AAC Journals.ASM.org

Molecular Features of Community-Associated Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Strains in the United States

Fupin Hu,[a,b,c] Jessica A. O'Hara,[a] Jesabel I. Rivera,[a] Yohei Doi[a]

Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA[a]; Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai, China[b]; Key Laboratory of Clinical Pharmacology of Antibiotics, Ministry of Health, Shanghai, China[c]

We characterized 30 community-associated extended-spectrum-β-lactamase-producing *Escherichia coli* isolates collected from five hospitals in the United States. Nineteen sequence types were identified. All sequence type 131 (ST131) isolates had the *fimH*30 allele. IncFII-FIA-FIB was the most common replicon type among the bla_{CTX-M}-carrying plasmids, followed by IncFII-FIA and IncA/C. Restriction analysis of the IncFII-FIA-FIB and IncFII-FIA plasmids yielded related profiles for plasmids originating from different hospitals. The plasmids containing bla_{CTX-M} or bla_{SHV} were stably maintained after serial passages.

Since its emergence in the 1980s, extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* has continued to spread worldwide (1). Besides the resistance to penicillins and cephalosporins conferred by the production of ESBL, these organisms are frequently resistant to other classes of agents, including aminoglycosides, trimethoprim-sulfamethoxazole, and fluoroquinolones, which limits the therapeutic options for infections caused by ESBL-producing organisms (2). In addition to the steadily rising overall prevalence of ESBL-producing *E. coli* strains, the last decade has observed a major epidemiological shift in these organisms, as they have escaped the health care environment to cause community-associated infections in individuals with minimal or no recent exposure to the health care system (3, 4). This change has coincided with the expansion of a single clonal lineage of *E. coli*, defined as sequence type 131 (ST131) by multilocus sequence typing (MLST), which often produces CTX-M-type ESBLs. ST131 accounted for an estimated 28% of *E. coli* iso-

Received 13 May 2014 Returned for modification 5 July 2014
Accepted 10 August 2014
Published ahead of print 18 August 2014
Address correspondence to Yohei Doi, yod4@pitt.edu.

doi:10.1128/AAC.03321-14

TABLE 1 Antimicrobial susceptibilities of community-associated ESBL-producing *E. coli* isolates[a]

Agent(s)	Resistance profile (%)			MIC (μg/ml)		
	Susceptible	Intermediate	Resistant	50%	90%	Range
Ampicillin-sulbactam	33.3	60	6.7	16/8	16/8	≤4/2 to >16/8
Piperacillin-tazobactam	100	0	0	≤8/4	≤8/4	≤8/4
Ticarcillin-clavulanic acid	53.3	43.3	3.3	16/2	64/2	≤8/2 to >64/2
Ampicillin	0	0	100	>16	>16	>16
Piperacillin	6.7	20	73.3	>64	>64	≤16 to >64
Cefazolin	0	0	100	>16	>16	>16
Ceftriaxone	3.3	3.3	93.3	>32	>32	≤0.5 to >32
Ceftazidime	60	16.7	23.3	4	>16	≤1 to >16
Cefepime	63.3	10[b]	26.7	≤4	>32	≤4 to >32
Aztreonam	33.3	13.3	53.3	16	>16	≤1 to >16
Doripenem	100	0	0	≤0.5	≤0.5	≤0.5
Ertapenem	100	0	0	≤0.25	≤0.25	≤0.25
Imipenem	100	0	0	≤0.5	≤0.5	≤0.5
Meropenem	100	0	0	≤0.5	≤0.5	≤0.5
Amikacin	93.3	3.3	3.3	≤8	16	≤8 to >32
Gentamicin	60.0	3.3	36.7	≤2	>8	≤2 to >8
Tobramycin	43.3	10	46.7	8	>8	≤2 to>8
Ciprofloxacin	16.7	3.3	80	>2	>2	≤0.5 to >2
Levofloxacin	16.7	0	83.3	>8	>8	≤1 to >8
Trimethoprim-sulfamethoxazole	33.3	0	66.7	>4/76	>4/76	≤2/38 to >4/76
Nitrofurantoin	100	0	0	≤32	≤32	≤32
Minocycline	76.7	16.7	6.7	2	8	≤1 to >8
Tetracycline	30	0	70	>8	>8	≤4 to >8
Tigecycline	100	0	0	≤1	≤1	≤1

[a] $n = 30$.
[b] Dose-dependent susceptibility.

TABLE 2 Molecular characteristics of community-associated ESBL-producing *E. coli* isolates

Isolate	Location	Source	β-Lactamase(s)	*fimH*[a]	ST	Replicon type of the plasmid carrying ESBL-encoding genes	Plasmid-mediated coresistance[d]
CA01	Pennsylvania	Urine	CTX-M-15, CMY-2	−	617	NT[b]	None
CA02	Pennsylvania	Urine	SHV-2	+	906	NA[c]	NA
CA03	Pennsylvania	Urine	CTX-M-15	+	131	N	None
CA04	Pennsylvania	Blood	SHV-7	+	101	L/M	None
CA05	Pennsylvania	Urine	CTX-M-14	+	131	FII	None
CA06	Pennsylvania	Urine	CTX-M-14	−	127	FII	None
CA07	Michigan	Blood	CTX-M-15	+	131	NA	NA
CA08	Michigan	Urine	CTX-M-14	+	131	FII-FIA-FIB	SXT, TET
CA09	Michigan	Urine	CTX-M-15	+	90	FII-FIA-FIB	GEN, TET
CA10	Michigan	Urine	SHV-5	+	12	A/C	None
CA11	Michigan	Urine	CTX-M-15	−	38	Y	None
CA12	Michigan	Abscess	CTX-M-15	+	131	FII-FIA	SXT
CA13	Michigan	Urine	CTX-M-15	+	448	NT	None
CA14	Texas	Urine	CTX-M-15	+	131	FII-FIA-FIB	SXT, TET
CA15	Texas	Urine	CTX-M-15	+	1280	NT	None
CA16	Texas	Blood	CTX-M-14	+	1193	NT	SXT, TET
CA17	Texas	Urine	CTX-M-15	+	410	FII-FIA-FIB	TET
CA18	Texas	Urine	CTX-M-15	+	44	FII-FIA-FIB	GEN, SXT, TET
CA19	New York	Ascites	CTX-M-15	+	131	FII-FIA	SXT
CA20	New York	Urine	CTX-M-14	+	405	FII-FIB	SXT, TET
CA21	New York	Urine	SHV-5	−	648	NT	GEN, SXT
CA22	New York	Urine	SHV-5	+	744	A/C	SXT, TET
CA23	New York	Urine	CTX-M-15	−	167	FII-FIA-FIB	GEN, SXT
CA24	New York	Urine	CTX-M-15	−	3554	FIA-FIB	SXT, TET
CA25	Iowa	Urine	CTX-M-14	+	38	NA	NA
CA26	Iowa	Urine	CTX-M-15	+	131	NA	NA
CA27	Iowa	Urine	SHV-5	+	131	FIA-FIB	None
CA28	Iowa	Urine	CTX-M-15	+	44	FII-FIA-FIB	GEN, SXT, TET
CA29	Iowa	Urine	CTX-M-15	+	205	NA	NA
CA30	Iowa	Urine	CTX-M-15	+	131	FII-FIA	SXT, TET

[a] +, present; −, not present.
[b] NT, nontypeable.
[c] NA, no transformant available.
[d] SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; GEN, gentamicin.

lates in one study (5), with an even higher prevalence among ESBL-producing isolates in another (6).

We recently conducted a multicenter cohort study to document the occurrence of community-associated ESBL-producing *E. coli* infections in the United States (7). In this study, we identified 107 cases of community-associated ESBL-producing *E. coli* infections from five health care systems in a 1-year period. Fifty-four percent of them were due to *E. coli* ST131, and 91% produced CTX-M-type ESBLs. The findings confirmed the predominance of CTX-M-producing ST131 isolates among community-associated ESBL-producing *E. coli* infections in the United States, but it also indicated that nearly half of the infections were not due to ST131. In addition, little is known about the nature of plasmids carrying genes encoding CTX-M-type or other ESBLs in ST131 and other clonal lineages associated with their spread in the community.

The objective of this follow-up study was to conduct further molecular analysis of representative community-associated ESBL-producing *E. coli* isolates and identify clones and plasmids involved in this emerging phenomenon in the United States. Thirty community-associated ESBL-producing *E. coli* isolates collected at hospitals and their affiliated clinics in 5 locations in the United States (New York, Pennsylvania, Michigan, Texas, and Iowa) between 2009 and 2010 were included. They were selected from the 107 community-associated ESBL-producing *E. coli* isolates reported earlier (8), in order to represent different locations (5 to 7 isolates per location), ESBLs (19 isolates with CTX-M-15, 5 isolates with CTX-M-14, and 6 isolates with SHV-type ESBL), and ST131 status (10 ST131 isolates and 20 non-ST131 isolates). Non-ST131 isolates were overrepresented, since the molecular epidemiology of *E. coli* ST131 isolates in the United States has been investigated in detail (5, 9, 10), whereas less is known about ESBL-producing non-ST131 isolates circulating in the community.

Antimicrobial susceptibility testing was performed by the broth microdilution method using Sensititre GN4F (Trek Diagnostics, Oakwood Village, OH) and interpreted according to the latest Clinical and Laboratory Standards Institute (CLSI) breakpoints (7, 11). For tigecycline, the breakpoint recommended by the U.S. Food and Drug Administration was used. Of the 30 isolates tested, 93.3% were resistant to ceftriaxone, while 60% and 63.3% were susceptible to ceftazidime and cefepime, respectively (Table 1). The rates of resistance to ciprofloxacin and levofloxacin were 80% and 83.3%, respectively. A total of 36.7%, 46.7%, and 3.3% of the isolates were resistant to gentamicin, tobramycin, and amikacin, respectively. Among other classes of agents, 66.7% were resistant to trimethoprim-sulfamethoxazole, 6.7% were resistant to minocycline, and 70% were resistant to tetracycline. All isolates

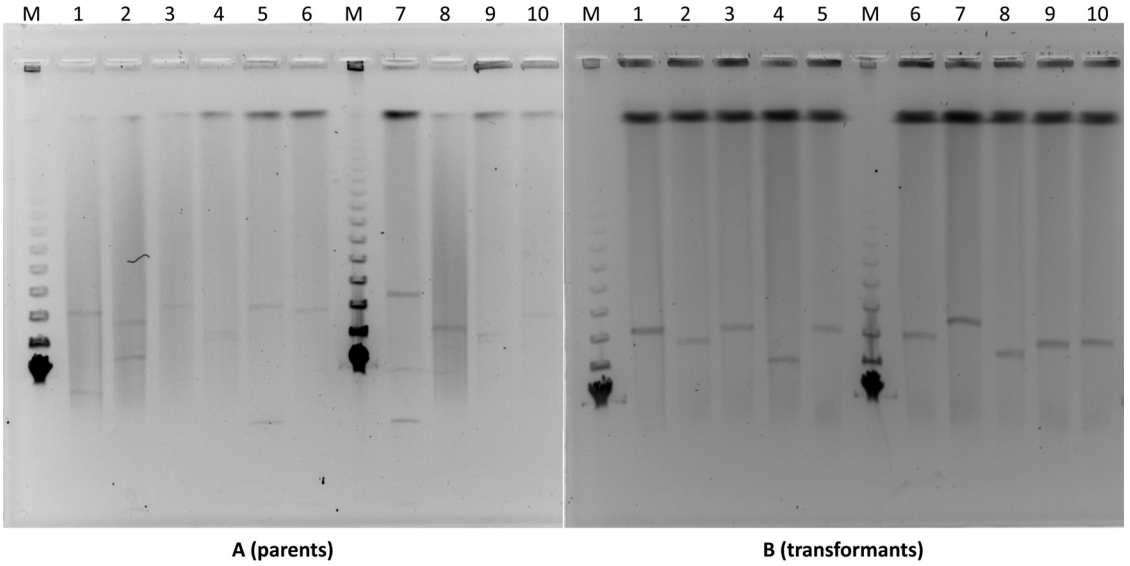

FIG 1 Plasmid analysis of IncFII plasmids carrying genes encoding CTX-M-type ESBLs in community-associated *E. coli* isolates and their transformants. IncFII plasmids represent the largest plasmids in the parents ranging approximately between 100 and 200 kb. Lane 1, CA08; lane 2, CA09; lane 3, CA14; lane 4, CA17; lane 5, CA18; lane 6, CA23; lane 7, CA28; lane 8, CA12; lane 9, CA19; lane 10, CA30; lane M, molecular marker.

were susceptible to carbapenems, piperacillin-tazobactam, nitrofurantoin, and tigecycline.

Multilocus sequence typing (MLST) was performed as previously described (12). New STs were registered through the *E. coli* MLST website (see http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). The ST131 isolates were further subtyped using the *fimH* sequence (13). MLST of the 30 ESBL-producing isolates identified 19 different STs (Table 2). As expected from the inclusion criteria (10 presumptive ST131 isolates based on PCR screening [7, 14]), 10 isolates belonged to ST131. Seven of them were associated with $bla_{\text{CTX-M-15}}$, while 2 isolates and 1 isolate were associated with $bla_{\text{CTX-M-14}}$ and $bla_{\text{SHV-5}}$, respectively. Two isolates each had ST38 and ST44 associated with $bla_{\text{CTX-M-15}}$ or $bla_{\text{CTX-M-14}}$, originating from hospitals in different states (ST38 from Michigan and Iowa and ST44 from Iowa and Texas). ST38 has been reported in CTX-M-producing *E. coli* worldwide, including isolates from community-associated infections, whereas less is known about ST44 (15–18). The remaining 16 STs only occurred in 1 isolate each. Twenty-four of the isolates were positive for *fimH*. All 10 ST131 isolates had *fimH*30, which assigned them to the highly virulent *H*30 subclone of ST131 (19). The high degree of clonal diversity of the non-ST131 isolates suggests that although the success of ST131 is clearly a major factor in the emerging epidemic of community-associated ESBL-producing *E. coli* isolates, nonmicrobiological factors may also be responsible for the spread of ESBL-producing non-ST131 isolates into the community.

Plasmid DNA was extracted from the clinical isolates using the standard alkaline lysis method (20). *E. coli* strain DH10B competent cells were transformed with the plasmid DNA by electroporation, and β-lactamase-producing transformants were selected on lysogenic agar plates containing 50 μg/ml ampicillin. Transfer of the plasmids carrying ESBL-encoding genes was confirmed by PCR of $bla_{\text{CTX-M}}$ or bla_{SHV} and reduced susceptibility to cefotaxime and/or ceftazidime. The plasmid replicons were determined using DNA extracted from the *E. coli* DH10B transformants according to the protocol by Carattoli et al. (21). The *E. coli* DH10B transformants with plasmids carrying ESBL-encoding genes were obtained for 25 isolates. The frequently occurring plasmid replicons included IncFII-FIA-FIB (7 isolates), IncFII-FIA (3 isolates), and IncA/C (2 isolates) (Table 2). IncFII-FIA-FIB was identified in various STs, including ST131, ST44, ST90, ST167, and ST410, whereas IncFII-FIA was identified in ST131 only. Co-

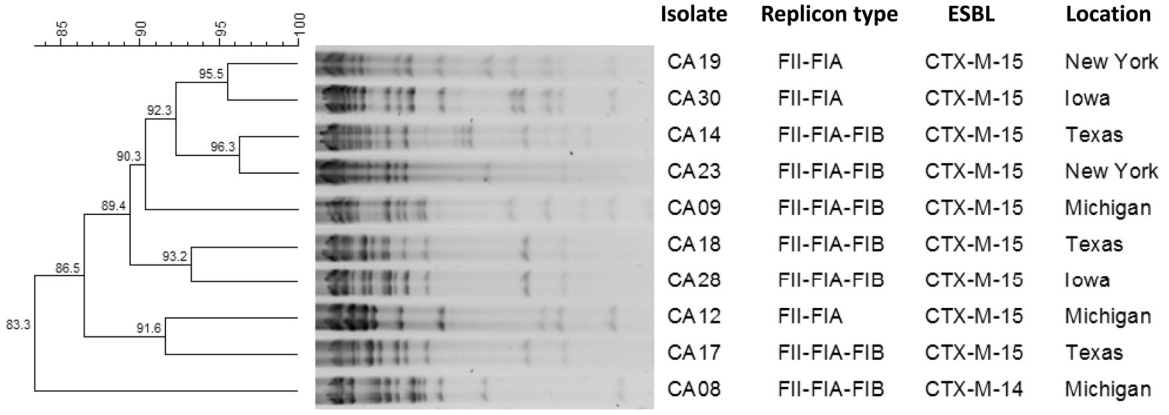

FIG 2 EcoRI plasmid restriction profiles of IncFII plasmids extracted from *E. coli* transformants.

TABLE 3 Stability of plasmids carrying ESBL-encoding genes in *E. coli* DH10 transformants

Isolate	β-Lactamase	Replicon type	No. in which bla_{CTX-M} or bla_{SHV} was detected by PCR (10 colonies/strain) by day[a]:							
			1	2	3	4	5	6	7	8
CA03TF	CTX-M-15	N	8 (+), 2 (−)	9 (+), 1 (−)	6 (+), 4 (−)	9 (+), 1 (−)	10 (−)	10 (−)	10 (−)	10 (−)
CA04TF	SHV-7	L/M	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA05TF	CTX-M-14	FII	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA06TF	CTX-M-14	FII	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA08TF	CTX-M-14	FII-FIA-FIB	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA09TF	CTX-M-15	FII-FIA-FIB	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA10TF	SHV-5	A/C	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA11TF	CTX-M-15	Y	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA12TF	CTX-M-15	FII-FIA	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA18TF	CTX-M-15	FII-FIA-FIB	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA20TF	CTX-M-14	FII-FIB	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA22TF	SHV-5	A/C	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA24TF	CTX-M-15	FIA-FIB	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)
CA28TF	CTX-M-15	FII-FIA-FIB	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	6 (+), 4 (−)
CA30TF	CTX-M-15	FII-FIA	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)

[a] +, positive; −, negative.

resistance to the non-β-lactam agents gentamicin, trimethoprim-sulfamethoxazole, or tetracycline was observed for 15 of the 25 transformants using the disk diffusion method (Table 2). Notably, all IncFII-FIA-FIB and IncFII-FIA plasmids conferred resistance to at least one non-β-lactam agent (trimethoprim-sulfamethoxazole, gentamicin, and/or tetracycline). Two IncFII-FIA-FIB plasmids conferred resistance to trimethoprim-sulfamethoxazole, tetracycline, and gentamicin. These multidrug resistance traits of IncFII-FIA-FIB and IncFII-FIA plasmids may provide them with a selective advantage. Furthermore, multireplicon IncF plasmids are considered to possess an ability to evolve the regulatory sequences for these replicons, and this versatility has been associated with the abrupt worldwide emergence of IncF plasmids carrying bla_{CTX-M} (22). In particular, IncFII plasmids carrying bla_{CTX-M} have been widely reported (23–25). The IncFII-FIA-FIB and IncFII-FIA plasmids were found across various STs, including ST131 isolates, suggesting that they may be serving as the vehicles for the spread of bla_{CTX-M} between ST131 isolates and non-ST131 isolates in the community.

For the 10 IncFII-FIA-FIB and IncFII-FIA plasmids, their sizes were estimated by S1 pulsed-field gel electrophoresis (PFGE) for the clinical isolates and their transformants (26). All of the tested clinical isolates possessed >1 plasmid, with some possessing up to 3 (Fig. 1). The sizes of the IncFII-FIA-FIB and IncFII-FIA plasmids varied, ranging from approximately 100 to 200 kb. They were then extracted from the transformants, digested with EcoRI, and profiled by an overnight electrophoresis on a 0.7% agarose gel. Dendrograms representing the similarities of the restriction patterns were constructed according to the unweighted-pair group method using average linkages (UPGMA) using BioNumerics version 6.01 (Applied Maths, Austin, TX). The plasmid restriction analysis yielded 4 related profiles using a similarity cutoff of 90%, with each profile containing 2 isolates from hospitals in different states (Fig. 2).

To determine the stability of the plasmids carrying ESBL-encoding genes, 15 representative transformants containing bla_{CTX-M} ($n = 12$) or bla_{SHV} ($n = 3$) with plasmids of various replicon types were grown separately in lysogenic broth free of antimicrobial agents at 37°C with shaking. After overnight growth, the cells were diluted into fresh lysogenic broth to a concentration of approximately 10^3 CFU/ml and incubated further for 8 successive days. The colonies were isolated on lysogenic agar plates, and 10 random colonies were subjected to PCR to determine the presence or absence of the ESBL genes (bla_{CTX-M} or bla_{SHV}, as appropriate) from each culture on each day (a total of 80 colonies for each transformant). One transformant completely lost its IncN plasmid by day 5, and another partially lost its IncFII-FIA-FIB plasmid on day 8 (Table 3). Otherwise, none of the remaining 13 transformants lost bla_{CTX-M} or bla_{SHV} during the 8 days of serial broth culture. Therefore, plasmids containing bla_{CTX-M} or bla_{SHV} were stably maintained in the majority of the isolates, even in the absence of selective pressure, suggesting that the cost of maintaining these plasmids carrying ESBL-encoding genes may be minimal for the host *E. coli*.

The emergence of community-associated ESBL-producing *E. coli* infections in the United States is driven by the epidemic ST131 clone and diverse non-ST131 clones. The most common IncFII-FIA-FIB and IncFII-FIA plasmids occur in ST131, as well as non-ST131 isolates in distant states, suggesting the potential role of plasmid-mediated dissemination of ESBL-producing *E. coli* into and within the community. Further genetic characterization of these key groups of plasmids is ongoing.

ACKNOWLEDGMENTS

We thank the participating sites for collecting the community-associated *E. coli* isolates used in the study.

Y.D. was supported by research grants from the National Institutes of Health (R21AI107302 and R01AI104895).

REFERENCES

1. **Kang CI, Cheong HS, Chung DR, Peck KR, Song JH, Oh MD, Choe KW.** 2008. Clinical features and outcome of community-onset bloodstream infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli*. Eur. J. Clin. Microbiol. Infect. Dis. **27**:85–88. http://dx.doi.org/10.1007/s10096-007-0401-6.
2. **Minarini LA, Gales AC, Palazzo IC, Darini AL.** 2007. Prevalence of community-occurring extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Brazil. Curr. Microbiol. **54**:335–341. http://dx.doi.org/10.1007/s00284-006-0307-z.
3. **Rupp ME, Fey PD.** 2003. Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. Drugs **63**:353–365. http://dx.doi.org/10.2165/00003495-200363040-00002.

4. **Rodríguez-Baño J, Alcalá JC, Cisneros JM, Grill F, Oliver A, Horcajada JP, Tórtola T, Mirelis B, Navarro G, Cuenca M, Esteve M, Peña C, Llanos AC, Cantón R, Pascual A.** 2008. Community infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*. Arch. Intern. Med. **168:**1897–1902. http://dx.doi.org/10.1001/archinte.168.17.1897.
5. **Colpan A, Johnston B, Porter S, Clabots C, Anway R, Thao L, Kuskowski MA, Tchesnokova V, Sokurenko EV, Johnson JR, VICTORY (Veterans Influence of Clonal Types on Resistance: Year 2011) Investigators.** 2013. *Escherichia coli* sequence type 131 (ST131) subclone *H*30 as an emergent multidrug-resistant pathogen among U.S. veterans. Clin. Infect. Dis. **57:**1256–1265. http://dx.doi.org/10.1093/cid/cit503.
6. **Johnson JR, Urban C, Weissman SJ, Jorgensen JH, Lewis JS, Jr, Hansen G, Edelstein PH, Robicsek A, Cleary T, Adachi J, Paterson D, Quinn J, Hanson ND, Johnston BD, Clabots C, Kuskowski MA, AMERECUS Investigators.** 2012. Molecular epidemiological analysis of *Escherichia coli* sequence type ST131 (O25:H4) and $bla_{\text{CTX-M-15}}$ among extended-spectrum-β-lactamase-producing *E. coli* from the United States, 2000 to 2009. Antimicrob. Agents Chemother. **56:**2364–2370. http://dx.doi.org/10.1128/AAC.05824-11.
7. **Doi Y, Park YS, Rivera JI, Adams-Haduch JM, Hingwe A, Sordillo EM, Lewis JS, Jr, Howard WJ, Johnson LE, Polsky B, Jorgensen JH, Richter SS, Shutt KA, Paterson DL.** 2013. Community-associated extended-spectrum β-lactamase-producing *Escherichia coli* infection in the United States. Clin. Infect. Dis. **56:**641-648. http://dx.doi.org/10.1093/cid/cis942.
8. **Kim YA, Qureshi ZA, Adams-Haduch JM, Park YS, Shutt KA, Doi Y.** 2012. Features of infections due to *Klebsiella pneumoniae* carbapenemase-producing *Escherichia coli*: emergence of sequence type 131. Clin. Infect. Dis. **55:**224-231. http://dx.doi.org/10.1093/cid/cis387.
9. **Johnson JR, Nicolas-Chanoine MH, DebRoy C, Castanheira M, Robicsek A, Hansen G, Weissman S, Urban C, Platell J, Trott D, Zhanel G, Clabots C, Johnston BD, Kuskowski MA, MASTER Investigators.** 2012. Comparison of *Escherichia coli* ST131 pulsotypes, by epidemiologic traits, 1967–2009. Emerg. Infect. Dis. **18:**598–607. http://dx.doi.org/10.3201/eid1804.111627.
10. **Johnson JR, Tchesnokova V, Johnston B, Clabots C, Roberts PL, Billig M, Riddell K, Rogers P, Qin X, Butler-Wu S, Price LB, Aziz M, Nicolas-Chanoine MH, Debroy C, Robicsek A, Hansen G, Urban C, Platell J, Trott DJ, Zhanel G, Weissman SJ, Cookson BT, Fang FC, Limaye AP, Scholes D, Chattopadhyay S, Hooper DC, Sokurenko EV.** 2013. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. J. Infect. Dis. **207:**919–928. http://dx.doi.org/10.1093/infdis/jis933.
11. **CLSI.** 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
12. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x.
13. **Weissman SJ, Johnson JR, Tchesnokova V, Billig M, Dykhuizen D, Riddell K, Rogers P, Qin X, Butler-Wu S, Cookson BT, Fang FC, Scholes D, Chattopadhyay S, Sokurenko E.** 2012. High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. Appl. Environ. Microbiol. **78:**1353–1360. http://dx.doi.org/10.1128/AEM.06663-11.
14. **Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, Mulvey MR, Nordmann P, Ruppé E, Sarthou JL, Frank T, Vimont S, Arlet G, Branger C, Woodford N, Denamur E.** 2009. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. J. Antimicrob. Chemother. **64:**274–277. http://dx.doi.org/10.1093/jac/dkp194.
15. **Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y.** 2009. Change in the prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* in Japan by clonal spread. J. Antimicrob. Chemother. **63:**72–79. http://dx.doi.org/10.1093/jac/dkn463.
16. **van der Bij AK, Peirano G, Goessens WH, van der Vorm ER, van Westreenen M, Pitout JDD.** 2011. Clinical and molecular characteristics of extended-spectrum-β-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam area, Netherlands. Antimicrob. Agents Chemother. **55:**3576–3578. http://dx.doi.org/10.1128/AAC.00074-11.
17. **Peirano G, van der Bij AK, Gregson DB, Pitout JD.** 2012. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J. Clin. Microbiol. **50:**294–299. http://dx.doi.org/10.1128/JCM.06025-11.
18. **Park SH, Byun JH, Choi SM, Lee DG, Kim SH, Kwon JC, Park C, Choi JH, Yoo JH.** 2012. Molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* in the community and hospital in Korea: emergence of ST131 producing CTX-M-15. BMC Infect. Dis. **12:**149. http://dx.doi.org/10.1186/1471-2334-12-149.
19. **Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, Nordstrom L, Billig M, Chattopadhyay S, Stegger M, Andersen PS, Pearson T, Riddell K, Rogers P, Scholes D, Kahl B, Keim P, Sokurenko EV.** 2013. The epidemic of extended-spectrum-β-lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, *H*30-Rx. mBio **4**(6):e00377-13. http://dx.doi.org/10.1128/mBio.00377-13.
20. **Sambrook J, Russell D.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
22. **Villa L, García-Fernández A, Fortini D, Carattoli A.** 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J. Antimicrob. Chemother. **65:**2518–2529. http://dx.doi.org/10.1093/jac/dkq347.
23. **Peirano G, Costello M, Pitout JD.** 2010. Molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* from the Chicago area: high prevalence of ST131 producing CTX-M-15 in community hospitals. Int. J. Antimicrob. Agents **36:**19–23. http://dx.doi.org/10.1016/j.ijantimicag.2010.02.016.
24. **Naseer U, Haldorsen B, Tofteland S, Hegstad K, Scheutz F, Simonsen GS, Sundsfjord A, Norwegian ESBL Study Group.** 2009. Molecular characterization of CTX-M-15-producing clinical isolates of *Escherichia coli* reveals the spread of multidrug-resistant ST131 (O25:H4) and ST964 (O102:H6) strains in Norway. APMIS **117:**526–536. http://dx.doi.org/10.1111/j.1600-0463.2009.02465.x.
25. **Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. J. Antimicrob. Chemother. **68:**2249–2254. http://dx.doi.org/10.1093/jac/dkt208.
26. **Bueno MF, Francisco GR, O'Hara JA, de Oliveira Garcia D, Doi Y.** 2013. Coproduction of 16S rRNA methyltransferase RmtD or RmtG with KPC-2 and CTX-M group extended-spectrum β-lactamases in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **57:**2397-2400. http://dx.doi.org/10.1128/AAC.02108-12.