The Uropathogenic *Escherichia coli* Subclone Sequence Type 131-H30 Is Responsible for Most Antibiotic Prescription Errors at an Urgent Care Clinic

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**Background.** The pandemic spread of antibiotic resistance increases the likelihood of ineffective empirical therapy. The recently emerged fluoroquinolone-resistant *Escherichia coli* sequence type (ST) 131-H30R subclone (H30) is a leading cause of multidrug-resistant urinary tract infection (UTI) and bloodstream infection worldwide.

**Methods.** We studied the relative impact of H30 on the likelihood that bacteria isolated from urine of urgent care patients would be resistant to the empirically prescribed antibiotic regimen for UTI.

**Results.** Of 750 urinalysis-positive urine samples from urgent care patients with suspected UTI, 306 (41%) yielded *E. coli*, from 35 different clonal groups (clonotypes). H30 predominated (14% prevalence overall), especially among older patients (age ≥70 years: 26%) and those with diabetes (43%) or urinary catheterization (60%). Resistance to the empirically selected antibiotic regimen occurred in 16% (40/246) of patients overall, 28% (20/71) of older patients, 30% (8/27) of patients with diabetes, 60% (3/5) of catheterized patients, and 71% (22/30) of those with H30. H30’s contribution to such mismatched antibiotic selection was 55% overall, 70% among older patients, and 100% among patients with diabetes or a urinary catheter. Among patients with ≥2 of these factors (older age, diabetes, or urinary catheter), 24% of all urinalysis-positive urine samples yielded H30, with a 92% likelihood of resistance to the selected empirical therapy.

**Conclusions.** The multidrug-resistant H30 subclone of *E. coli* ST131 is responsible for the great majority of mismatched empirical antibiotic prescriptions for suspected UTI at an urgent care clinic among patients ≥70 years old or with diabetes or urinary catheterization.

**Keywords.** Urinary tract infections; *Escherichia coli* ST131-H30; empiric antibiotic therapy; urgent care; clonal diagnostics.
Despite the widespread occurrence of H30, its relative contribution to mismatched empirical antibiotic prescribing has not been evaluated. Moreover, the prevalence of H30 has not been evaluated in patients presenting to an emergency department or urgent care clinic with UTI, which is one of the leading reasons for emergency department visits and subsequent hospitalization. Here, we investigated the prevalence of H30 among patients with suspected UTI in an urgent care setting, its association with a wide range of relevant host characteristics, and its contribution to antibiotic/pathogen mismatch with empirical therapy.

METHODS

E. coli Urine Isolates

The 306 E. coli study isolates were collected within a previously reported prospective clonal diagnostic study [16], which was done from July 2014 to November 2015 at the Kaiser Permanente Washington (KPWA) urgent care clinic (UCC, Capitol Hill, Seattle), assisted by the KPWA central laboratory (Tukwila, Washington) and the Department of Microbiology, University of Washington (UW; Sokurenko laboratory). The KPWA Institutional Review Board granted a waiver of consent for collection and use of the samples.

Study participants were men and women aged 18 and older, admitted to the UCC with symptoms that prompted a dipstick urinalysis (Bayer Multistix strips) for suspected UTI. During the study, 750 total urinalysis-positive samples underwent standardized quantitative culture, species identification, and susceptibility testing at both the KPWA central laboratory and UW (research laboratory).

Laboratory Analysis

Quantitative urine culture and species identification was done in the research laboratory using HardyCHROM UTI selective agar plates (Hardy Diagnostics). Escherichia coli-positive urine samples were analyzed as follows. From each sample, 2-4 arbitrarily selected single colonies underwent clonotyping using a previously described 7-SNP test [17]: In brief, this polymerase chain reaction–based test determines the presence/absence of 7 single-nucleotide polymorphisms (ie, 7-SNP) in fumC and fimH (used in the fumC/fimH [CH] sequence typing scheme for E. coli [18, 19]), thus assigning an isolate to a distinct clonotype (CT) with a triple-number designator (eg, CT561). The identity of putative ST131-H30 isolates (per the 7-SNP test) was further confirmed by CH sequence typing. Susceptibility to 12 antibiotics (representing 8 drug classes) and production of ESBLs was tested by disk diffusion according to Clinical and Laboratory Standards Institute guidelines [20]. Multidrug resistance was defined as resistance to ≥3 antimicrobial classes.

Medical Records Review

Source patients’ electronic medical records were reviewed to extract age, gender, and all diagnosis codes and prescriptions from 1 month before to 2 months after the index UCC visit. Urinary catheter use was inferred from diagnoses entered at or before the index visit, diabetes from any corresponding diagnosis, and prior antibiotic use from prescription of any antibiotic between 30 and 2 days before the index visit.

Statistical Analysis

Statistical analysis was performed using Stata/IC 14.0 software (StataCorp, College Station, Texas). Two-by-two comparisons were performed using the χ2 test or, if appropriate, Fisher exact test. The 2-sample Kolmogorov-Smirnov (K-S) test was performed to evaluate differences in the age distribution function for patients with H30 vs non-H30 E. coli bacteriuria. The limited number of patients in this study precluded meaningful analysis of the clinical data using bin-type age strata. Accordingly, we selected an age cutoff to dichotomize age for 2 × 2 comparisons, and chose 70 years because this corresponded to the largest K-S statistic [21]. Univariate logistic regression was used to estimate the association of host factors with the urine isolate’s H30 status, and identified the following host factors as being associated with H30 bacteriuria: older age (≥70 years old), male gender, diabetes mellitus, urine catheter use, and prior prescription of antibiotics. To adjust for possible confounding by host characteristics, a multivariable logistic regression model was constructed that included all H30-associated host characteristics identified in the univariate model.

RESULTS

Prevalence of E. coli H30 Among E. coli Urine Isolates From Urgent Care Patients

Of 750 urinalysis-positive urine samples from patients seen at the KPWA UCC, 460 (61%) yielded bacteria, and 306 of those (66.5%) yielded E. coli, at a median concentration of 10⁵ colony-forming units (CFU)/mL (range, 10² to >10⁷ CFU/mL). According to the 7-SNP test, the 306 E. coli isolates were from 35 clonotypes, which were represented by 1–43 isolates each (Figure 1A).

The most prevalent clonotype was fluorquinolone-resistant CT561, corresponding to the H30 subclone, which accounted for 43 (14.1%) of all E. coli isolates. The next 6 most prevalent clonotypes (and the corresponding ST-fimH subclones) collectively comprised 122 of 306 (39.9%) isolates and included CT620 (ST73-H9/H10: 28 [9.2%]), CT760 (ST95-H41: 24 [7.8%]), CT530 (ST127-H2: 23 isolates [7.5%]), CT271 (ST69-H27: 19 [6.2%]), CT661 (ST73-H30: 14 isolates [4.6%]), and CT571 (ST14-H27/H64: 14 [4.6%]). Collectively, these 6 clonotypes plus H30 comprised 54.0% of all isolates. H30 was significantly more prevalent than any other clonotype (P < 0.02) except for the second largest, CT620 (P = 0.06). H30 did not differ significantly from other clonotypes for average urine E. coli CFU load (means, 6.5 × 10⁴ [95% confidence interval, 2.0–20 × 10⁴] CFU/mL vs 4.6 × 10⁴ [95% confidence interval, 3.0–6.9 × 10⁴] CFU/mL, respectively; P = .50). Thus, H30 subclone was the
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**most prevalent clonal group, occurring in approximately 1 of 7 patients with E. coli bacteriuria.**

**H30 Subclone Prevalence in Relation to Age**

To analyze H30 in relation to age, we plotted the cumulative age distribution functions for patients with H30 and non-H30 urinary E. coli, performing a K-S test. The K-S test showed that patients with H30 tend to be older (P = .001), with the largest K-S statistic (D = 0.37) calculated for the age of 70 years (Figure 1B). H30 was significantly more prevalent among older patients (aged ≥70) than younger patients (aged 18–69) with E. coli bacteriuria (26% vs 9%, P < .001). Thus, among UCC patients with E. coli bacteriuria, the H30 subclone was closely associated with older age, occurring in 1 of 4 of patients ≥70 years old.

**Association of H30 With Patient Characteristics**

We next determined whether the association of H30 with older age involved other patient characteristics, including gender and clinical factors such as diabetes, urinary catheter use, and prior antibiotic prescriptions (Table 1). H30 was associated significantly with diabetes and urinary catheter use in both age groups, and with male gender and prior antibiotic use among younger patients only. After multivariable adjustment, older age, diabetes, and urinary catheter use remained significant predictors of H30 isolation, whereas male gender and antibiotic use lost statistical significance (Table 2). Thus, among UCC patients with E. coli bacteriuria, older age, diabetes, and urinary catheter use were independent predictors of having H30.

**Contribution of H30 to Antimicrobial Resistance**

The prevalence of antibiotic resistance was generally higher among E. coli isolates from older as opposed to younger patients (Table 3). This difference was statistically significant for ciprofloxacin (32% vs 16%, P < .01) and multidrug resistance (19% vs 10%, P = .03), borderline for cefazolin (13% vs 6%, P = .09) and fosfomycin (5% vs 1%, P = .09), and numerically evident for ESBL production and all other agents except trimethoprim-sulfamethoxazole (TMP-SMX).

Nearly all resistance phenotypes were significantly more prevalent among H30 isolates than non-H30 isolates, both overall and within each age group (Table 3). The most dramatic difference involved ciprofloxacin resistance: Although accounting

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**Table 1. Association of H30 With Host Characteristics Among Younger (Aged 18–69) and Older (Aged ≥70) Urgent Care Patients With Escherichia coli Bacteriuria**

<table>
<thead>
<tr>
<th>Host Characteristic</th>
<th>All Patients</th>
<th>Younger</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total, No.</td>
<td>H30, No. (%)</td>
<td>P Value</td>
</tr>
<tr>
<td>Male gender</td>
<td>306</td>
<td>43 (14)</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes</td>
<td>35</td>
<td>9 (26)</td>
<td>.04</td>
</tr>
<tr>
<td>Urinary catheter</td>
<td>35</td>
<td>15 (43)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Prior antibiotics</td>
<td>10</td>
<td>6 (60)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

*Significance of differences in prevalence of H30 vs non-H30 Escherichia coli among patients with or without a host characteristic was evaluated using the $\chi^2$ test.

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**Figure 1.** A, Distribution by size, in ascending order, of all 35 clonotypes identified in 306 Escherichia coli urine isolates from urgent care patients. The 6 most prevalent clonotypes are listed, with their most prevalent sequence type–fimH subclone(s) shown in parenthesis. B, Cumulative function of age distribution for patients with H30 and non-H30 urinary E. coli. Arrow indicates the 2-sample Kolmogorov-Smirnov statistic. Abbreviations: CT, clonotype; ST, sequence type.
for only 16% of all isolates, H30 comprised 68% (43/63) of ciprofloxacin-resistant isolates, 62% (8/14) of ESBL-producing isolates, and 55% (21/38) of multidrug-resistant isolates.

Although differences did not achieve statistical significance, for all agents except fosfomycin resistance prevalence tended to be numerically higher among H30 isolates from older as opposed to younger patients (Table 3). Interestingly, the age-vs-resistance relationship among non-H30 isolates was the opposite, with resistance being numerically more prevalent among younger patients for 7 of the 10 studied resistance phenotypes. Consequently, the fold-difference in resistance prevalence between H30 and non-H30 isolates was most dramatic among older patients, where it was 2-fold for ampicillin, 2.5-fold for TMP-SMX, 5-fold for amoxicillin-clavulanate, and 9-fold for multidrug resistance. Furthermore, among older patients ESBL production was limited to H30 isolates. Thus, H30 was by far the main contributor to antimicrobial resistance among E. coli urine isolates from UCC patients and was solely responsible for the higher overall resistance prevalence among isolates from older vs younger patients.

Table 3. Comparison of Antibiotic Resistance in Younger (Aged 18–69) and Older (Aged ≥70) Adult Patients With H30 or Non-H30 Urinary Escherichia coli

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>All (n = 306)</th>
<th>Younger (n = 218)</th>
<th>Older (n = 88)</th>
<th>Older (n = 263)</th>
<th>Younger (n = 198)</th>
<th>Older (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>21</td>
<td>16</td>
<td>32</td>
<td>100</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>37</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>8</td>
<td>6</td>
<td>13</td>
<td>35</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Cefpodoximeb</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>19</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Ceftriaxoneb</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>19</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>48</td>
<td>45</td>
<td>55</td>
<td>81</td>
<td>75</td>
<td>42</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>21</td>
<td>20</td>
<td>25</td>
<td>56</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>ESBL producersb</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>19</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Multidrug-resistant E. coli</td>
<td>12</td>
<td>10</td>
<td>19</td>
<td>49</td>
<td>40</td>
<td>6</td>
</tr>
</tbody>
</table>

For comparisons of older vs younger patients, statistically significant higher prevalence (P < .05) is indicated by boldface text. For comparisons of H30 vs non-H30 E. coli, P values are listed.

Abbreviations: ESBL, extended-spectrum β-lactamase; TMP-SMX, trimethoprim-sulfamethoxazole.

aThe difference in resistance prevalence between urine isolates from older vs younger patients was estimated for all E. coli and, separately, H30 and non-H30 isolates, by using Fisher exact test.

bIn this dataset, all isolates resistant to second-generation cephalosporins (eg, cefpodoxime) were also resistant to third-generation cephalosporins (eg, ceftriaxone) and produced ESBLs.
(Table 4). That is, regardless of patient age, antibiotic/pathogen mismatch was nearly 10 times more likely with H30 isolates (71% overall; 78% among older patients, 62% among younger patients) than with non-H30 isolates (8.4% overall; 11.3% among older patients, 7.4% among younger patients) (P < .001, overall and within each age group). Furthermore, H30 was responsible for 55% of antibiotic/pathogen mismatch overall, including 40% among younger and 70% among older patients.

Antibiotic/pathogen mismatches were also associated with the 2 other host risk factors for H30, both singly and in combination with one another and/or older age. Specifically, antibiotic/pathogen mismatch occurred in 30% (8/27) of patients with diabetes and 60% (3/5) of those with a urinary catheter (Table 4), all due to H30. It also occurred in 26% (21/82) of those with at least 1 such host risk factor (older age, diabetes, and/or catheter use), of which 71% (15/21) were due to H30, and 45% (9/20) of those with at least 2 such risk factors, of which all (9/9) were due to H30. Thus, H30 was the single largest contributor to antibiotic/pathogen mismatches overall, and was responsible for most mismatches among older patients and those with diabetes and/or urinary catheter use.

H30 Prevalence Among All Urinalysis-positive Patients With Risk Factors for H30

The above analyses were limited to urinalysis-positive patients who proved later to have E. coli bacteriuria, yet at the time of presentation these patients were indistinguishable from other urinalysis-positive patients. For a more realistic operational perspective, we estimated the probability of H30 occurrence among all urinalysis-positive urine samples for UCC patients with 1 or more of the identified risk factors for H30 (older age, diabetes, and catheter use; Table 5). Although among the source patients for the 750 urinalysis-positive urine samples H30 was present in only 6% overall, it was isolated from 11% of older patients, 17% of those with diabetes, and 19% of those with a urinary catheter. The likelihood of H30 doubled from baseline (from 6% to 11%) if at least 1 such risk factor was present, and doubled again (to 24%) if 2 or more risk factors were present. Thus, in certain subsets of patients with a positive urinalysis, H30 occurs in 1 in 4 cases.

DISCUSSION

We demonstrate here that the recently emerged H30 subclone of E. coli ST131 is associated with mismatched empirical antimicrobial prescriptions for treatment of E. coli bacteriuria in an urgent care population, especially in patients with readily identifiable risk factors such as older age, diabetes, and urinary catheter use. Notably, a recent study demonstrated that the ≥70 years age group has had the largest increase since 1998 in UTI-related hospitalizations in the United States [22]. This could, at least partially, reflect the fact that this age group is the primary target of H30 bacteriuria can be attributed to this subclone’s notoriously extensive antibiotic resistance profile [15, 23]. Our study confirms that among UCC patients antibiotic resistance is significantly more prevalent for several antibiotic classes among H30 isolates as compared to other E. coli, usually independent of age group. Although H30 is best known for its uniform resistance...
to fluoroquinolones, many of its members are also resistant to other drugs commonly prescribed for UTI such as TMP-SMX and β-lactams, including third-generation cephalosporins. Indeed, 20% of the present H30 isolates were ESBL producers, as compared with <3% of non-H30 isolates. While resistance to nitrofurantoin and fosfomycin also was more common for H30, its overall resistance to those antibiotics was generally well below 10%.

Despite recommendations against fluoroquinolones as first- or second-choice regimens for uncomplicated UTI [6], their overuse is common [24–26], possibly due to a perception that these drugs are both safe and more effective than other drugs. Nitrofurantoin, an antibiotic of choice for uncomplicated UTI [6], here was prescribed for only 6% of patients. Nitrofurantoin is used relatively infrequently in the United States and abroad [24, 27], possibly due to its longer treatment durations (as compared with fluoroquinolones or TMP-SMX), lower efficacy and greater toxicity in patients with renal insufficiency [28], and relatively narrow spectrum of activity. Our data suggest that nitrofurantoin would be attractive for use in UCC patients with suspected UTI if a rapid test were to detect H30. This applies also to fosfomycin, which is not used widely [24, 29, 30], possibly due to limited experience in the United States, high cost, and mixed opinion about efficacy against UTI relative to nitrofurantoin [31, 32]. Preferential use of nitrofurantoin or fosfomycin for infections likely due to H30 is especially important, considering that H30 exhibits uniform resistance to fluoroquinolones and a 30% resistance prevalence for TMP-SMX and narrow-spectrum cephalosporins. Thus, the persistence of empirical use of fluoroquinolones for UTI in the United States is not only ineffective but harmful, leading to the co-selection of H30 in particular and multidrug resistance in general; it could well have been one of the major reasons for the above-mentioned increased hospitalization rate among UTI patients that was associated with the fluoroquinolone resistance of H30.

In view of the significant impact of H30 on mismatched antimicrobial selection for UCC patients, a point-of-care diagnostic test that could identify H30 directly in patients’ urine could be highly beneficial. Development of such a rapid ST131-H30-specific urine test was reported previously [15, 33]. Here, we estimated how often H30 could be isolated in different categories of patients presenting to urgent care with suspected UTI. We found previously that the urinalysis test used here (see Methods) was 98% sensitive for E. coli bacteriuria [16]. Thus, H30 detection is relevant for patients with a positive urinalysis. By contrast, urinalysis was only 41% specific for E. coli bacteriuria, presumably due to other uropathogens or nonbacterial causes of an abnormal urinalysis result. We found that among UCC patients with positive urinalysis, certain patient subsets had a significantly higher risk of having H30 than did the overall population (6%), including patients ≥70 years of age (11%) or with diabetes (17%), a urinary catheter (19%), or 2 or more risk factors (23%). The latter value is twice that among all older patients and 8 times that among “no risk factor” patients. Thus, certain risk groups—some of which are sizeable—have a high chance of having urinary H30, detection of which could meaningfully reduce the mismatched prescription rate.

The practical significance of point-of-care detection of urinary H30 strains would depend on the benefits and harms of reducing mismatched empiric prescription and of fluoroquinolone use, including less selection for resistance. In recent studies of patients with suspected UTI, inappropriate drug selection was strongly associated with return visits to an emergency department or urgent care clinic [34]. Emergency department visits are significantly more expensive than outpatient office visits, costing on average more than $2000 [35, 36]. Because ineffective UTI treatment may also result in higher hospital admission rates or prolonged hospital stays, as demonstrated specifically for H30 [23], the cost of mismatched prescription could be very high, which could justify the introduction of point-of-care precision diagnostic tests to minimize their occurrence. Finally, because H30 is associated with adverse clinical outcomes independent of its resistance [15], recognition of its presence conceivably could directly benefit the clinical management of high-risk patients, although this remains to be demonstrated. Moreover, even in the absence of specific diagnostic tests, it would be appropriate to treat patients who belong to the risk groups described here (elderly and/or suffering from diabetes and/or using urinary catheter) as likely exposed to H30 infection and, thus, if possible, to prescribe nitrofurantoin, fosfomycin or, with caution, third-generation cephalosporins—but not fluoroquinolones, to which resistance is universal, or TMP-SMX, to which resistance is very common. Considering H30’s tendency to cause repeated infection and severe complications independently of drug–bug mismatch, such patients also might need closer surveillance for these sequelae.

The only other clonally related bacterial isolates that have been shown to contribute to mismatched empirical antibiotic prescriptions in similar proportions is methicillin-resistant Staphylococcus aureus (MRSA). While specific treatments, tests, and surveillance measures have been developed for MRSA, there has not been such in-depth focus on H30, likely because H30 emerged much more recently than MRSA and its tight clonal nature was recognized only within the past decade.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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