Interaction between Antibiotic Resistance, Resistance Genes, and Treatment Response for Urinary Tract Infections in Primary Care

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ABSTRACT
Given increasing antimicrobial resistance, we aimed to determine antibiogram susceptibility and presence of resistance genes in uropathogens in primary care, factors associated with resistance to commonly prescribed antibiotics, and effect of treatment on early symptom resolution. We conducted a prospective study of primary care patients with urinary tract infection (UTI) symptoms and culture-confirmed UTI in Singapore from 2015 to 2016. Cohort characteristics and antimicrobial susceptibility of cultured isolates were analyzed. Among Enterobacteriaceae isolates, early symptom resolution (within 3 days) according to antibiotic prescribed and isolate susceptibility and factors associated with antibiotic resistance were evaluated. Of 695 symptomatic patients, 299 were urine culture positive; of these 299 patients, 259 (87%) were female. Escherichia coli was the most common uropathogen (76%). Enterobacteriaceae isolates (n = 283) were highly susceptible to amoxicillin-clavulanate (86%), nitrofurantoin (87%), and fosfomycin (98%), but >20% were resistant to ciprofloxacin and co-trimoxazole. Isolates resistant to appropriate indicator antibiotics were further tested to determine proportions positive for blaCTX-M (14/26, 54%), plasmid-mediated ampC (12/24, 50%), qnr (7/69, 10%), and fos (1/6, 17%) resistance genes. A total of 67% of patients given antibiotics with susceptible isolates reported early resolution versus 45% given antibiotics with nonsusceptible isolates (P = 0.001) and 27% not treated (P = 0.018). On multivariable analysis, Indian ethnicity and diabetes mellitus were associated with amoxicillin-clavulanate resistance. Genitourinary abnormalities, UTI in the past 12 months, and hospitalization in the past 6 months were associated with ciprofloxacin and co-trimoxazole resistance. Patients given active empirical antibiotics were most likely to report early symptom resolution, but correlation with in vitro susceptibility was imperfect. Factors associated with resistance may guide the decision to obtain initial urine culture.

KEYWORDS antibacterial agents, drug resistance, microbial, primary health care, therapeutics, urinary tract infection

Annually, about 150 million urinary tract infections (UTI) are diagnosed worldwide (1), and over half of adult women have at least one episode in their lifetime (2). UTI frequently presents in primary care, where treatment is usually empirical without urine culture or antibiotic susceptibility testing to guide treatment. This is appropriate given
the practical necessity to treat without waiting for culture results, as long as causative uropathogens and their antimicrobial susceptibility profile remain predictable (3).

*Escherichia coli* is the major pathogen isolated in community-acquired UTIs (4), accounting for 75% of UTIs in primary care in Singapore (5). In a 2009 study of UTI in Singapore, 39% and 24% of *E. coli* isolates were resistant to co-trimoxazole and ciprofloxacin, respectively (5). However, antimicrobial resistance in community-acquired uropathogens has been increasing across many settings (6, 7) and is associated with poorer outcomes, including delayed symptom resolution, repeat medical consults, and disease progression due to ascending infection (8). Periodic reassessment of antimicrobial susceptibility patterns helps to identify groups where resistance should be suspected, determine optimal empirical antibiotics, and determine whether it remains appropriate to forgo susceptibility testing.

Our study evaluated the etiology, antibiotic susceptibility patterns, and prevalence of common hospital-associated resistance genes in uropathogens in primary care in Singapore. We assessed the effect of appropriate treatment on early symptom resolution and evaluated factors associated with resistance to commonly prescribed antibiotics in our setting.

**MATERIALS AND METHODS**

**Setting.** We conducted a prospective cohort study in three public primary care clinics (polyclinics) in Singapore from 2015 to 2016. Polyclinics provide subsidized care for residents residing in urban housing estates, serving high proportions of elderly and patients requiring chronic disease care (9).

**Participants.** Adult male and female clinic patients aged 21 years and above and identified by the physician to have UTI-related symptoms were invited to participate and referred to a research assistant stationed on site. Informed consent was taken from all participants prior to entry into the study. All demographic and clinical data for the study were collected using a standardized questionnaire. This was either self-completed or administered by the research assistant, who then checked to ensure that there was no missing data. All recruited patients submitted urine samples for cultures. Those unable to give consent or provide a midstream urine sample were excluded.

We used an established standard for UTI diagnosis, i.e., detection of the uropathogen in the presence of clinical symptoms (10). Laboratory cutoffs for positive urine culture were isolation of $\geq 10^5$ CFU/ml of primary urinary pathogens (*E. coli*, *Klebsiella* sp., or *Staphylococcus saprophyticus*) in pure culture or isolation of $\geq 10^6$ CFU/ml of these organisms as predominant growth, in accordance with the European guidelines for urinalysis (11). The significance cutoff for other potential pathogens was $\geq 10^5$ CFU/ml.

**Pathogen identification and antibiotic susceptibility testing.** Uropathogens were identified using conventional phenotypic methods, with antibiotic susceptibility determined by Vitek N257 cards (bio-Mérieux, France), with supplemental disc susceptibility testing for fosfomycin, trimethoprim, and cefpodoxime. Categorical susceptibility breakpoints followed current guidelines from the Clinical and Laboratory Standards Institute (12), except for *Klebsiella pneumoniae* susceptibility to fosfomycin, where breakpoints from a published study were used (13).

**Antibiotic resistance genotyping.** Molecular testing for selected plasmid-mediated resistance genes commonly found in the local hospital setting was performed for isolates with resistance to appropriate indicator antibiotics as follows: CTX-M extended-spectrum beta-lactamases (ESBL) and plasmid-mediated *ampC* genes for ceftriaxone-resistant isolates of *E. coli* and *K. pneumoniae* (14, 15), *qnr* genes for ciprofloxacin-resistant isolates (16), and *fosA* genes for fosfomycin-resistant isolates (*fosA* being plasmid-mediated in *E. coli*) (17).

**Follow-up of participants.** Participants were followed up using a standardized telephone-administered questionnaire at least 4 days after their consultation and asked about resolution of urinary symptom(s), including when each symptom resolved. The study endpoint was early symptom resolution, defined as having all symptoms resolved within 3 days of initial consultation date.

**Statistical analysis.** Cohort characteristics and antimicrobial susceptibility patterns of uropathogens are described. Among patients with uropathogens from the *Enterobacteriaceae* family, we assessed the distribution of prescribed antibiotics among treated patients and the number of patients not treated with antibiotics (as per the clinical assessment of the attending physician). We then compared early symptom resolution outcomes according to antibiotic prescribed (if any) and the uropathogen’s susceptibility pattern. We also evaluated patient characteristics associated with early symptom resolution and factors correlated with resistance to amoxicillin-clavulanate, ciprofloxacin, co-trimoxazole, and ceftriaxone.

Pearson’s chi-square or Fisher’s exact test were used to evaluate differences in proportions for categorical variables. We calculated crude odds ratios (ORs) with 95% confidence intervals (CIs) for demographic and clinical factors associated with antibiotic resistance and early symptom resolution and adjusted ORs for multivariable logistic regression analyses using key clinical variables (with $P$ values of $<0.10$ on univariate analysis) and adjusting for potential demographic confounders (age, gender, and ethnicity). All analyses were performed using Stata version 15 (StataCorp, College Station, TX). A $P$ value of $<0.05$ was considered statistically significant.
RESULTS

Participant characteristics. Over the 2-year study period, 743 patients with UTI-related symptoms were screened across the three study sites, of whom 695 (94%) were eligible and agreed to participate. A total of 299 had positive urine culture according to the predefined laboratory cutoffs and were included in our analyses. This comprised 259 (87%) females and 40 males (Table 1), who were generally elderly (mean age, 60.8 ± 17.3 years) and predominantly of Chinese ethnicity (n = 222, 74%). Substantial proportions reported physician-diagnosed UTI in the past 12 months (n = 115, 39%), diabetes mellitus (DM) (n = 76, 25%), genitourinary (GU) abnormalities (n = 42, 14%), antibiotic use in the past 4 weeks (n = 49, 16%), and hospitalization in the past 6 months (n = 49, 16%). A total of 270 (90%) participants completed follow-up, with 164 (55%) reporting early symptom resolution.

Distribution of uropathogens and antibiotic susceptibility patterns. We detected 266 isolates from females and 40 from males. Overall, the most common pathogen was *Escherichia coli* (n = 231, 76%), followed by *Klebsiella pneumoniae* (n = 20, 7%) and *Proteus mirabilis* (n = 14, 5%). A further 18 (6%) isolates were other *Enterobacteriaceae*, with remaining isolates being *Streptococcus agalactiae* (n = 8, 3%), *Enterococcus faecalis* (n = 6, 2%), and *Staphylococcus saprophyticus* (n = 3, 1%). This overall distribution was similar for females. Among males, *E. coli* was the most common pathogen detected (n = 25, 63%), with small numbers of other isolates including *E. faecalis* (n = 3, 8%), *K. pneumoniae* (n = 2, 5%), and *Citrobacter koseri* (n = 2, 5%).

Among *Enterobacteriaceae* (Table 2), high proportions of isolates were susceptible to amoxicillin-clavulanate (86%), cefixime (91%), nitrofurantoin (87%), and fosfomycin (98%). However, <80% were susceptible to ciprofloxacin (76%), levofloxacin (76%), and co-trimoxazole (74%). Isolates also demonstrated high susceptibility to parenterally administered antibiotics (minimum of 89% for gentamicin). Using only data from female participants gave similar results (see Table S1 in the supplemental material), but numbers were insufficient for a corresponding assessment in males.

| Characteristic | Total no. (%) | No. female (%) | No. male (%) |
|---------------|--------------|---------------|--------------|
| Mean age (yr) (SD) | 60.8 (17.3) | 59.9 (17.6) | 66.5 (14.1) |
| Age group (yr) | | | |
| 21–29 | 25 (8) | 23 (9) | 2 (5) |
| 30–39 | 17 (6) | 16 (6) | 1 (3) |
| 40–49 | 23 (8) | 23 (9) | 0 (0) |
| 50–59 | 61 (20) | 56 (22) | 5 (13) |
| 60–69 | 75 (25) | 62 (24) | 13 (33) |
| 70–79 | 63 (21) | 48 (19) | 15 (38) |
| 80 and above | 35 (12) | 31 (12) | 4 (10) |
| Ethnicity | | | |
| Chinese | 222 (74) | 198 (76) | 24 (60) |
| Malay | 31 (10) | 26 (10) | 5 (13) |
| Indian | 29 (10) | 20 (8) | 9 (23) |
| Others | 17 (6) | 15 (6) | 2 (5) |
| Medical history | | | |
| Physician-diagnosed UTI in past 12 mo | 115 (39) | 102 (39) | 13 (33) |
| Hospitalization within the last 6 mo | 37 (12) | 28 (11) | 9 (23) |
| Diabetes mellitus | 76 (25) | 59 (23) | 17 (43) |
| Genitourinary abnormalities | 42 (14) | 32 (12) | 10 (25) |
| Use of antibiotics within last 4 wks | 49 (16) | 43 (17) | 6 (15) |
| Outcomes during telephone follow-up | | | |
| Successfully contacted for follow-up | 270 (90) | 235 (91) | 35 (88) |
| Symptoms resolved within 3 days | 164 (55) | 151 (58) | 13 (33) |
Figure 1 shows factors associated with resistance. Amoxicillin-clavulanate resistance was positively associated with Indian ethnicity (versus Chinese), physician-diagnosed UTI in the past 12 months, hospitalization in the past 6 months, and DM. Ciprofloxacin, co-trimoxazole, and ceftriaxone resistance were positively associated with recent UTI, hospitalization, or GU abnormalities (all three); age group (ciprofloxacin and ceftriaxone); use of antibiotics in the past 4 weeks (ciprofloxacin and co-trimoxazole); and history of DM (ceftriaxone only).

### TABLE 2 Antibiotic susceptibility profiles of Enterobacteriaceae isolated from urine cultures (n = 283)

| Antibiotic         | Escherichia coli (n = 231) | Other Enterobacteriaceae* (n = 52) |
|--------------------|----------------------------|------------------------------------|
|                    | Total no. tested | No. susceptible | % | Total no. tested | No. susceptible | % |
| **Oral**           |                |                |   |                |                |   |
| Amoxicillin        | 231            | 114            | 49 | 52             | 13             | 25 |
| Amoxicillin-clavulanate | 231            | 206            | 89 | 52             | 37             | 71 |
| Ceftibuten         | 87             | 77             | 89 | 21             | 21             | 100 |
| Cefuroxime         | 231            | 173            | 75 | 52             | 41             | 79 |
| Cephalexin         | 231            | 60             | 26 | 52             | 36             | 69 |
| Ciprofloxacin      | 231            | 165            | 71 | 52             | 50             | 96 |
| Co-trimoxazole     | 231            | 164            | 71 | 52             | 46             | 89 |
| Fosomycin          | 231            | 227            | 98 | 20             | 19             | 95 |
| Levofloxacin       | 143            | 102            | 71 | 31             | 30             | 97 |
| Nitrofurantoin     | 230            | 228            | 99 | 52             | 17             | 33 |
| Trimethoprim       | 231            | 161            | 70 | 52             | 44             | 85 |
| **Parenteral**     |                |                |   |                |                |   |
| Amikacin           | 231            | 231            | 100 | 52             | 52             | 100 |
| Aztreonam          | 231            | 206            | 89 | 52             | 51             | 98 |
| Cefepime           | 231            | 213            | 92 | 52             | 52             | 100 |
| Cefotaxime         | 143            | 127            | 89 | 31             | 30             | 97 |
| Cefotaxine         | 231            | 218            | 94 | 52             | 39             | 75 |
| Ceftazidime        | 231            | 205            | 89 | 52             | 51             | 98 |
| Ceftriaxone        | 231            | 205            | 89 | 52             | 51             | 98 |
| Ertapenem          | 231            | 231            | 100 | 52             | 51             | 98 |
| Gentamicin         | 231            | 201            | 87 | 52             | 50             | 96 |
| Imipenem           | 231            | 231            | 100 | 51             | 35             | 69 |
| Meropenem          | 231            | 231            | 100 | 52             | 52             | 100 |
| Piperacillin-tazobactam | 231            | 218            | 94 | 51             | 50             | 98 |

*Includes Klebsiella pneumoniae (20), Proteus mirabilis (14), Enterobacter aerogenes (7), Citrobacter koseri (6), Serratia marcescens (2), Citrobacter werkmanii (1), Enterobacter cloacae (1), and Morganella morganii (1).

Figure 1 Univariate and multivariable analysis of factors associated with resistance to various antibiotics for urinary tract infections caused by Enterobacteriaceae. Diamonds indicate univariate odds ratios, and triangles indicate multivariable odds ratios. Error bars indicate 95% confidence intervals. Shaded diamonds and triangles denote statistically significant variables. UTI, urinary tract infection; LL, lower limit; UL, upper limit; AUC, area under the curve.
A multivariable analysis identified factors independently associated with amoxicillin-clavulanate resistance as Indian ethnicity (adjusted odds ratio [AOR] = 4.14; 95% CI, 1.43 to 11.99; P = 0.01) and DM (AOR = 2.54; 95% CI, 1.09 to 5.88; P = 0.03). Factors independently associated with ciprofloxacin, co-trimoxazole, and ceftriaxone resistance were recent UTI (all three) as well as GU abnormalities and recent hospitalization (for ciprofloxacin only).

**Antibiotic treatment, resistance, and early symptom resolution.** Of 278 participants with *Enterobacteriaceae* isolates, 265 (95%) were given empirical antibiotics during the initial consultation, most commonly amoxicillin-clavulanate (63%), ciprofloxacin (26%), and co-trimoxazole (6%). A total of 83% of antibiotics prescribed were active against the isolates for that patient as follows: 88% for nitrofurantoin and 87% for amoxicillin-clavulanate but only 76% for ciprofloxacin and 69% for co-trimoxazole (P value across treatment groups = 0.047).

Among patients with follow-up data (Fig. 2), 152/240 (63%) treated with any empirical antibiotics at initial consultation reported early symptom resolution, which was significantly higher than 3/11 (27%) patients not given empirical antibiotics (P = 0.024). Within the treated group, 135/202 (67%) patients with susceptible isolates reported early resolution versus 17/38 (45%) with nonsusceptible isolates (P = 0.001). Corresponding proportions for those prescribed amoxicillin-clavulanate were 94/134 (70%) versus 8/18 (44%) (P = 0.036); for co-trimoxazole, proportions were 9/10 (90%) versus 0/5 (0%) (P = 0.002); and for nitrofurantoin, proportions were 7/8 (88%) versus 1/1 (100%) (P = 1.000). For ciprofloxacin, however, only 23/48 (48%) treated patients with susceptible isolates reported early resolution versus 7/13 (54%) treated patients with nonsusceptible isolates (P = 0.762).

We further evaluated possible reasons for the observed outcomes with ciprofloxacin. Table S2 in the supplemental material shows that patient characteristics across different antibiotic treatment groups did not have major differences, except that the proportion of patients with DM varied across patients treated with ciprofloxacin (36%), co-trimoxazole (40%), nitrofurantoin (50%), and amoxicillin-clavulanate (20%). On stratification by dosage of ciprofloxacin prescribed (250 mg twice a day versus 500 mg twice a day), 5/16 (31%) patients given 250 mg twice a day reported early resolution versus 25/45 (56%) patients given 500 mg twice a day (P = 0.095).
On multivariable analysis of factors associated with early symptom resolution (Table 3), male patients were significantly less likely to report resolution. Compared to no antibiotic treatment, treatment of nonsusceptible isolates with any antibiotic was not significantly associated with symptom resolution. Treatment of susceptible isolates with amoxicillin-clavulanate or “other antibiotics” (co-trimoxazole or nitrofurantoin) was significantly associated with resolution, but treatment with ciprofloxacin remained nonsignificant.

Prevalence of resistance genes and early symptom resolution. A total of 14/26 (54%) E. coli and K. pneumoniae isolates with reduced susceptibility to ceftriaxone were positive for bla\textsubscript{CTX-M} alleles. A total of 12/24 (50%) E. coli and K. pneumoniae isolates screened for ampC genes were positive. Four E. coli isolates were positive for both ampC and bla\textsubscript{CTX-M} genes, while another 4 had neither cephalosporinase detected. qnr resistance genes were detected in 7 (10%) of 69 isolates with reduced quinolone susceptibility. Finally, only 1/6 (17%) tested positive for fos genes (fosA96). In total, 28 isolates were positive for at least one of these resistance genes (common in the local hospital setting), but only 7 (25%) belonged to patients reporting hospitalization in the past 6 months.

Among patients treated using amoxicillin-clavulanate, 3/3 (100%) patients with isolates negative for bla\textsubscript{CTX-M} and ampC genes reported early resolution versus 3/5 (60%) with bla\textsubscript{CTX-M} only, 2/5 (40%) with ampC only, and 0/3 (0%) when both ampC and bla\textsubscript{CTX-M} were detected (P = 0.091).

**DISCUSSION**

In our study of a predominantly female cohort, with a high proportion of elderly, we observed high levels of resistance to ciprofloxacin and co-trimoxazole, which were still being prescribed. For amoxicillin-clavulanate, which was most commonly prescribed, those with resistant isolates were less likely to report early symptom resolution. Moreover, genotypic markers of plasmid-mediated resistance were identified in a substantial number of isolates.

When compared with proportions of E. coli isolates from the study in 2009 (5), similar proportions were susceptible to amoxicillin-clavulanate (89% versus 91% in 2009) and ciprofloxacin (71% versus 76%), but the proportion susceptible to co-trimoxazole was slightly higher (71% versus 62%; P = 0.049). However, the 2009 study

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**TABLE 3** Univariate and multivariable analyses of factors associated with early resolution of symptoms

| Factor                                      | Univariate |            |          | Multivariable |            |          |
|---------------------------------------------|------------|------------|----------|---------------|------------|----------|
| Gender, male                                | 0.34       | 0.15–0.76  | 0.008    | 0.34          | 0.14–0.83  | 0.018    |
| Age, per 10-yr increase                     | 0.96       | 0.82–1.11  | 0.563    | 1.03          | 0.86–1.24  | 0.745    |
| Ethnicity (vs Chinese)                      |            |            |          |               |            |          |
| Malay                                       | 0.70       | 0.30–1.59  | 0.39     | 0.75          | 0.29–1.93  | 0.547    |
| Indian                                      | 0.75       | 0.33–1.69  | 0.481    | 1.09          | 0.42–2.86  | 0.859    |
| Others                                      | 1.19       | 0.35–4.11  | 0.780    | 1.44          | 0.36–5.65  | 0.605    |
| Medical history                             |            |            |          |               |            |          |
| Physician-diagnosed UTI in past 12 mo       | 0.66       | 0.39–1.11  | 0.116    |               |            |          |
| Hospitalization within the last 6 mo        | 0.83       | 0.37–1.84  | 0.646    |               |            |          |
| Diabetes mellitus                           | 0.55       | 0.31–0.98  | 0.042    | 0.77          | 0.38–1.53  | 0.448    |
| Genitourinary abnormalities                 | 0.51       | 0.25–1.06  | 0.073    | 0.69          | 0.30–1.58  | 0.385    |
| Use of antibiotics within last 4 wks         | 0.72       | 0.37–1.39  | 0.324    |               |            |          |
| Antibiotic given and susceptibility (vs no antibiotic) |            |            |          |               |            |          |
| Given amoxicillin-clavulanate, not susceptible | 2.13   | 0.42–10.78 | 0.359    | 2.27          | 0.43–12.10 | 0.337    |
| Given ciprofloxacin, not susceptible         | 3.11       | 0.56–17.33 | 0.195    | 2.94          | 0.50–17.16 | 0.232    |
| Given other antibiotic, not susceptible      | 0.53       | 0.04–6.65  | 0.625    | 0.62          | 0.05–8.51  | 0.723    |
| Given amoxicillin-clavulanate, susceptible   | 6.27       | 1.58–24.85 | 0.009    | 6.11          | 1.49–25.12 | 0.012    |
| Given ciprofloxacin, susceptible             | 2.45       | 0.58–10.38 | 0.223    | 2.38          | 0.54–10.39 | 0.250    |
| Given other antibiotic, susceptible         | 20.00      | 2.75–145.48| 0.003    | 22.37         | 2.89–173.19| 0.003    |

On multivariable analysis of factors associated with early symptom resolution (Table 3), male patients were significantly less likely to report resolution. Compared to no antibiotic treatment, treatment of nonsusceptible isolates with any antibiotic was not significantly associated with symptom resolution. Treatment of susceptible isolates with amoxicillin-clavulanate or “other antibiotics” (co-trimoxazole or nitrofurantoin) was significantly associated with resolution, but treatment with ciprofloxacin remained nonsignificant.
relied on retrospective chart reviews of urine cultures. Since physicians may selectively test more complicated patients or those who fail treatment (18), that study may have overestimated antibiotic resistance levels compared to those in our current study, which prospectively obtained urine cultures from all UTI patients and arguably provides a more accurate assessment of current antibiotic susceptibility profiles.

Globally, antibiotic susceptibility of uropathogens varies widely (19). We observed less resistance of *E. coli* to amoxicillin-clavulanate compared to that of some other community settings (7, 20). Ciprofloxacin and co-trimoxazole resistance was comparable to that of data from primary care in Hong Kong (21) and less prevalent than in India, Iran, and Turkey (20). Resistance in uncomplicated UTIs may be correlated with antibiotic consumption (22). Antibiotics cannot be obtained without prescription by licensed physicians in Singapore. However, one study suggests large variations in primary care prescriptions of antibiotics for upper respiratory tract infection (from 0% to 70% of episodes) (23). The moderate levels of resistance observed in Singapore should hence motivate additional legislative (24) and physician education interventions to improve antibiotic stewardship (25).

Among *Enterobacteriaceae*, factors independently associated with resistance to amoxicillin-clavulanate were Indian ethnicity and DM. Potential explanations may lie in travel-related exposures to the high prevalence of antibiotic-resistant bacteria in South Asia for the former (26) and greater exposure of DM patients to health care interventions for the latter (27). Associations between ciprofloxacin and co-trimoxazole resistance with past UTI diagnosis is consistent with extant literature (28, 29), and the association between ciprofloxacin resistance and hospitalization may be related to the substantially higher resistance prevalence in hospital settings (30).

Regardless of the specific patterns of resistance, we believe that the findings with wider relevance were our follow-up data on how matching empirical antibiotic to uropathogen susceptibility affects early symptom resolution, but also how this relationship was imperfect. The small proportion (27%) reporting early resolution despite not receiving empirical antibiotics was consistent with the clinical course of untreated uncomplicated cystitis, where 25% to 42% may experience resolution (4). In those given antibiotics, the “90/60” rule has been proposed, where infections due to susceptible and resistant isolates would correspondingly respond 90% and 60% of the time (31). In our study, 67% of treated patients with susceptible isolates and 45% of treated patients with nonsusceptible isolates reported early resolution. Our short follow-up duration may account for the lower proportions reporting symptom resolution (67/45 instead of 90/60), though interestingly, the ratio between groups appeared to be consistent with the 90/60 rule.

However, while we demonstrated the overall impact of appropriate empirical antibiotic therapy on early symptom resolution, our findings also caution us about the validity of extrapolating efficacy purely based on *in vitro* susceptibility testing alone. In patients prescribed amoxicillin-clavulanate, symptoms appeared less likely to resolve when genotypic resistance markers were present than when they were absent. For those prescribed ciprofloxacin, the “90/60” rule has been proposed, where infections due to susceptible and resistant isolates would correspondingly respond 90% and 60% of the time (31). In our study, 67% of treated patients with susceptible isolates and 45% of treated patients with nonsusceptible isolates reported early resolution. Our short follow-up duration may account for the lower proportions reporting symptom resolution (67/45 instead of 90/60), though interestingly, the ratio between groups appeared to be consistent with the 90/60 rule.

The effectiveness of ciprofloxacin is a function of peak concentration divided by MIC (32). Particularly in the elderly, where there is decreased renal excretion, the drug may not reach sufficient concentrations in the urine with a 250-mg twice daily prescription. A 500-mg once a day dosing regimen has been shown to deliver higher drug concen-
trations in the urine, and this regimen at minimum should be prescribed to optimize outcomes (32).

Overall, with regard to empirical therapy, the vast majority of isolates remained susceptible to fosfomycin and nitrofurantoin. Both of these are recommended antibiotics for uncomplicated UTIs in international guidelines (over amoxicillin-clavulanate, which is thought to have lower efficacy, more adverse effects, and broader spectrum of cover) (19) and are viable alternatives that should be considered for Singapore. Given the levels of resistance observed (>20%), ciprofloxacin and co-trimoxazole are less preferred in our setting without ordering urine cultures to guide treatment. In turn, the need for urine cultures could be guided by the risk factors highlighted in this study. Furthermore, appropriate dosing of ciprofloxacin, as discussed above, is vital to ensuring a favorable response to therapy.

Only a low proportion of isolates (<10%) in our primary care setting carried the bla$_{CTX-M}$, ampC, and qnr resistance genes, which are highly prevalent among hospital Enterobacteriaceae isolates in Singapore (33, 34). However, of these, 75% were not associated with hospitalization in the past 6 months, and acquisition may thus be occurring within the community. The rising prevalence of resistance, including strains carrying key resistance genes with possible clinical significance, compounded by the imperfect correlation between susceptibility testing results with actual clinical effectiveness, is likely an emerging and important issue which will increasingly complicate management of UTI in primary care settings around the world.

In the face of rapidly changing resistance profiles, strategies are urgently needed for rationalizing how best to determine guidelines for UTI treatment outside of the hospital, where there is typically no routine surveillance or follow up with patients. We argue that a robust approach may need to go beyond surveillance of antimicrobial susceptibility testing results and include routine monitoring of a sample of patients for symptom resolution and possibly the use of genotypic markers of resistance.

The strengths of our study include using a prospective (rather than a potentially biased retrospective) approach to determine the distribution of uropathogens presenting in the primary care setting and their antibiotic susceptibility profiles, with the cohort design demonstrating how antibiotic choices potentially interact with susceptibility profiles and genotypic markers of resistance to affect a clinically relevant endpoint. However, our study has some limitations. Our cohort was predominantly elderly and reflective of the catchment of polyclinics, which provide subsidized primary care services, though this may also reflect the ageing of the population in Singapore (35). We did not split and analyze patients according to different types of UTI (from uncomplicated cystitis to acute pyelonephritis). However, our intent in this paper was to provide generalizable results for UTI in primary care. Some culture results obtained may have been false positives, which would have affected the accuracy of our study, although this number is likely to be small, since the criteria for recruitment were initial presentation with symptoms of UTI. Our symptom resolution endpoint may have been too brief to document complications and risk of recurrent UTI known to occur with inappropriate treatment (8), and we had only a small number of patients untreated with antibiotics for comparison. Some subgroup analyses were also limited by the sample size. Our study only focused on patients with culture-positive UTIs, although a recent study showed that a high proportion of symptomatic women with negative urine cultures did actually have UTI, most commonly due to *E. coli* (36). Further studies are needed to evaluate whether treatment outcomes in culture-negative patients are similar to culture-positive patients.

In conclusion, our study profiled antibiotic susceptibilities for uropathogens in our primary care setting and identified risk factors for resistance, which may guide the decision to obtain initial urine culture to guide therapy. While patients given active empirical antibiotics were most likely to report early symptom resolution, the correlation with *in vitro* susceptibility was imperfect. Strategies are needed to monitor clinical effectiveness of treatment options for UTI in primary care in the face of rapidly changing resistance patterns.
SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00143-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We thank Nur Afiqah Binte Abdul Rahman from the National Centre for Infectious Diseases, Singapore, for administrative support for this study.

This study was funded by the Communicable Disease Public Health Research Grant, administered by the National Medical Research Council under the Ministry of Health, Singapore (grant CDPHRG/0013/2014). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Ethics approval was obtained from the National Healthcare Group Domain Specific Review Board (2014/01224).

We declare no conflicts of interest.

H.J.H. analyzed and interpreted the data and prepared the manuscript with inputs from all authors. M.X.T. and T.Y.T. assisted in acquisition, analysis, and interpretation of the study data and drafting of the manuscript. M.I.C. provided overall direction and planning for the study, analyzed and interpreted the data, and revised the manuscript content critically. All authors approved the final version of the submitted manuscript.

REFERENCES

1. Stamm WE, Norrby SR. 2001. Urinary tract infections: disease panorama and challenges. J Infect Dis 183:S1–S4. https://doi.org/10.1086/318850.

2. Foxman B. 2002. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 113:S5–S13.

3. Stamm WE, Hooton TM. 1993. Management of urinary tract infections in adults. N Engl J Med 329:1326–1334. https://doi.org/10.1056/NEJM199310283291808.

4. Hooton TM. 2012. Clinical practice. Uncomplicated urinary tract infection. N Engl J Med 366:1028–1037. https://doi.org/10.1056/NEJMcp1104429.

5. Bahadin J, Teo SS, Mathew S. 2011. Aetiology of community-acquired urinary tract infection and antimicrobial susceptibility patterns of uropathogens isolated. Singapore Med J 52:415–420.

6. Cullen IM, Manecksha RP, McCullagh E, Ahmad S, O’Kelly F, Flynn R, McDermott TED, Murphy P, Grainger R, Fennell JP, Thornhill JA. 2013. An 11-year analysis of the prevalent uropathogens and the changing pattern of Escherichia coli antibiotic resistance in 38,530 community urinary tract infections, Dublin 1999–2009. Ir J Med Sci 182:81–89. https://doi.org/10.1007/s11845-012-0834-5.

7. Yılmaz N, Ağus N, Bayram A, Samıoğlu P, Şirin MC, Derici YK, Hano SY. 2016. Antimicrobial susceptibilities of Escherichia coli isolates as agents of community-acquired urinary tract infection (2008–2014). Turk J Urol 42:32–36. https://doi.org/10.5152/tud.2016.90836.

8. McNulty CA, Richards J, Livermore DM, Little P, Charlett A, Freeman E, Harvey I, Thomas M. 2006. Clinical relevance of laboratory-reported antibiotic resistance in acute uncomplicated urinary tract infection in primary care. J Antimicrob Chemother 58:1000–1008. https://doi.org/10.1093/jac/dkl318.

9. Chow WL, Wang VW, Low YS, Tse DW, Lim JF. 2012. Factors that influence the choice of seeking treatment at polyclinics. Singapore Med J 53;109–115.

10. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. 2010. The diagnosis of urinary tract infection: a systematic review. Dtsch Arztebl Int 107:361–367. https://doi.org/10.3238/arztebl.2010.0361.

11. Aspevall O, Hallander H, Gant V, Kouri T. 2001. European guidelines for urinalysis: a collaborative document produced by European clinical microbiologists and clinical chemists under ECLM in collaboration with ESCMID. Clin Microbiol Infect 7:173–178. https://doi.org/10.1046/j.1198-743x.2001.00237.x.

12. Clinical and Laboratory Standards Institute. 2015. Performance standards for antimicrobial susceptibility testing: 25th informational supplement. CLSI M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.

13. Lu CL, Liu CY, Huang YT, Liao CH, Teng LJ, Turnidge JD, Hsieh PR. 2011. Antimicrobial susceptibilities of commonly encountered bacterial isolates to fosfomycin determined by agar dilution and disk diffusion methods. Antimicrob Agents Chemother 55:4295–4301. https://doi.org/10.1128/AAC.00349-11.

14. Woodford N, Fagan EJ, Ellington MJ. 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. J Antimicrob Chemother 57:154–155. https://doi.org/10.1093/jac/dkl368.

15. Pérez-Pérez FJ, Hanson ND. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162. https://doi.org/10.1128/jcm.40.6.2153-2162.2002.

16. Guillard T, Moret H, Brasme L, Carlier A, Vernet-Garnier V, Cambau E, de Champs C. 2011. Rapid detection of qnr and qepA plasmid-mediated quinolone resistance genes using real-time PCR, Diagn Microbiol Infect Dis 70:253–259. https://doi.org/10.1016/j.diagmicrobio.2011.07.004.

17. Ho PL, Chan J, Lo WU, Lai EL, Cheung YY, Lau TC, Chow KH. 2013. Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary Escherichia coli isolates. J Med Microbiol 62:1707–1713. https://doi.org/10.1099/jmm.0.062653-0.

18. Ti TY, KumaraSinghe G, Taylor MB, Tan SL, Ee A, Chua C, Low A. 2003. What is true community-acquired urinary tract infection? Comparison of pathogens identified in urine from routine outpatient specimens and from community clinics in a prospective study. Eur J Clin Microbiol Infect Dis 22:242–245.

19. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ, Soper DE. 2011. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 52:e103–e120. https://doi.org/10.1093/cid/ciq257.
20. Shaifali I, Gupta U, Mahmood SE, Ahmed J. 2012. Antibiotic susceptibility patterns of urinary pathogens in female outpatients. N Am J Med Sci 4:163–169. https://doi.org/10.4103/1947-2714.94940.

21. Wong CKM, Kung K, Au-Doung PLW, Ip M, Lee N, Fung A, Wong S. 2017. Antibiotic resistance rates and physician antibiotic prescription patterns of uncomplicated urinary tract infections in southern Chinese primary care. PLoS One 12:e0177266. https://doi.org/10.1371/journal.pone.0177266.

22. Kahlmeter G. 2003. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis in Europe. The ECO.SENS study. Int J Antimicrob Agents 22(Suppl):49–52. https://doi.org/10.1016/S0924-8579(03)00229-2.

23. Lee MHM, Pan DST, Huang JH, Chen MI, Chong JWC, Goh EH, Jiang L, Leo YS, Lee TH, Wong CS, Loh VWK, Lim FS, Poh AZ, Tham TY, Wong WM, Yu Y. 2017. Results from a patient-based health education intervention in reducing antibiotic use for acute upper respiratory tract infections in the private sector primary care setting in Singapore. Antimicrob Agents Chemother 61:e02257-16. https://doi.org/10.1128/AAC.02257-16.

24. Llor C, Bjerrum L. 2014. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf 5:229–241. https://doi.org/10.1177/2042098614554919.

25. Lee TH, Wong JG, Lye DC, Chen MI, Loh VW, Leo YS, Lee LK, Chow AL. 2017. Medical and psychosocial factors associated with antibiotic prescribing in primary care: survey questionnaire and factor analysis. Br J Gen Pract 67:e168–e177. https://doi.org/10.3399/bjgp17X688885.

26. Kumar SG, Adithan C, Harish BN, Sujatha S, Roy G, Malini A. 2013. Antimicrobial resistance in India: a review. J Nat Sci Biol Med 4:286–291. https://doi.org/10.4103/0976-9668.116970.

27. Nitzan O, Elias M, Chazan B, Saliba W. 2015. Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management. Diabetes Metab Syndr Obes 8:129–136. https://doi.org/10.2147/DMSO.S51792.

28. den Heijer CD, Beerepoot MA, Prins JM, Geerlings SE, Stobberingh EE. 2012. Determinants of antimicrobial resistance in Escherichia coli strains isolated from faeces and urine of women with recurrent urinary tract infections. PLoS One 7:e49909. https://doi.org/10.1371/journal.pone.0049909.