17* (118 isolates), *tetA* (119), *sul1* (138), and efflux pumps *qacE* (138) and/or *yphE* (228)
  - Transferrable cephalosporinases (AmpCs) were present in a minority of isolates (bla_{CMY}-like in 4 isolates and $bla_{DHA\text{-}1}$ in 2)
- The prevalence of type 1 fimbrin D-mannose specific adhesin, FimH30, was similar among isolates belonging to ST131 (173/231; 74.9%) and non-ST131 (20/28; 71.4%) types (data not shown)
  - Overall, a smaller number of isolates belonged to FimH41 (n=22) and FimH27 (n=13)
- A significant number of isolates belonging to ST131 clades I, II, III, VI, and VII (64.8-80.3%) were identified as ST131-025b serotype, while only 1 of 28 (3.6%) non-ST131 belonged to this serotype (Figure 1)

Figure 1 Characteristics of ST131 clades identified using high resolution phylogenetic tree generated by 1928D



[a] qnr-like includes *qnrA*, *qnrB*, *qnrE*, and *qnrS* and subtypes
[b] Includes outliers of the clade III-a and b; 6 isolates each

Table 1 *E. coli* ST131 isolates distributed among clades based on the cutoff (≤50 allelic distance) criteria and relevant characteristics

Clade	Allele distance[a]	Geographic distribution	No. of isolates	Predominant resistance mechanism (no. positive)	
				ESBL	Quinolone R
I	43	9 countries including USA	43	$bla_{CTX\text{-}M\text{-}15}$ (39), $bla_{OXA\text{-}1}$ (35)	*aac(6')-Ib-cr* (39)
II	47	12 countries including USA	71	$bla_{CTX\text{-}M\text{-}14}$-like[b] (44)	*aac(6')-Ib-cr* (3), *qnrB19* (1)
III	49 (53)	13 countries including USA	66	$bla_{CTX\text{-}M\text{-}15}$ (61), $bla_{OXA\text{-}1}$ (43)	*aac(6')-Ib-cr* (45)
IV	40	Australia and USA	5	$bla_{CTX\text{-}M\text{-}15}$ (3), $bla_{CTX\text{-}M\text{-}14}$-like[b] (1)	*qnrS1* (1)
V	45 (55)	8 countries including USA	16	$bla_{CTX\text{-}M\text{-}15}$-like (6)	—
VI	49	Costa Rica and USA	6	$bla_{CTX\text{-}M\text{-}214}$ and $bla_{CTX\text{-}M\text{-}3}$ (1)	—
VII	28	Costa Rica and USA	6	$bla_{CTX\text{-}M\text{-}15}$ (4)	—

[a]Number in parentheses is allele distance (cgMLST) for outliers of the clade.
[b]Includes $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}64}$, $bla_{CTX\text{-}M\text{-}65}$ and $bla_{CTX\text{-}M\text{-}91}$
ESBL, extended-spectrum β-lactamase; R, resistant

Table 2 Allelic differences between ST131 clades I, II, and III

Gene identifier	Clade I allele	Occurrence % in clade I	Clade II allele	Occurrence % in clade II	Clade III allele	Occurrence % in clade III	Different clade
RS00050	22	100	25	100	25	100	Clade I
RS00065	45	100	46	100	46	98.1	Clade I
RS00070	45	97.7	44	98.6	44	100	Clade I
RS00090	59	100	57	100	57	98.1	Clade I
RS00100	42	100	44	100	44	100	Clade I
RS00105	33	100	35	100	35	100	Clade I
RS00110	53	100	58	98.6	58	98.1	Clade I
RS00120	68	100	63	98.6	63	98.1	Clade I
RS05065	41	100	38	100	38	100	Clade I
RS15715	19	95.3	6	98.6	6	98.1	Clade I
RS23945	27	100	17	100	17	100	Clade I
RS02515	61	97.7	59	100	61	98.1	Clade II
RS02720	52	100	44	100	52	98.1	Clade II
RS17410	62	100	64	100	62	100	Clade II
RS23535	55	97.7	56	100	55	98.1	Clade II
RS24430	78	100	77	100	78	100	Clade II
RS25820	82	97.7	81	98.6	82	100	Clade II

Allele occurrence of ≥95.0% was selected to differentiate clades.
Rows highlighted in orange represent alleles that distinguish clade I from clades II and III. Blue rows represent alleles that distinguish clade II from clades I and III. Allele numbers are consecutive numbers assigned by the software for more than 2,500 gene sequences compared.

Conclusions

- The cgMLST 1928D is a robust platform for epidemiological analysis of *E. coli* isolates, providing additional granularity when compared to MLST
  - A threshold of ≤50 cgMLST distance was useful for classifying isolates into clades
- Clades II and III were closely related, but carried different $bla_{CTX\text{-}M}$ genes, while clades I and III were not as closely related, but both carried $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, and *aac(6')-Ib-cr*
  - These findings suggest that these clades might have acquired resistance genes at different points in their genetic evolution
- *E. coli* ST131 clone has a global presence with $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}14}$ as the major β-lactamases and *aac(6')-Ib-cr* as the aminoglycoside/quinolone resistance mechanism
- FimH30 and serotype 025b are predominant in ST131 isolates

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