# Epidemiology of the multidrug-resistant ST131-*H30* subclone among extraintestinal *Escherichia coli* collected from US children

Arianna Miles-Jay[1,3], Scott J. Weissman[2,3], Amanda L. Adler[3], Veronika Tchesnokova[4], Evgeni V. Sokurenko[4], Janet G. Baseman[1], Danielle M. Zerr[2,3]

**Affiliations**
1. Department of Epidemiology, University of Washington, Seattle, WA
2. Department of Pediatrics, University of Washington, Seattle, WA
3. Seattle Children's Research Institute, Seattle, WA
4. Department of Microbiology, University of Washington, Seattle, WA

**Running Title:** *E. coli* ST131-*H30* in US children

**Summary:** ST131-*H30* was responsible for 5.3% of all extraintestinal *E. coli* infections and 44% of ESBL-producing extraintestinal *E. coli* infections among US children. The clinical and demographic correlates of infection with ST131-*H30* differed between extended-spectrum cephalosporin-resistant and -sensitive isolates.

**Corresponding author:**

Danielle M Zerr, MD, MPH
Seattle Children's Hospital
4800 Sand Point Way, NE
Seattle, WA 98105
206-987-2653
danielle.zerr@seattlechildrens.org

**Alternate Corresponding author:**

Arianna Miles-Jay
University of Washington School of Public Health
1959 NE Pacific St
Health Sciences Building
Box 357236
Seattle, WA 98105
206-884-1565
admiles@uw.edu

## ABSTRACT

**Background:** *E. coli* ST131-*H30* is a globally important pathogen implicated in rising rates of multidrug resistance, especially fluoroquinolone resistance and extended-spectrum beta-lactamase (ESBL) production, among *E. coli* causing extraintestinal infections. Previous studies have focused on adults, especially the elderly, leaving the epidemiology of *H30* among children undefined.

**Methods:** We used clinical data and extraintestinal *E. coli* isolates from a case-control study conducted at four US freestanding children's hospitals from 2009-2013 to estimate the burden and identify clinical and demographic correlates of infection with *H30. H30* isolates were identified using *fumC/fimH* genotyping. Host correlates of *H30* infection were examined using univariable and multivariable log-binomial regression models stratified by extended-spectrum cephalosporin resistance status.

**Results:** The estimated prevalence of ST131-*H30* was 5.3% among all extraintestinal *E. coli* isolates and 44% among ESBL-producing isolates. The host correlates of infection with *H30* differed by extended-spectrum cephalosporin resistance status: among resistant isolates, age ≤5 years was positively associated with *H30* infection (relative risk [RR] 1.80, 95% confidence interval [CI] 1.18-2.86); among sensitive isolates, age ≤5 years was negatively associated with *H30* (RR 0.48, 95% CI 0.26-0.86), while presence of an underlying medical condition was positively associated with *H30* (RR 4.19, 95% CI 2.32-8.00).

**Conclusions:** ST131-*H30* is less common among extraintestinal *E. coli* collected from children compared to what has been reported among adults, possibly reflecting infrequent fluoroquinolone use in pediatrics; however, it is similarly dominant among ESBL-producing isolates. The *H30* subclone appears to disproportionately affect young children relative to other extended-spectrum cephalosporin-resistant *E. coli*.

## INTRODUCTION

Extraintestinal *Escherichia coli* are a common cause of urinary tract and bloodstream infections across all ages, and have displayed worrisome increasing rates of antimicrobial resistance in the past two decades.[1] This increase has been attributed to the emergence and rapid clonal expansion of *E. coli* Sequence Type (ST) 131, which has transformed the population structure of extraintestinal *E. coli* infections worldwide.[2–5] Molecular epidemiologic studies have shown that a subclone of ST131, termed *H30* or clade C, has driven the global dissemination of ST131.[6–8] There are two dominant antimicrobial resistant sublineages nested within ST131-*H30*/clade C: *H30R* or clade C1, which is characterized by resistance to fluoroquinolones, and *H30Rx* or clade C2, which is characterized by resistance to fluoroquinolones as well as production of a CTX-M-15-type extended-spectrum beta-lactamase (ESBL) that confers resistance to extended-spectrum cephalosporins (Supplementary Fig 1).[7–10]

Although *E. coli* ST131-*H30* (hereafter, *H30*) has been recognized as a high-risk clone of significant public health importance,[5,11] there is a lack of data about its epidemiology in the pediatric population. Most studies that have included *H30* isolates from children have occurred over short time periods at single centers and have accumulated few *H30* isolates.[12–14] Among adults in the US, *H30* is estimated to comprise about 50% of ESBL-producing *E. coli* infections and between 10% and 20% and of all clinical *E. coli* infections, and has been linked to host factors including older age, healthcare contact, local or systemic compromise, and recent antibiotic use.[6,12–15] Associations with adverse outcomes such as persistent infections, hospitalization, new infections, and sepsis have also been reported in adult populations.[7,12,16] Understanding the epidemiology of *H30* in pediatric populations is important, as its dominance among multidrug-resistant (MDR) extraintestinal *E. coli* makes it a likely culprit of many difficult-to-treat infections in children. Proper treatment of urinary tract infections – the most common type of infection caused by extraintestinal *E. coli* – is especially

critical in pediatric populations, as young children are more prone to upper tract infection with potential short- and long-term complications such as renal scarring and decreased renal function.[17,18]

We sought to address this knowledge gap using data from a multiyear, multicenter prospective study of extraintestinal *E. coli* infections to quantify the burden and identify clinical and demographic correlates of infection with *H30* among children. In addition, we describe and compare the antimicrobial resistance characteristics of *H30*, *H30Rx*, and all other (non-*H30*) *E. coli* isolates in a US pediatric population.

## METHODS

### Patients and isolates

All isolates and clinical data came from a multicenter case-control study that conducted prospective isolate and data collection and is described in detail elsewhere.[19] In brief, between September 1, 2009 and September 30, 2013, four freestanding US children's hospitals (referred to here as West, Midwest 1, Midwest 2, and East) used standard clinical microbiology techniques to identify and collect all extended-spectrum cephalosporin-resistant (ESC-R) *E. coli* collected from urine or other normally sterile sites during routine clinical care of both inpatient and outpatient children < 22 years of age. ESC-R isolates were defined as those non-susceptible to ceftriaxone, cefotaxime, ceftazidime, cefepime, or aztreonam. For each resistant isolate, three consecutive *E. coli* isolates that were susceptible to the aforementioned agents, referred to here as extended-spectrum cephalosporin-susceptible (ESC-S) isolates, were also collected. Isolates were archived and shipped to the coordinating study hospital quarterly. The Institutional Review Board at each hospital approved the study protocol.

### Laboratory methods

Methods for antibiotic susceptibility and typing of resistance phenotypes and determinants were also described previously.[19] Briefly, after arriving at the coordinating study hospital, all isolates were tested for antimicrobial susceptibility using disk diffusion, and ESC-R or ESC-S status was confirmed. ESC-R phenotypes (ESBL vs. AmpC) were characterized using a combination of disk diffusion and E-tests. Genetic determinants of extended-spectrum cephalosporin resistance were identified by PCR using primers for genes encoding common extended-spectrum cephalosporinases.[19] *H30* isolates were identified using the *fumC/fimH* genotyping scheme.[20] *H30Rx* isolates were identified by PCR detection of sublineage-specific single nucleotide polymorphisms.[7]

**Clinical data**

Demographic and clinical data were collected from the medical records of patients using a standardized data abstraction form. Methods for categorizing underlying medical conditions and capturing antibiotic exposure were also described previously.[19,21]

**Prevalence estimates**

The overall prevalence of *H30* was estimated using the prevalence of *H30* among ESC-R isolates (which were captured completely), the prevalence of *H30* among the collected ESC-S isolates (which were captured partially), and the total number of *E. coli* isolates collected from each clinical microbiology laboratory during the study period, excluding repeat isolates from a given patient if they were collected within 15 days of the first isolate. The 15-day cut point was chosen in an effort to identify isolates that represented a new infection episode. The total number of *E. coli* isolates from each study hospital was only available between October 1, 2009 and September 30, 2013, so any isolates collected during September 2009 were excluded. After calculating the prevalence of *H30* among ESC-R and ESC-S isolates separately, the overall prevalence among all clinical *E. coli* isolates was estimated using a weighted average of the

stratified prevalence estimates, with the weights being the relative proportions of the two mutually exclusive groups among the total number of *E. coli* isolates reported. This approach makes two assumptions: 1) all ESC-R isolates were captured; and 2) the collected ESC-S isolates, which were collected without respect to any patient or microbiological characteristics beyond ESC-S status and temporal proximity to the ESC-R isolates, are representative of all ESC-S isolates. The same approach was applied to calculate the prevalence of *H30Rx.*

**Statistical analyses**

Host correlates of infection

Host factors were compared between patients with *H30* isolates vs. non-*H30* isolates, stratified by ESC-R status. Only the first isolate from each unique individual was considered. Variables were first compared using Chi-squared or Fisher's Exact tests to identify potential predictors of interest. Factors with a p-value of <0.05 were considered candidate predictors of interest. The magnitude of the association between each predictor of interest and *H30* infection was then assessed using univariable and multivariable log-binomial regression models. For each predictor of interest, the relative risk (RR) and 95% confidence intervals (CIs) from three models are presented: 1) a univariable model that estimates the crude (unadjusted) total effect of the predictor of interest on the outcome; 2) a multivariable model that estimates the total effect of the predictor of interest on the outcome, adjusted for potential confounders; and 3) a multivariable model that estimates the direct effect of the predictor of interest on the outcome, adjusted for potential confounders as well as for potential mediating variables. Potential confounders and mediators were selected according to the conceptual framework found in the supplementary material (Supplementary Fig 2).

Antimicrobial resistance characteristics

Isolates were stratified by ESC-R and ESC-S status and *H30* isolates were additionally stratified

into *H30Rx* and *H30*-non-*Rx*; only the first isolate from each patient was considered. Antimicrobial resistance characteristics were compared to those of non-*H30* isolates using Chi-squared or Fisher's Exact tests. All analyses were conducted using R version 3.3.1 (R Core Team, 2016).

## RESULTS

### Isolates and prevalence estimates

A total of 1347 eligible *E. coli* isolates were collected from 1286 unique patients: 339 ESC-R isolates from 278 unique patients and 1008 ESC-S isolates from 1008 patients. The estimated prevalence of *H30* among all clinical *E. coli* isolates at all study hospitals was 5.3%, while the study hospital-specific prevalence ranged from 2.7% to 6.2% (Figure 1). The estimated overall prevalence of *H30Rx* was 0.87%.

### Host correlates of infection by ESC-R status

Among ESC-R isolates, and when only considering the first isolate per patient, patient age was associated with *H30* infection and further examined as a predictor of interest (Table 1). Our sample size precluded multilevel predictors, so age was categorized into ages 0-5 and 6-21 years in regression models. In our data, young age was strongly associated with Asian race (data not shown), and Asian race also displayed an association with *H30* infection. Therefore, we included Asian race (yes/no) as a potential confounder of the association between patient age and *H30* infection. After adjusting for Asian race, age 0-5 was associated with an 80% increased risk of the infecting organism being *H30* (RR 1.80, 95%CI 1.18-2.86). There was no evidence that this association was mediated through factors related to underlying illness (Table 3). When restricting the outcome to *H30Rx* infection only (vs. non-*H30* infection) and adjusting for Asian race, the effect size was stronger (RR 2.21, 95%CI 1.33-3.87).

Among ESC-S isolates, patient age and several factors associated with underlying illness were associated with *H30* infection (Table 1). Each of these variables was examined as a predictor of interest except for: (i) history of transplantation, due to small numbers, and (ii) type of infection acquisition, since it is a composite variable and previous hospitalization and underlying medical conditions were examined independently. Underlying medical condition and indwelling device categories were collapsed into any vs. none. In a univariate analysis, patient age ≤ 5 years was negatively associated with *H30* infection (RR 0.48, 95% CI 0.26-0.86). Of the variables related to underlying illness, after adjusting for potential confounders, only presence of an underlying medical condition (RR 4.19, 95%CI 2.32-8.00) remained as an independent predictor of *H30* infection. When including potential mediators in the models, the magnitude of the associations between age ≤ 5 years and presence of an underlying medical condition with *H30* infection decreased, but the associations remained statistically significant (Table 3).

Since patient age was a strong predictor in both the analyses of ESC-R and ESC-S isolates, we visually inspected the distributional differences of age measured continuously. While the non-*H30* age distributions are very similar, the *H30* age distributions display marked differences between ESC-R and ESC-S isolates (Figure 2).

**Antimicrobial resistance characteristics by ESC-R and *H30Rx* status**

Among ESC-R isolates, nearly all *H30Rx* (96.9%) and *H30*-non-*Rx* (94.7%) isolates were non-susceptible to fluoroquinolones, compared to 39% of non-*H30* isolates. Similarly, nearly all ESC-R *H30Rx* (98.4%) and *H30*-non-*Rx* (89.5%) isolates were ESBL-producing, while non-*H30* isolates were more evenly split between ESBL producers and AmpC producers (Table 2). Overall, *H30* was responsible for 44.0% of all ESBL-producing isolates, with *H30Rx* alone accounting for 34.6%. The vast majority (88.9%) of ESBL-producing *H30Rx* isolates had a CTX-M-15 beta-lactamase, while ESBL-producing *H30*-non-*Rx* isolates were dominated by the CTX-

M-27 beta-lactamase (58.8%); ESBL-producing non-*H30* isolates were more evenly split between CTX-M-15 and CTX-M-14 beta-lactamases. Among ESC-S isolates, fluoroquinolone non-susceptibility was dominant (85.7%) among *H30*-non-*Rx* isolates, while only 2.6% of non-*H30* ESC-S isolates were non-susceptible to fluoroquinolones (Table 2).

## DISCUSSION

We utilized a multiyear, multicenter, prospective study of extraintestinal *E. coli* infections in children's hospitals to address a critical knowledge gap about the epidemiology of the globally important ST131-*H30* subclone among US children. Our results can be summarized into three main findings. First, the estimated prevalence of *H30* among pediatric clinical *E. coli* isolates of 5.3% was lower than the 10-20% that has been observed in US adults.[6,12,13] However, *H30* was nearly as dominant among ESBL-producing isolates in children (44%) as has been reported in adults (about 50%).[14,15] Second, patient age was associated with infection due to *H30*, but the nature of this association contrasted sharply between ESC-R and ESC-S infections. Among ESC-R infections, *H30* was associated with young age (≤5 years). Conversely, among ESC-S infections, *H30* was associated with older age (6-21 years), as well as with the presence of an underlying medical condition. Third, the antimicrobial resistance characteristics of *H30* and *H30Rx* collected from children were consistent with what has been previously reported.[14–16,22,23] ESC-R *H30* isolates were almost always fluoroquinolone-resistant and ESBL-producing, and ESBL-producing *H30Rx* isolates were associated with the CTX-M-15 beta-lactamase, while ESBL-producing *H30*-non-*Rx* isolates were associated with the CTX-M-27 beta-lactamase.

Other studies have suggested that *H30* is less prevalent among children than adults; however, very few pediatric isolates were included in these studies.[13,14] Interestingly, we observed that *H30* was nearly as dominant among ESBL-producing *E. coli* infections in children as has been

reported in adults.[14,15] These findings are consistent with a recent study from a pediatric setting conducted in the Midwestern US.[24] However, in the context of all clinical extraintestinal *E. coli* infections, ESBL-producing organisms are still relatively rare in both adults and children. The bulk of the *H30* isolates circulating in the population are non-ESBL-producing but fluoroquinolone-resistant, and the prevalence of such organisms was much lower in our study than estimates from adult populations.[13,14] This observation may be explained by differential antibiotic use in these populations. Fluoroquinolones are infrequently prescribed to children due to historical concerns about toxicity;[25] in our study, about 5% of patients received fluoroquinolones in the year before collection of their first isolate, while 46% of patients received any antibiotic in that same time period (data not shown). Lower rates of fluoroquinolone use likely translate to less selective pressure on fluoroquinolone-resistant organisms such as *H30*. Interestingly, a recent study conducted in adults in Australia and New Zealand, a population that also has low rates of fluoroquinolone use, reported an overall prevalence of *H30* of 3.5%, but a prevalence of *H30* among ESC-R *E. coli* of 39%, which is similar to our findings.[26]

Although not explicitly previously reported, the association between *H30* and young age among ESC-R isolates is consistent with the findings of a recent longitudinal study showing that among children, the prevalence of ESBL-producing *Enterobacteriaceae* was highest and increasing most rapidly in children aged 1-5.[27] Why *H30/H30Rx* is more frequently found among young children with ESC-R infections compared to older children with ESC-R infections, as well as where young children are acquiring this pathogen, deserves further investigation. Previous studies have portrayed *H30* as an opportunistic pathogen that favors compromised hosts,[12] and the developing immune systems of young children could be a factor in colonization or infection. While transmission of *H30* between children within healthcare facilities has not been documented, there are reports of transmission of, and persistent colonization with, *H30/H30Rx* among healthy children within daycares and households.[28–31] Future studies might focus on

systematic sampling in the community setting in order to better elucidate the reservoirs and transmission dynamics of *H30/H30Rx* among young children.

The association we observed between ESC-S *H30* infections and older children is not consistent with the limited existing data.[13,32] This association could be driven by different selective pressures in older children: specifically, fluoroquinolones may be prescribed more frequently to older children than younger children due to less concern about toxicity. This prescribing pattern was borne out in our data; the median age was 12.6 years among patients that received fluoroquinolones in the year prior to their infection, whereas the median overall age was 5.4 years (data not shown). Our multivariable models that included potential mediators suggested that factors associated with healthcare contact and antibiotic use may partially explain the observed association between older patient age and antibiotic use, but not completely. A more refined examination of antibiotic exposure, specifically focusing on fluoroquinolones, was not feasible here due to small numbers, but is warranted.

Notably, previous studies have described *H30* as being associated with healthcare contact and compromised hosts;[12,13] however, we found those associations only among ESC-S *H30* infections. Even without ESBL production, ESC-S *H30* pathogens are more antimicrobial-resistant than other ESC-S isolates, and factors associated with compromised hosts and healthcare contact are consistently associated with antimicrobial resistant infections.[33] We observed that when compared to other ESC-R organisms, there is no evidence of an association between *H30* and underlying illness. This observation raises the question of whether some of the host correlates observed in previous studies are specific to the *H30* subclone, or just reflect risk factors for MDR extraintestinal *E. coli* in general. Future studies should consider comparing *H30* to other MDR *E. coli* where possible.

A number of limitations need to be considered in the interpretation of these data. First, because of the case-control design of the parent study, the prevalence of *H30* and *H30*Rx among clinical *E. coli* isolates could not be calculated directly. However, we believe the assumptions employed in our prevalence estimates are reasonable, and that these data provide the best estimate of the prevalence of *H30* in children to date. The design of the parent study was also beneficial, as it allowed us to enrich the collection with the less common MDR isolates and examine risk factors for infection with *H30* among those with ESC-R *E. coli* isolates specifically. Second, because this study was meant to be descriptive and exploratory, any significant statistical associations could be an artifact of multiple testing, and should be interpreted cautiously. To mitigate this, we attempted to make thoughtful model building decisions and interpretations by using conceptual models rather than taking a purely data-driven approach. Third, although this was a multicenter study, our data were collected from freestanding children's hospitals between 2009 and 2013, so the results may not be generalizable to other settings, and epidemiologic patterns may have shifted slightly during the subsequent several years. Despite these limitations, this study significantly improves our understanding of the impact of *H30* in children, and is one of the most robust examinations of the clinical burden of, and risk factors for, *H30* infections to date.

**Conclusion**

Although *E. coli* ST131-*H30* is not as prevalent among children as has been reported in adults, perhaps as a result of low rates of fluoroquinolone use in pediatrics, this high-risk clone is dominant among ESC-R extraintestinal *E. coli* infections in children. In particular, the ESBL-producing type of *H30*, dominated by the *H30Rx* subclone, disproportionately affects young children relative to other MDR *E. coli*, even when accounting for other underlying host factors. More densely sampled studies are needed to investigate the reservoirs and transmission dynamics of this serious pathogen in a pediatric population in order to determine where these

infections are coming from, and ultimately to develop interventions designed to reduce the burden of this difficult-to-treat pathogen in children.

**FUNDING**

Research reported in this publication was supported by the National Institutes of Health via the National Institute for Allergies and Infectious Diseases [grant number R01AI083413], and via the National Center for Advancing Translational Sciences [grant number TL1TR000422]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## CONFLICTS OF INTEREST

E.V.S has patent applications to detect *E. coli* strains and is a major shareholder in IDGenomics. The other authors report no conflicts of interest.

**ACKNOWLEDGEMENTS**

The authors thank Carey-Ann Burnham, Alexis Elward, Jason Newland, Rangaraj Selvarangan, Kaede Sullivan, Theoklis Zaoutis, and Xuan Qin for their provision of bacterial isolates and associated clinical data. Additionally, they thank Jeff Myers and Huxley Smart for their assistance with molecular typing of isolates.

## REFERENCES

1. World Health Organization. Antimicrobial resistance: global report on surveillance. Available at: http://www.who.int/drugresistance/documents/surveillancereport/en/. Accessed 15 April 2015.
2. Johnson JR, Nicolas-Chanoine MH, Deb Roy C, et al. Comparison of Escherichia coli ST131 pulsotypes, by epidemiologic traits, 1967-2009. Emerg Infect Dis, **2012**; 18:598–607.
3. Nicolas-Chanoine M-H, Blanco J, Leflon-Guibout V, et al. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother, **2008**; 61:273–81.
4. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. Escherichia coli sequence type ST131 as the major cause of serious multidrug-resistant E. coli infections in the United States. Clin Infect Dis, **2010**; 51:286–294.
5. Mathers AJ, Peirano G, Pitout JDD. Escherichia coli ST131: The Quintessential Example of an International Multiresistant High-Risk Clone. Adv Appl Microbiol, **2015**; 90:109 – 154.
6. Johnson JR, Tchesnokova V, Johnston B, et al. Abrupt emergence of a single dominant multidrug-resistant strain of Escherichia coli. J Infect Dis, **2013**; 207:919–928.
7. Price LB, Johnson JR, Aziz M, et al. The epidemic of extended-spectrum-β-lactamase-producing Escherichia coli ST131 is driven by a single highly pathogenic subclone, H30-Rx. MBio, **2013**; 4:e00377-13.
8. Petty NK, Ben Zakour NL, Stanton-Cook M, et al. Global dissemination of a multidrug resistant Escherichia coli clone. Proc Natl Acad Sci USA, **2014**; 111:5694–9.
9. Stoesser N, Sheppard AE, Pankhurst L, et al. Evolutionary History of the Global Emergence of the Escherichia coli Epidemic Clone ST131. MBio, **2016**; 7:e02162-15.
10. Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM, et al. Sequential acquisition of

virulence and fluoroquinolone resistance has shaped the evolution of Escherichia coli ST131. MBio, **2016**; 7:1–12.

11. Baquero F, Tedim AP, Coque TM. Antibiotic resistance shaping multi-level population biology of bacteria. Front Microbiol, **2013**; 4:1–15.
12. Johnson JR, Thuras P, Johnston BD, et al. The Pandemic H 30 Subclone of Escherichia coli Sequence Type 131 (ST131) is Associated with Persistent Infections and Adverse Outcomes Independent from Its Multi-Drug Resistance and Associations with Compromised Hosts. Clin. Infect. Dis. **2016**; 62:1529–1536.
13. Banerjee R, Johnston B, Lohse C, et al. The clonal distribution and diversity of extraintestinal Escherichia coli isolates vary according to patient characteristics. Antimicrob Agents Chemother, **2013**; 57:5912–5917.
14. Drawz SM, Porter S, Kuskowski M a., et al. Variation in resistance traits, phylogenetic background, and virulence genotypes among Escherichia coli clinical isolates from adjacent hospital campuses serving distinct patient populations. Antimicrob Agents Chemother, **2015**; 59:5331–5339.
15. Banerjee R, Robicsek A, Kuskowski M a., et al. Molecular epidemiology of Escherichia coli sequence type 131 and its H30 and H30-Rx subclones among extended-spectrum-β -lactamase-positive and -negative E. coli clinical isolates from the Chicago region, 2007 to 2010. Antimicrob Agents Chemother, **2013**; 57:6385–6388.
16. Peirano G, Pitout JDD. Fluoroquinolone-resistant Escherichia coli sequence type 131 isolates causing bloodstream infections in a canadian region with a centralized laboratory system: rapid emergence of the H30-Rx sublineage. Antimicrob Agents Chemother, **2014**; 58:2699–703.
17. Shaikh N, Ewing AL, Bhatnagar S, Hoberman A. Risk of Renal Scarring in Children With a First Urinary Tract Infection: A Systematic Review. Pediatrics, **2010**; 126:1084–1091.
18. Jacobson SH, Eklöf O, Eriksson CG, Lins LE, Tidgren B, Winberg J. Development of

hypertension and uraemia after pyelonephritis in childhood: 27 year follow up. BMJ, **1989**; 299:703–6.

19. Zerr DM, Miles-Jay A, Kronman MP, et al. Previous antibiotic exposure increases risk of infection with extended spectrum beta lactamase- and AmpC-producing Escherichia coli and Klebsiella pneumoniae in pediatric patients. Antimicrob Agents Chemother, **2016**; 60:4237–4243.
20. Weissman SJ, Johnson JR, Tchesnokova V, et al. High-resolution two-locus clonal typing of extraintestinal pathogenic Escherichia coli. Appl Environ Microbiol, **2012**; 78:1353–1360.
21. Das S, Adler AL, Miles-Jay A, et al. Antibiotic prophylaxis is associated with subsequent resistant infections in children with an initial extended-spectrum cephalosporin-resistant *Enterobacteriaceae* infection. Antimicrob Agents Chemother, **2017**; Advanced online publication. doi:10.1128/AAC.02656-16.
22. Olesen B, Frimodt-Moller J, Leihof RF, et al. Temporal Trends in Antimicrobial Resistance and Virulence-Associated Traits within the Escherichia coli Sequence Type 131 Clonal Group and Its H30 and H30-Rx Subclones, 1968 to 2012. Antimicrob Agents Chemother, **2014**; 58:6886–6895.
23. Matsumura Y, Johnson JR, Yamamoto M, et al. CTX-M-27- and CTX-M-14-producing, ciprofloxacin-resistant Escherichia coli of the H30 subclonal group within ST131 drive a Japanese regional ESBL epidemic. J Antimicrob Chemother, **2014**; 70:1639–1649.
24. Logan LK, Hujer AM, Marshall SH, et al. Analysis of β-Lactamase Resistance Determinants in Enterobacteriaceae from Chicago Children: A Multicenter Survey. Antimicrob Agents Chemother, **2016**; 60: 3462– 3469
25. Bradley JS, Jackson M a. The Use of Systemic and Topical Fluoroquinolones. Pediatrics **2011**; 128:e1034–e1045.
26. Rogers BA, Ingram PR, Runnegar N, et al. Sequence type 131 fimH30 and fimH41

subclones amongst Escherichia coli isolates in Australia and New Zealand. Int J Antimicrob Agents, **2015**; 45:351–358.

27. Logan LK, Braykov NP, Weinstein R a., Laxminarayan R. Extended-Spectrum β-Lactamase-Producing and Third-Generation Cephalosporin-Resistant Enterobacteriaceae in Children: Trends in the United States, 1999-2011. J Pediatric Infect Dis Soc, **2014**; 3:320-328.
28. Madigan T, Johnson JR, Clabots C, et al. Extensive Household Outbreak of Urinary Tract Infection and Intestinal Colonization due to Extended-Spectrum β -Lactamase-Producing Escherichia coli Sequence Type 131. Clin. Infect. Dis. **2015**; 61:e5–e12.
29. Blanc V, Leflon-Guibout V, Blanco J, et al. Prevalence of day-care centre children (France) with faecal CTX-M-producing Escherichia coli comprising O25b:H4 and O16:H5 ST131 strains. J Antimicrob Chemother, **2014**; 69:1231–1237.
30. Johnson JR, Davis G, Clabots C, et al. Household Clustering of Escherichia coli Sequence Type 131 Clinical and Fecal Isolates According to Whole Genome Sequence Analysis. Open Forum Infect. Dis. **2016**; 3:ofw129.
31. Gurnee E a., Ndao IM, Johnson JR, et al. Gut Colonization of Healthy Children and Their Mothers With Pathogenic Ciprofloxacin-Resistant Escherichia coli. J Infect Dis, **2015**; 212:1862–1868.
32. Banerjee R, Johnston B, Lohse C, Porter SB, Clabots C, Johnson JR. Escherichia coli Sequence Type 131 is a Dominant, Antimicrobial-Resistant Clonal Group Associated with Healthcare and Elderly Hosts. Infect Control Hosp Epidemiol, **2013**; 34:361–9.
33. Safdar N, Maki DG. The Commonality of Risk Factors for Nosocomial Colonization and. Ann. Intern. Med. **2002**; 136:834–844.

## TABLES

**Table 1: Selected demographic and clinical characteristics of *H30* and non-*H30* isolates, stratified by extended-spectrum cephalosporin resistance status.**

| | ESC-R n=278 | | | ESC-S n=1008 | | |
|---|---|---|---|---|---|---|
| | ***H30*** **n=83** | **non-*H30*** **n=195** | **p-value** | ***H30*** **n=47** | **non-*H30*** **n=961** | **p-value** |
| **Age** | | | 0.008* | | | <0.001* |
| 0-5 | 60 (72.3) | 98 (50.3) | | 16 (34.0) | 504 (52.5) | |
| 6-10 | 10 (12.1) | 40 (20.5) | | 4 (8.5) | 190 (19.8) | |
| 11-15 | 6 (7.2) | 31 (15.9) | | 12 (25.5) | 126 (13.1) | |
| 16-21 | 7 (8.4) | 26 (13.3) | | 15 (31.9) | 141 (14.7) | |
| **Female** | 65 (78.3) | 142 (72.8) | 0.417 | 39 (83.0) | 831 (86.5) | 0.643 |
| **Hispanic ethnicity** | 8 (10.0) | 36 (19.3) | 0.092 | 4 (8.9) | 135 (14.6) | 0.397 |
| **Race** | | | 0.087 | | | 0.314 |
| White/Caucasian | 39 (49.4) | 116 (62.0) | | 29 (63.0) | 629 (68.2) | |
| African-American | 12 (15.2) | 29 (15.5) | | 12 (26.1) | 219 (23.7) | |
| Asian | 22 (27.9) | 32 (17.1) | | 2 (4.4) | 51 (5.5) | |
| Native American | 4 (5.1) | 2 (1.1) | | 1 (2.2) | 6 (0.7) | |
| Pacific Islander | 2 (2.5) | 7 (3.7) | | 1 (2.2) | 10 (1.1) | |
| More than one race | 0 (--) | 1 (0.5) | | 1 (2.2) | 8 (0.9) | |
| **Site of culture** | | | 0.233 | | | 0.753 |
| Urine | 78 (94.0) | 173 (88.7) | | 45 (95.7) | 923 (96.1) | |
| Blood | 2 (2.4) | 15 (7.7) | | 2 (4.3) | 32 (3.3) | |
| Other | 3 (3.6) | 7 (3.6) | | 0 (0.0) | 6 (0.6) | |
| **Type of acquisition**[a] | | | 0.921 | | | <0.001* |
| Community-associated | 28 (33.7) | 63 (33.3) | | 14 (29.8) | 601 (62.5) | |
| Healthcare-associated | 45 (54.2) | 103 (52.8) | | 30 (63.8) | 299 (31.1) | |
| Hospital-associated | 10 (12.0) | 27 (13.8) | | 3 (6.4) | 61 (6.4) | |
| **Hospitalized in past 6 months** | 25 (30.1) | 69 (35.4) | 0.477 | 13 (27.7) | 143 (14.9) | 0.031* |
| **Underlying medical condition** | | | 0.888 | | | <0.001* |
| Urologic | 30 (36.1) | 75 (38.7) | | 26 (55.3) | 185 (19.3) | |
| Malignancy | 4 (4.8) | 13 (6.7) | | 1 (2.1) | 26 (2.7) | |
| Other condition | 16 (19.3) | 35 (18.0) | | 6 (12.8) | 104 (10.8) | |
| No condition | 33 (39.8) | 71 (36.6) | | 14 (29.8) | 644 (67.2) | |
| **Antibiotic use in the past 30 days** | 34 (41.0) | 85 (43.6) | 0.785 | 16 (34.0) | 178 (18.5) | 0.014* |
| **History of transplantation** | 3 (3.6) | 19 (9.7) | 0.136 | 5 (10.6) | 22 (2.3) | 0.007* |
| **Received immunosuppressants in last year** | 9 (10.8) | 37 (19.0) | 0.135 | 6 (12.8) | 62 (6.5) | 0.125 |
| **Device type** | | | 0.153 | | | <0.001* |
| Central venous catheter | 7 (8.4) | 28 (14.4) | | 3 (6.4) | 53 (5.5) | |
| Foley catheter | 6 (7.2) | 5 (2.6) | | 3 (6.4) | 11 (1.1) | |
| Other device | 14 (16.9) | 26 (13.3) | | 10 (21.3) | 55 (5.7) | |
| No device | 56 (67.5) | 136 (69.7) | | 31 (66.0) | 842 (87.6) | |

| | | | | | |
|---|---|---|---|---|---|
| **Hospital** | | | 0.156 | | 0.349 |
| West | 22 (26.5) | 78 (40.0) | | 13 (27.7) | 341 (35.5) |
| East | 24 (28.9) | 51 (26.2) | | 16 (34.0) | 284 (29.6) |
| Midwest 1 | 11 (13.3) | 23 (11.8) | | 3 (6.4) | 108 (11.2) |
| Midwest 2 | 26 (31.3) | 43 (22.1) | | 15 (31.9) | 228 (23.7) |

[a] Type of acquisition defined as follows: community associated, culture obtained in an outpatient setting or < 48 hours after hospital admission from an otherwise healthy patient without hospitalization in the previous 6 months; healthcare associated, culture obtained in an outpatient setting or < 48 hours after hospital admission from a patient who had been hospitalized in the previous 6 months and/or had a chronic medical condition requiring frequent health care or prolonged/recurrent antibiotic courses; and hospital associated, culture obtained > 48 hours after hospital admission or < 48 hours after hospital discharge from a patient without signs or symptoms of infection on admission

* p-value < 0.05

**Table 2: Selected antimicrobial resistance characteristics of *H30*Rx, *H30-non-Rx,* and non-*H30* isolates stratified by extended-spectrum cephalosporin resistance status**

| | ESC-R n=278 | | | | | ESC-S n=1008 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *H30* n = 83 | | | p-value vs. non-*H30*: | | *H30* n = 47 | | | p-value vs. non-*H30*: | |
| | Rx n=64 | *non-Rx* n=19 | non-*H30* n=195 | Rx | *non-Rx* | Rx n=5 | *non-Rx* n=42 | non-*H30* n=961 | Rx | *non-Rx* |
| **Co-resistance** | | | | | | | | | | |
| Ciprofloxacin | 62 (96.9) | 18 (94.7) | 76 (39.0) | <0.001* | <0.001* | 5 (100) | 36 (85.7) | 25 (2.6) | <0.001* | <0.001* |
| Gentamycin | 28 (43.8) | 6 (31.6) | 73 (37.4) | 0.453 | 0.798 | 0 (--) | 13 (31.0) | 34 (3.5) | 1.00 | <0.001* |
| TMP/SMX[a] | 43 (67.2) | 15 (78.9) | 121 (62.1) | 0.555 | 0.226 | 1 (20.0) | 26 (61.9) | 240 (25.0) | 1.00 | <0.001* |
| TMP/SMX & ciprofloxicin | 41 (64.1) | 15 (78.9) | 64 (32.8) | <0.001* | <0.001* | 1 (20.0) | 23 (54.8) | 15 (1.6) | 0.080 | <0.001* |
| All three | 19 (29.7) | 5 (26.3) | 36 (18.5) | 0.084 | 0.374 | 0 (--) | 8 (19.0) | 2 (0.2) | 1.00 | <0.001* |
| **ESC-R type** | | | | <0.001* | 0.007* | | | | | |
| ESBL only | 64 (100)[b] | 17 (89.5) | 102 (52.6) | | | -- | -- | -- | -- | -- |
| AmpC only | 0 (--) | 2 (5.4) | 88 (45.4) | | | -- | -- | -- | -- | -- |
| ESBL & AmpC | 0 (--) | 0 (--) | 4 (2.06) | | | -- | -- | -- | -- | -- |
| Undetermined | 0 (--) | 0 (--) | 1 (0.5) | | | | | | | |
| **ESBL determinants[c]** | **n=64** | **n=17** | **n = 106** | | | | | | | |
| CTX-M-15 | 60 (93.8) | 3 (17.6) | 48 (45.3) | <0.001* | 0.060 | -- | -- | -- | -- | -- |
| CTX-M-14 | 0 (--) | 2 (11.8) | 44 (41.5) | <0.001* | 0.037* | -- | -- | -- | -- | -- |
| CTX-M-27 | 1 (1.6) | 10 (58.8) | 1 (0.9) | 1.000 | <0.001* | -- | -- | -- | -- | -- |
| CTX-M others | 0 (--) | 1 (5.3) | 7 (6.6) | 0.046* | 1.000 | -- | -- | -- | -- | -- |
| ESBL SHV | 0 (--) | 0 (--) | 3 (2.8) | 0.292 | 1.000 | -- | -- | -- | -- | -- |
| ESBL TEM | 0 (--) | 0 (--) | 0 (--) | -- | -- | -- | -- | -- | -- | -- |
| None identified | 3 (4.7) | 1 (5.3) | 4 (3.8) | 1.000 | 0.531 | -- | -- | -- | -- | -- |
| **AmpC determinants[c]** | **n=0** | **n=2** | **n=92** | | | | | | | |
| CMY-2 | -- | 1 (50.0) | 79 (96.3) | -- | 0.277 | -- | -- | -- | -- | -- |
| DHA | -- | 0 (--) | 2 (2.2) | -- | 1.000 | -- | -- | -- | -- | -- |
| FOX | -- | 0 (--) | 2 (2.2) | -- | 1.000 | -- | -- | -- | -- | -- |
| None identified | -- | 1 (50.0) | 10 (10.9) | -- | 1.000 | -- | -- | -- | -- | -- |

[a] TMP/SMX = trimethoprim-sulfamethoxazole [b] One of these isolates was also resistant to meropenem and produced a KPC-3 carbapenemase.

[c] Total exceeds 100% as isolates could have more than one determinant identified. * p-value < 0.05

**Table 3: Total and direct effect of selected factors on risk of *H30* infection vs. infection with other *E. coli* types using log-binomial regression models stratified by extended-spectrum cephalosporin resistance status**

| | ESC-R | | | ESC-S | | |
|---|---|---|---|---|---|---|
| | Total effect RR (95% CI) | | Direct effect RR (95% CI) | Total effect RR (95% CI) | | Direct effect RR (95% CI) |
| | Crude | Adjusted | Adjusted | Crude | Adjusted | Adjusted |
| **Age 0-5 years** | 1.98 (1.33-3.09)* | 1.80 (1.18-2.86)[a]* | 1.87 (1.21-2.97)[b]* | 0.48 (0.26-0.86)* | -- | 0.54 (0.29-0.97)[c] |
| **Antibiotics in preceding 30 days** | -- | -- | -- | 2.17 (1.18-3.81)* | 1.15 (0.60-2.13)[d] | -- |
| **Underlying medical condition** | -- | -- | -- | 4.46 (2.47-8.49)* | 4.19 (2.32-8.00)[e]* | 3.25 (1.60-6.74)[f]* |
| **Hospitalization in past 6 months** | -- | -- | -- | 2.09 (1.08-3.77)* | 1.25 (0.64-2.33)[g] | 1.01 (0.49-2.00)[h] |
| **Presence of indwelling device** | -- | -- | -- | 3.34 (1.83-5.84)* | 1.62 (0.80-3.22)[i] | 1.61 (0.79-3.20)[c] |

[a] Covariates: Asian race (yes/no)

[b] Covariates: Asian race (yes/no), underlying medical condition (yes/no), antibiotics in the last 30 days (yes/no), hospitalization in the past 6 months (yes/no)

[c] Covariates: underlying medical condition (yes/no), antibiotics in the last 30 days (yes/no), hospitalization in the past 6 months (yes/no)

[d] Covariates: age (0-5 or 6-21, hospitalization in the past 6 months (yes/no), underlying medical condition (yes/no), indwelling device (yes/no)

[e] Covariates: age (0-5 or 6-21)

[f] Covariates: age (0-5 or 6-21, hospitalization in the past 6 months (yes/no), antibiotics in the last 30 days (yes/no), indwelling device (yes/no)

[g] Covariates: age (0-5 or 6-21), underlying medical condition (yes/no)

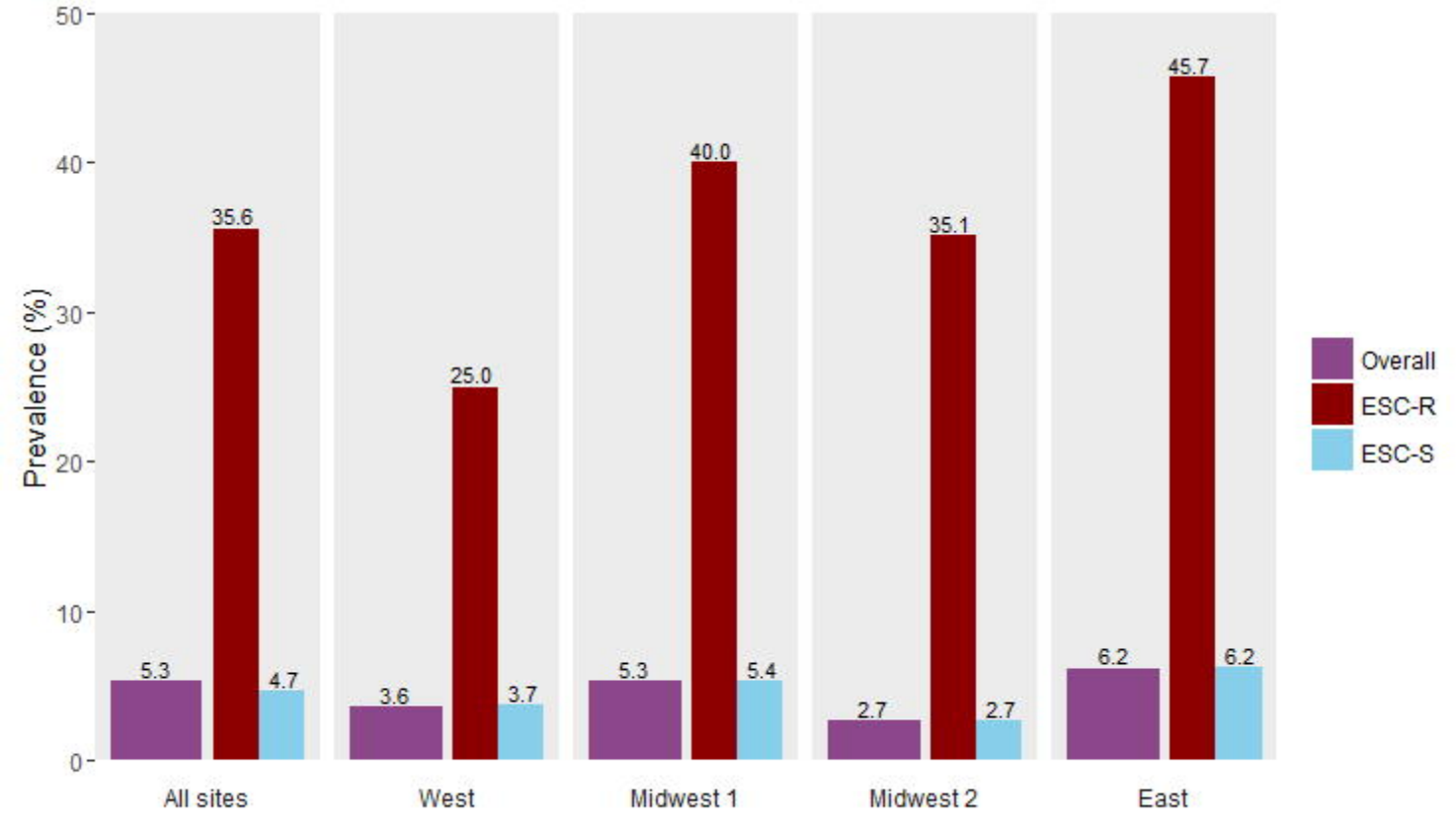
[h] Covariates: age (0-5 or 6-21), underlying medical condition (yes/no), antibiotics in the last 30 days, indwelling device (yes/no)

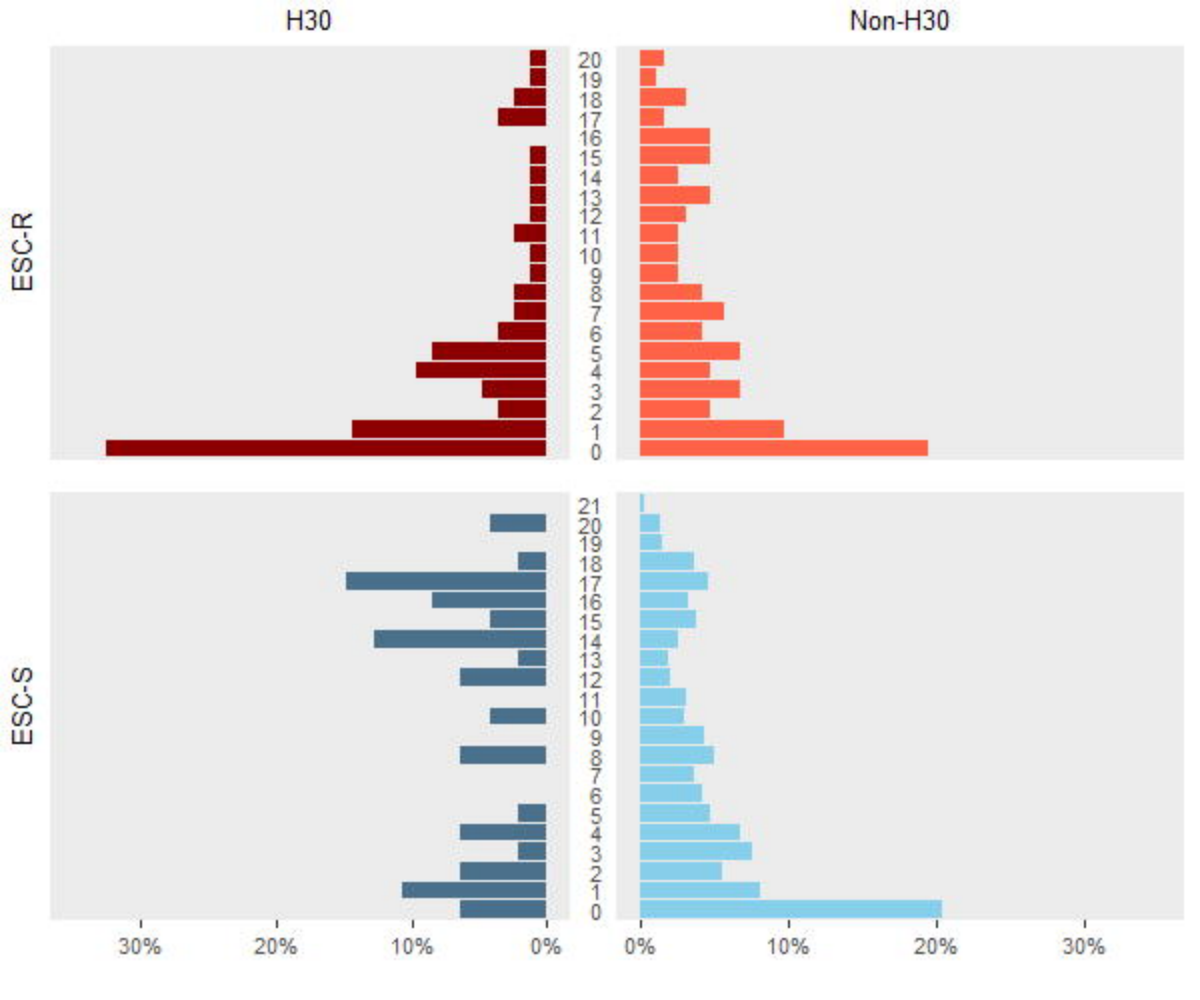
[i] Covariates: underlying medical condition (yes/no), hospitalization in the past 6 months (yes/no)

## FIGURE LEGENDS

**Figure 1: Estimated prevalence of ST131-*H30* among extraintestinal *E. coli* infections overall and by study hospital. ESC-R = extended-spectrum cephalosporin-resistant. ESC-S = extended-spectrum cephalosporin-sensitive. The raw numbers that generated these estimates can be found in Supplementary Table 1.**

**Figure 2: Distributions of age (in years) by ST131-*H30* and non-ST131-*H30* status and extended-spectrum cephalosporin resistance status. ESC-R = extended-spectrum cephalosporin-resistant. ESC-S = extended-spectrum cephalosporin-sensitive.**


Prevalence (%)
50
40
30
20
10
0
5.3
35.6
4.7
3.6
25.0
3.7
5.3
40.0
5.4
2.7
35.1
2.7
6.2
45.7
6.2
All sites
West
Midwest 1
Midwest 2
East
Overall
ESC-R
ESC-S


H30
Non-H30
ESC-R
ESC-S
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
30%
20%
10%
0%
0%
10%
20%
30%