Journal of Global Antimicrobial Resistance 13 (2018) 81–84

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Association of *Escherichia coli* ST131 lineage with risk of urinary tract infection recurrence among young women

Miriam D. Ismail[1], Ihsan Ali[2], Savannah Hatt, Elizabeth A. Salzman, Anna W. Cronenwett, Carl F. Marrs, Alexander H. Rickard, Betsy Foxman*

The Center for Molecular and Clinical Epidemiology of Infectious Diseases (MAC-EPID), Department of Epidemiology, University of Michigan, School of Public Health, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form 30 October 2017
Accepted 10 December 2017
Available online 16 December 2017

Keywords:
Escherichia coli
ST131
ESBL
Urinary tract infection
Recurrence

ABSTRACT

Objectives: The aim of this study was to test the hypothesis that urinary tract infections (UTIs) caused by *Escherichia coli* of the sequence type 131 (ST131) lineage are more likely to recur than UTIs caused by other *E. coli* lineages.
Methods: Isolates from 221 young women with UTI caused by *E. coli* participating in a randomised controlled trial were used. Participants were followed for 6 months or until UTI recurrence.
Results: Sequence type was not associated with risk of recurrence. Isolates in the ST131 lineage were more resistant than other STs to quinolones (6.2% vs. 1.3%) but not trimethoprim/sulfamethoxazole (15.4% vs. 15.0%).
Conclusions: These results do not support an increased risk of recurrent UTI among otherwise healthy women with UTI caused by *E. coli* ST131.
© 2017 International Society for Chemotherapy of Infection and Cancer. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Urinary tract infection (UTI) is the second most common bacterial infection, after otitis media, and is a major reason for antibiotic therapy [1]. Women aged 18–24 years are at especially high risk of UTI, with 1 in 5 women in this age group having a symptomatic UTI annually [2]. *Escherichia coli* causes three-quarters of all UTIs among outpatients [1]; however, certain *E. coli* lineages are more likely to cause UTI than others [3]. Uropathogenic lineages are generally more resistant to antibiotics than commensal *E. coli* [4], and one uropathogenic lineage, namely sequence type 131 (ST131), particularly sublineage ST131:O25b:H30, is associated with multidrug resistance [5]. It has also been suggested that *E. coli* ST131 is more persistent, as the median duration of rectal carriage of *E. coli* ST131 carrying extended-spectrum β-lactamase (ESBL) among 41 residents in a long-term care facility was 13 months compared with 2–3 months for 33 residents carrying other ESBL-producing *E. coli* [6]. Therefore, it is plausible that UTI caused by *E. coli* ST131 may be more likely to recur than UTI caused by other sequence types.

Recurrent UTI is a major cause of morbidity among young women [1]; the risk of a second UTI within 6 months following a first UTI is 24% [7]. Antibiotic resistance complicates treatment: as UTI in women is usually treated empirically, two or three courses of different antibiotics might be tried before cure is effective. Approximately one-half of recurrent UTIs (those that follow effective treatment) are caused by the same strain causing the previous UTI [7]. Recurrence is associated with various virulence factors, particularly Dr adhesins [8], but it is generally believed that a combination of host factors [9] and persistence of the uropathogen in the bowel or vaginal cavity [10] explain most recurrences.

The aim of this study was to test the hypothesis that UTIs caused by uropathogenic *E. coli* ST131 are more likely to recur than UTIs caused by other uropathogenic *E. coli* by comparing recurrence rates (excluding relapse) among otherwise healthy young women with UTI caused by *E. coli* of the ST131 lineage (including seven H30 isolates) with all other *E. coli*. Typing of *fumC*/*fimH* (CH), a high-resolution two-locus clonal typing system that maps to multilocus sequence types (MLSTs) with 95% accuracy and corresponds

* Corresponding author.
E-mail address: bfoxman@umich.edu (B. Foxman).
[1] Present address: Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD 21201, USA.
[2] Permanent address: Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

https://doi.org/10.1016/j.jgar.2017.12.006
2213-7165/© 2017 International Society for Chemotherapy of Infection and Cancer. Published by Elsevier Ltd. All rights reserved.

closely to MLST-based clonal groupings, was used [11]. Isolates and data were collected as part of a randomised controlled clinical trial, where the 6-month recurrence rate was 16.9% [12].

2. Materials and methods

Escherichia coli isolates ($n = 221$) were obtained from women participating in a 2005–2008 prospective, randomised, placebo-controlled trial of the therapeutic efficacy of cranberry juice in preventing recurrent UTIs [12]. Informed consent was obtained from the participating subjects, and the study protocol was approved by the Institutional Review Board of the University of Michigan (Ann Arbor, MI).

2.1. Inclusion/exclusion criteria

Inclusion criteria were women aged 18–40 years, with at least three urinary symptoms (painful and frequent urination, and report of either urgency, haematuria or suprapubic pressure) and a urine culture positive for *E. coli* (≥1000 CFU/mL urine of *E. coli*). Women were excluded if they had received antibiotic treatment in the previous 48 h, reported recent hospitalisation or catheterisation (previous 2 weeks), or had kidney stones, diabetes, pregnancy or a cranberry allergy. The initial UTI was treated with antibiotics (usually trimethoprim/sulfamethoxazole). Participants were followed for up to 6 months or until they developed a culture-confirmed UTI.

2.2. Definition of recurrence

Participants were followed until they experienced a second UTI or for 6 months, whichever came first. Recurrence was defined as a UTI that met the enrolment criteria and occurred >14 days after the treated enrolment UTI, or a UTI <15 days post-enrolment of a different *E. coli* strain by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR).

Of the 319 women participating in the original study, 252 had an initial UTI due to *E. coli*. Isolates had been saved and stored for 228 women, but enrolment isolates could not be located for 3 women, and 4 enrolment isolates could not be cultured from the freezer stock, leaving a total of 221 urinary *E. coli* isolates for study.

2.3. Antimicrobial susceptibility testing

Isolates were tested for susceptibility to ciprofloxacin, levofloxacin, ampicillin, cefazolin, ceftriaxone, ceftazidime, cefepime, aztreonam, piperacillin, nitrofurantoin and trimethoprim/sulfamethoxazole using a VITEK®2 system (bioMérieux, Durham, NC). ESBL production was determined using the Clinical and Laboratory Standards Institute (CLSI) confirmatory test [13].

2.4. CH typing

CH typing was performed using published conditions [11]. PCR reactions were carried out with 12.5 μL of Go*Taq*® DNA Polymerase (Promega, Madison, WI), 2.5 μL of forward and reverse primers, 2.5 μL of template DNA (extracted using QIAcube; QIAGEN, Hilden, Germany) and 2.5 μL of water. PCR products were run on agarose gels to confirm the presence of a band of the expected size. The products were subsequently purified (QIAquick PCR Purification Kit; QIAGEN) and were sequenced with forward and reverse primers (University of Michigan DNA Sequencing Core). The resulting sequences were trimmed and aligned using CodonCode Aligner. *fumC* and *fimH* types were assigned using the available web services (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli and https://cge.cbs.dtu.dk/services/FimTyper-1.0/) and NCBI BLAST and were compared with published CH types [11]. Three isolates could not be grown, therefore the CH type was not determined.

2.5. Data analysis

The χ^2 test was used to test for differences in recurrence. The 6-month risk of recurrence by sequence type was estimated and 95% confidence intervals were calculated using the binomial. SAS v.9.4 (SAS Institute Inc., Cary, NC) was used to perform all data analyses. Figures were prepared using R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/).

3. Results

Using CH typing [11], *E. coli* isolated from 221 young women with a physician-diagnosed UTI were typed and classified into ST complexes. The women were followed from time of UTI diagnosis for 6 months to estimate the risk of recurrence [12]. Overall, the mean participant age was 21.2 years (range 18–40 years). Prior to the index UTI, more than one-half (61.8%) of the women had a history of UTI; each participant had a mean of 3.9 previous UTIs. The most common ST complex among the initial infecting *E. coli* was ST131 (65/221; 29.4%), of which 7/65 (10.8%) were ST131:O25b:H30. ST95 (33/221; 14.9%) and ST69 (21/221; 9.5%) also occurred frequently (Fig. 1).

There were no statistically significant differences in the demographics or medical history of participants infected with *E. coli* ST131 complex versus non-ST131. Resistance to fluoroquinolones was higher among ST131 than non-ST131 isolates (6.2% vs. 1.3%), and the one isolate that exhibited an ESBL phenotype was ST131, but these effects were also not statistically significant (Table 1). None of the seven ST131:O25b:H30 isolates were resistant to any of the antibiotics. Among 221 women, 24 (10.9%) culture-confirmed recurrent UTI episodes were observed. There were no statistically significant differences in risk of recurrence by ST complex (Fig. 2).

4. Discussion

This study investigated whether a UTI caused by *E. coli* ST131 was more likely to cause recurrent UTI than other *E. coli* STs among 221 young women. Although multiple studies exist on the epidemiology of uropathogenic *E. coli* ST131 infections (see, e.g., [14]), limited information exists on its clinical relevance in recurring UTIs among healthy young women. At least in this population, UTI caused by ST131:O25b:H30 and others in the ST131 complex were no more likely to recur than *E. coli* of other STs.

Only 6.2% of the ST131 isolates in this study were resistant to quinolones and only one was ESBL-positive; ampicillin resistance was higher in ST131 isolates (41.5% vs. 30.1%) but other resistances occurred at similar rates to the non-ST131 isolates. Seven ST131:O25b:H30 *E. coli* were obtained, none of which were resistant to levofloxacin, ciprofloxacin or any other antibiotic.

The prevalence of antibiotic resistance reported here is consistent with that reported in a 2016 French population-based study of UTI outpatients where all urines were cultured, but which did not report sequence types [15]. However, the prevalence of ST131:O25b in the current study is lower than reported in 322 Australian female cystitis patients (13% ST131, most (88%) were ST131:O25b and 12% were ESBL-producers [16]), in 271 Hong Kong female UTI patients (8.5% were O25b-ST131; 69.6% of these isolates were ciprofloxacin-resistant but only 1 was an ESBL-producer [17]), and in a Turkish study of 294 men and women with acute UTI (12% were ST131:O25b:H30, and 64% of the 35 ST131 isolates

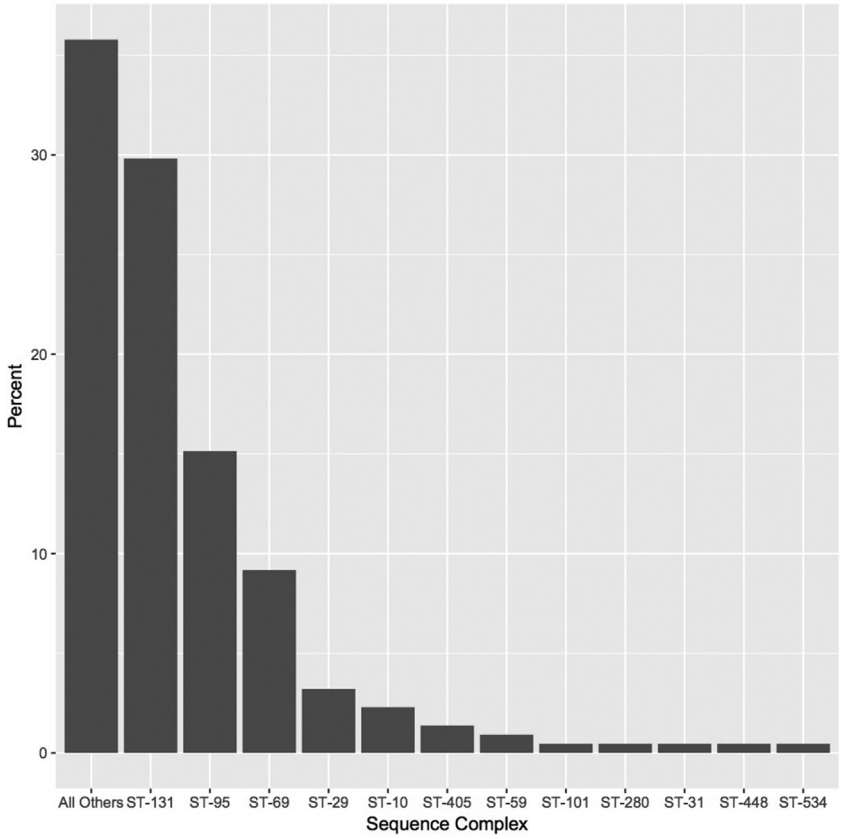


Fig. 1. Distribution (%) of 218 *Escherichia coli* isolates with selected sequence type (ST) complexes determined by *fumC*|*fimH* (CH) typing [11] among isolates from young women with culture-confirmed, physician-diagnosed urinary tract infection, 2005–2008.

produced ESBL [18]). These differences may reflect variation by time and space and study population. This may limit the generalisability to other populations, such as those in hospital, that are inherently more susceptible to adverse outcomes of infection.

The vast majority of UTIs occur among otherwise healthy young women. In this population, recurrence and treatment failure due to antibiotic resistance are major concerns. The current results suggest that among young women neither ST131:O25b:H30 nor other isolates for the ST131 lineage are associated with increased recurrence risk. Among young women with UTI, concerns about ST131:O25b:H30 and ST131 should focus on the association with antibiotic resistance since this has major therapeutic and economic implications.

Table 1
Characteristics of 221 women with urinary tract infections (UTIs) caused by *Escherichia coli* with associated antibiotic susceptibility profiles.[a]

	ST131 isolates ($n = 65$)	Non-ST131 isolates ($n = 156$)	All isolates ($n = 221$)	*P*-value[b]
Patient characteristics				
Age (years) (mean ± S.D.)	20.9 ± 3.20	21.4 ± 3.82	21.2 ± 3.65	0.41
UTI ever (%)[c]	41 (63.1)	93 (61.2)	134 (61.8)	0.79
No. of previous UTIs ever (mean ± S.D.)	3.3 ± 2.72	4.2 ± 4.41	3.9 ± 3.97	0.19
No. of UTIs in the past 12 months (mean ± S.D.)	1.2 ± 1.28	1.2 ± 2.19	1.2 ± 1.95	0.82
Intercourse ever (%)[c]	63 (98.4)	149 (97.4)	212 (97.7)	0.64
Frequency of intercourse past 4 weeks (mean ± S.D.)	9.0 ± 6.84	11.2 ± 9.84	10.5 ± 9.08	0.07
Recurrence within 6 months (%)	7 (10.8)	17 (10.9)	24 (10.9)	0.98
Antibiotic susceptibility [no. (%) resistant][d]				
Ciprofloxacin	4 (6.2)	2 (1.3)	6 (2.7)	0.07
Levofloxacin	4 (6.2)	2 (1.3)	6 (2.7)	0.07
Ampicillin	27 (41.5)	46 (30.1)	73 (33.5)	0.07
Nitrofurantoin	0 (0)	0 (0)	0 (0)	N/A
SXT	10 (15.4)	23 (15.0)	33 (15.1)	0.95
ESBL-positive	1 (1.5)	0 (0.00)	1 (0.5)	0.30

S.D., standard deviation; SXT, trimethoprim/sulfamethoxazole; ESBL, extended-spectrum β-lactamase.
[a] Isolates and data were collected as part of a randomised controlled clinical trial [12].
[b] χ^2 test for categorical variables, and Student *t* test for continuous variables.
[c] Data on UTI history was missing for 4 women ST131 negative; and on history of sexual intercourse for 3 women ST131 negative and 1 ST131 positive.
[d] Isolates were additionally tested for susceptibility to cefazolin, ceftriaxone, ceftazidime, cefepime, aztreonam and piperacillin. Only one isolate, which was subsequently determined to be an ESBL-producer, was resistant to these antibiotics. Resistance information for ampicillin, nitrofurantoin and SXT was not available for 3 ST131 negative isolates.

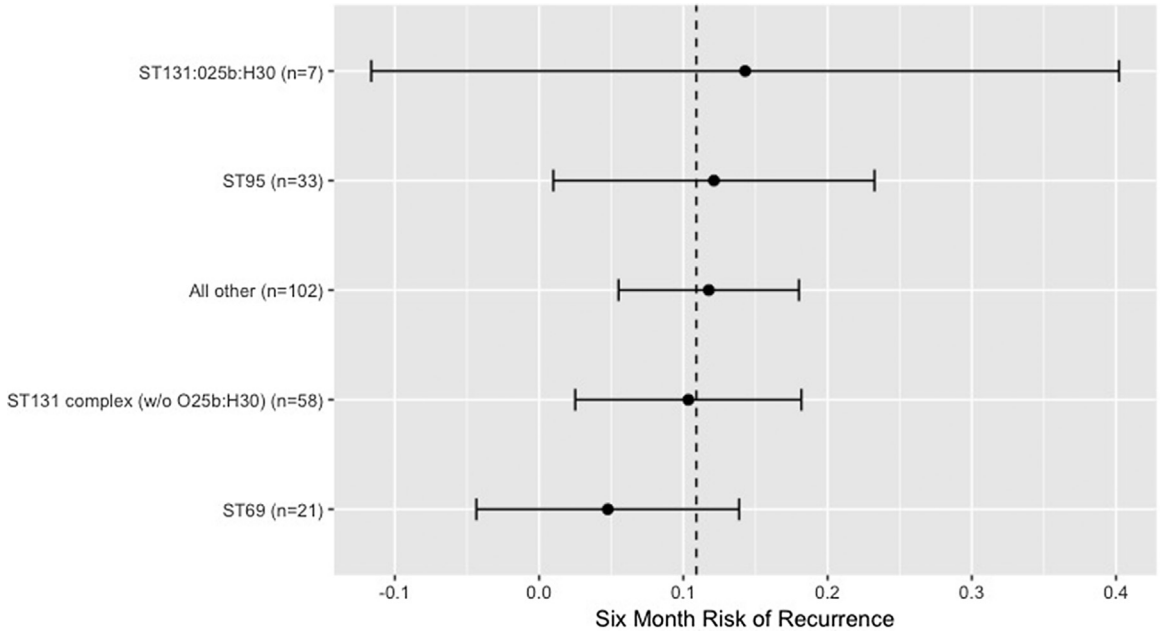


Fig. 2. Forest plot of the 6-month risk of a second urinary tract infection (UTI) by sequence type (ST) of initial infecting *Escherichia coli* among 221 otherwise healthy young women with UTI, 2005–2008. Bars show 95% confidence intervals around the risk estimates. Dotted line corresponds to average risk of recurrence in the population (10.9%).

Acknowledgments

The authors thank all of the members of the MAC-EPID team for technical assistance and for their editing contribution.

References

[1] Foxman B.. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am 2014;28:1–13.
[2] Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. Ann Epidemiol 2000;10: 509–15.
[3] Lo Y, Zhang L, Foxman B, Zöllner S. Whole-genome sequencing of uropathogenic *Escherichia coli* reveals long evolutionary history of diversity and virulence. Infect Genet Evol 2015;34:244–50.
[4] Dang TND, Zhang L, Zöllner S, Srinivasan U, Abbas K, Marrs CF, et al. Uropathogenic *Escherichia coli* are less likely than paired fecal *E. coli* to have CRISPR loci. Infect Genet Evol 2013;19:212–8.
[5] Graham SE, Zhang L, Ali I, Cho YK, Ismail MD, Carlson HA, et al. Prevalence of CTX-M extended-spectrum β-lactamases and sequence type 131 in Korean blood, urine, and rectal *Escherichia coli* isolates. Infect Genet Evol 2016;41:292–5.
[6] Overdevest I, Haverkate M, Veenemans J, Hendriks Y, Verhulst C, Mulders A, et al. Prolonged colonisation with *Escherichia coli* O25:ST131 versus other extended-spectrum β-lactamase-producing *E. coli* in a long-term care facility with high endemic level of rectal colonisation, the Netherlands, 2013 to 2014. Euro Surveill 2016;21, doi:http://dx.doi.org/10.2807/1560-7917.ES.2016.21.42.30376.
[7] Foxman B, Gillespie B, Koopman J, Zhang L, Palin K, Tallman P, et al. Risk factors for second urinary tract infection among college women. Am J Epidemiol 2000;151:1194–205.
[8] Foxman B, Zhang L, Tallman P, Palin K, Rode C, Bloch C, et al. Virulence characteristics of *Escherichia coli* causing first urinary tract infection predict risk of second infection. J Infect Dis 1995;172:1536–41.
[9] O'Brien VP, Hannan TJ, Schaeffer AJ, Hultgren SJ. Are you experienced? Understanding bladder innate immunity in the context of recurrent urinary tract infection. Curr Opin Infect Dis 2015;28:97–105.
[10] Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. Cell Microbiol 2009;11:1034–43.
[11] Weissman SJ, Johnson JR, Tchesnokova V, Billig M, Dykhuizen D, Riddell K, et al. High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. Appl Environ Microbiol 2012;78:1353–60.
[12] Barbosa-Cesnik C, Brown MB, Buxton M, Zhang L, DeBusscher J, Foxman B. Cranberry juice fails to prevent recurrent urinary tract infection: results from a randomized placebo-controlled trial. Clin Infect Dis 2011;52:23–30.
[13] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. Wayne, PA: CLSI; 2008 Document M100-S18.
[14] Hertz FB, Nielsen JB, Schønning K, Littauer P, Knudsen JD, Løbner-Olesen A, et al. Population structure of drug-susceptible, -resistant and ESBL-producing *Escherichia coli* from community-acquired urinary tract. BMC Microbiol 2016;16:63.
[15] Rossignol L, Vaux S, Maugat S, Blake A, Barlier R, Heym B, et al. Incidence of urinary tract infections and antibiotic resistance in the outpatient setting: a cross-sectional study. Infection 2017;45:33–40.
[16] Kudinha T, Johnson JR, Andrew SD, Kong F, Anderson P, Gilbert GL. *Escherichia coli* sequence type 131 as a prominent cause of antibiotic resistance among urinary *Escherichia coli* isolates from reproductive-age women. J Clin Microbiol 2013;51:3270–6.
[17] Ho P-L, Lo WU, Lai EL, Chow K-H, Yam W-C. *Escherichia coli* O25b-ST131 is an important cause of antimicrobial-resistant infections in women with uncomplicated cystitis. J Antimicrob Chemother 2012;67:2534–5.
[18] Can F, Azap OK, Seref C, Ispir P, Arslan H, Ergonul O. Emerging *Escherichia coli* O25b/ST131 clone predicts treatment failure in urinary tract infections. Clin Infect Dis 2015;60:523–7.