Geographical distribution of antimicrobial resistance among *Escherichia coli* causing acute uncomplicated pyelonephritis in the United States

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Abstract

The susceptibility to 12 antimicrobial agents of 165 *Escherichia coli* isolates from women with acute uncomplicated pyelonephritis of mild to moderate severity was analyzed by geographic region in the US. Ampicillin, trimethoprim, and trimethoprim/sulfamethoxazole resistance exhibited a descending prevalence gradient from west to east. Composite antimicrobial resistance phenotypes also exhibited significant regional differences, with a greater prevalence of most combined resistance profiles seen in the Pacific region of the US, but with significant north–south variation for combined ampicillin/sulfisoxazole resistance. These findings suggest geographical segregation of resistant clones and/or resistance elements among uropathogenic *E. coli* within the US, which is relevant both to clinical practice and to understanding the basis for the current epidemic of antimicrobial resistance in *E. coli*.

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1. Introduction

The treatment of uncomplicated urinary tract infection (UTI) has become more challenging because of the rising prevalence in *Escherichia coli* of resistance to traditional first-line antimicrobial agents such as trimethoprim/sulfamethoxazole (TMP/SMZ). In vitro resistance to TMP/SMZ is associated with treatment failure in both cystitis [1–3] and pyelonephritis [4], making the use of alternative therapy (usually fluoroquinolones) necessary in areas where the prevalence of resistance among uropathogens exceeds an arbitrary critical threshold [5]. Therefore, it is important to follow local susceptibility patterns when selecting empirical therapy for either of these conditions.

An inhomogeneous geographical distribution of resistance to TMP/SMZ in the United States among uropathogens has been reported [6]. This phenomenon may reflect a differential distribution of resistant clones (e.g., recently emerged *E. coli* clonal group A) or the associated resistance elements [6,7]. However, the available geographical surveys are limited by an absence of data regarding host characteristics. This leaves open the possibility that the observed locale-specific susceptibility differences are due to different admixtures of clinical syndromes (including asymptomatic bacteriuria, cystitis, and pyelonephritis), or different degrees of host compromise (e.g., complicated UTI), rather than true geographical variation [8]. They also are limited by their focus on single-drug resistance phenotypes, whereas antimicrobial resistance is often to multiple agents, in
patterns that reflect the varied combinations of resistance elements present in the isolates.

Herein, we report antimicrobial susceptibility data for E. coli isolates from a well-characterized patient population (i.e., otherwise healthy women with acute pyelonephritis of mild-to-moderate severity), stratified by geographic region within the US. Trimethoprim and sulfonamides were examined both separately, and in combination, since resistance to these drugs is encoded by distinct, albeit often linked, resistance genes [9]. Additionally, to better characterize the distribution of antibiotic resistance phenotypes across the US, we analyzed composite resistance profiles and explored alternative geographical stratifications of the dataset.

2. Methods

2.1. Subjects and strains

All available pre-therapy E. coli urine isolates (n = 170) from women with acute uncomplicated pyelonephritis of mild-to-moderate severity from a multi-center treatment trial (conducted in 1994–1996) were obtained from the study sponsor [4,6]. Subjects were female outpatients at least 18 years of age and were enrolled if they demonstrated flank pain/tenderness, temperature greater than 38 °C, and pyuria. Exclusion criteria included recent antimicrobial use (i.e., self-reported consumption of any antimicrobial agent within 72 h of presentation), hospital admission, severe sepsis, immunocompromised condition, diabetes, urologic abnormalities, or antimicrobial allergy. Only one isolate per subject was analyzed. Isolates were assigned to one of four geographical regions within the US (i.e., Pacific, South Central, Northeast/Mid-Atlantic, and South Atlantic) based on their locale of origin, similar to previous geographic analyses [2]. Five isolates from Minnesota were excluded since they did not correspond with these geographical regions, and were too few for separate analysis.

2.2. Susceptibility testing

Susceptibility to 12 antimicrobial agents, including those most relevant for the therapy of acute pyelonephritis and/or cystitis (amoxicillin/clavulanate, ampicillin, cefoxitin, ceftazidime, ciprofloxacin, gentamicin, nitrofurantoin, piperacillin/tazobactam, sulfisoxazole, ticarcillin/clavulanate, trimethoprim, and TMP/SMZ), was determined by standard disk diffusion methods, with E. coli strain ATCC 25955 used as the control [10]. Zone sizes were interpreted according to National Committee for Clinical Laboratory Standards criteria [11]. Isolates with intermediate susceptibility to any of the antimicrobials tested were considered susceptible. Data were analyzed both as individual drug resistance phenotypes and as composite antibiograms, which were defined for each isolate according to the observed pattern of resistance to individual agents.

2.3. Statistical methods

Comparisons of proportions were tested using Pearson’s χ² Test or Fisher’s Exact Test, with statistical significance defined by P < 0.05. Comparisons involving the prevalence of individual drug resistance phenotypes and composite resistance profiles were made across the four geographic regions, between each region and all other regions combined, for all pair-wise combinations of individual regions, and between different sub-divisions of the population, i.e., (Pacific + Northeast/Mid-Atlantic) versus (South Central + South Atlantic), a “north-south” split, and (Pacific + South Central) versus (Northeast/Mid-Atlantic + South Atlantic), an “east-west” split. Finally, assessments were made for a linear trend from Pacific to South Central to Northeast/Mid-Atlantic to South Atlantic.

3. Results

3.1. Geographical distribution of individual resistance markers

Among the 12 antimicrobial agents against which the 165 E. coli pyelonephritis isolates were tested, resistance was most prevalent (% resistant) to ampicillin (40%), sulfisoxazole (36%), trimethoprim (21%), and TMP/SMZ (18%), whereas ≤ 1 isolate each (<1%) was resistant to amoxicillin/clavulanate, cefoxitin, ceftazidime, ciprofloxacin, gentamicin, nitrofurantoin, piperacillin/tazobactam, or ticarcillin/clavulanate (Table 1). Accordingly, only the former drugs were analyzed for geographical segregation.

The prevalence of resistance to ampicillin, trimethoprim, and TMP/SMZ was significantly geographically distributed, exhibiting a descending gradient from the Pacific, through the South Central and Northeast/Mid-Atlantic regions, to the South Atlantic region (for ampicillin, overall P > 0.05, linear trend P = 0.03; for trimethoprim, overall P = 0.004, linear trend P < 0.001; and for TMP/SMZ, overall P = 0.002, linear trend P < 0.001). For resistance to sulfisoxazole, a similar but nonsignificant trend was evident for this geographical gradient (Table 2).

When the Pacific region (high prevalence) and South Atlantic region (low prevalence) were compared individually with all other regions combined or with one another, for resistance to individual drugs, significant differences were seen for ampicillin, trimethoprim, and TMP/SMZ (Table 2). Likewise, for comparisons in-
Table 1
Prevalence of antimicrobial resistance by region among 165 E. coli pyelonephritis isolates

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regional prevalence of resistant, no. (%)</th>
<th>P values for selected comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 165)</td>
<td>Pacific (PAC) (n = 45)</td>
</tr>
<tr>
<td>amp</td>
<td>66 (40)</td>
<td>24 (53)</td>
</tr>
<tr>
<td>sul</td>
<td>59 (36)</td>
<td>20 (44)</td>
</tr>
<tr>
<td>tmp</td>
<td>34 (21)</td>
<td>16 (36)</td>
</tr>
<tr>
<td>sxt</td>
<td>30 (18)</td>
<td>16 (36)</td>
</tr>
</tbody>
</table>

*Note: < 1 isolate each was resistant to amoxicillin/clavulanate, cefotaxim, ceftriaxone, ciprofloxacin, gentamicin, nitrofurantoin, piperacillin/tazobactam, or ticarcillin/clavulanate. Geographic regions and no. of isolates by state: Pacific (California, n = 22; Washington, n = 23), South Central (Mississippi, n = 3; Tennessee, n = 4; Texas, n = 25), Northeast/Mid-Atlantic (Connecticut, n = 1; Pennsylvania, n = 13), South Atlantic (District of Columbia, n = 2; Florida, n = 68; Georgia, n = 4).

b P values (by Pearson’s χ² Test and Fisher’s Exact Test) are shown if P < 0.05. Comparisons of individual regions versus all others were not significant (P > 0.05) for South Central versus All, or for Northeast/Mid-Atlantic versus All. Pair-wise comparisons between regions were not significant (P > 0.05), except for Pacific vs. South Atlantic, for trimethoprim and TMP/SMZ (as shown) and for South Central vs. South Atlantic for trimethoprim (P = 0.02; not shown). Abbreviations are as follows: PAC, Pacific region and SA, South Atlantic region.

c Abbreviations are as follows: amp, ampicillin; sul, sulfisoxazole; tmp, trimethoprim; and sxt, trimethoprim-sulfamethoxazole (TMP/SMZ).

volving the larger two-region geographical zones, significant differences were seen between “North” versus “South” and “East” versus “West”, for trimethoprim and TMP/SMZ (Table 2).

3.2. Antibiotics

The statistically significant west-to-east descending gradient of resistance prevalence observed for ampicillin, trimethoprim, and TMP/SMZ, but not sulfisoxazole, and the north-south gradient observed for trimethoprim and TMP/SMZ, but not ampicillin or sulfisoxazole, suggested that the various resistance phenotypes exhibited distinctive patterns of geographical distribution, which we predicted would lead to a geographic segregation of composite resistance profiles. To explore this possibility, we next combined individual resistance phenotypes into a composite antibiotic for each isolate and analyzed these by region.

Overall, 12 distinct antibiograms were encountered in the population; these ranged in prevalence from 1% to 54% each. Susceptibility to all 12 antimicrobial agents was the most common antibiogram, both overall (54% of isolates) and within each geographical region (40–64%) (Table 3). The three next most common antibiograms overall were ampicillin/sulfisoxazole/TMP-SMZ/trimethoprim (hereafter referred to as ASTW) (15%), ampicillin/sulfisoxazole (15%), and isolated ampicillin resistance (8%). The remaining eight antibiograms occurred in only 1–2% of isolates each and collectively accounted for only 8% of the population (Table 3).

Accordingly, only the four most prevalent antibiograms were analyzed for geographical segregation.

For the overall prevalence of individual antibiograms by region, only the comparison involving the ASTW antibiogram was statistically significant (P = 0.005) (Table 4). However, in comparisons of each region versus all other regions, pairwise comparisons between individual regions, and pairwise comparisons between the larger two-region geographical zones, varying statistically significant differences were noted for both the all-susceptible and the ASTW antibiograms (Table 4). Additionally, comparisons between the North and South zones yielded the unique finding of a significantly higher prevalence of the ampicillin/sulfisoxazole antibiogram in the South (Table 4).

Table 2
Comparisons of antimicrobial resistance prevalence between two-region geographic zones among 165 E. coli pyelonephritis isolates

<table>
<thead>
<tr>
<th>Drug</th>
<th>Geographical zone prevalence of resistant, no. (%)</th>
<th>P values for selected comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 165)</td>
<td>North zone (n = 59)</td>
</tr>
<tr>
<td>amp</td>
<td>66 (40)</td>
<td>29 (49)</td>
</tr>
<tr>
<td>sul</td>
<td>59 (36)</td>
<td>23 (39)</td>
</tr>
<tr>
<td>tmp</td>
<td>34 (21)</td>
<td>18 (31)</td>
</tr>
<tr>
<td>sxt</td>
<td>30 (18)</td>
<td>18 (31)</td>
</tr>
</tbody>
</table>

*North, Pacific + Northeast/mid-Atlantic regions; South, South Central + South Atlantic regions; West, Pacific + South Central regions; and East, Northeast/Mid-Atlantic + South Atlantic regions.

b P values (by Pearson’s χ² Test and Fisher’s Exact Test) are shown if P < 0.05. n.s., not significant.

c Abbreviations are as follows: amp, ampicillin; sul, sulfisoxazole; tmp, trimethoprim; and sxt, trimethoprim-sulfamethoxazole (TMP/SMZ).
4. Discussion

We found that among *E. coli* isolates causing acute uncomplicated pyelonephritis in women in the US, the prevalence of resistance to ampicillin, trimethoprim, and TMP/SMZ, as well as of certain composite resistance profiles, varies significantly by geographical region. For most individual and combined resistance phenotypes, the prevalence was greatest in the Pacific region and least in the South Atlantic region, yielding a significant west-to-east gradient across the US. However, there was a significant north-south difference for several individual resistance phenotypes, which were more prevalent in the North, and for combined ampicillin/sulfisoxazole resistance, which was most prevalent in the South.

The Pacific region had the highest prevalence of the multi-drug resistance phenotype ASTW of any region, while the South Atlantic region had the lowest. Interestingly, however, although the majority of individual drug and composite resistance profiles exhibited a descending gradient of prevalence from west-to-east, not all of the observed geographical differences were best described by, or were even consistent with, this pattern. Notably, when comparing the North versus the South, the ampicillin/sulfisoxazole antibiogram was significantly more prevalent in the South, even though the ASTW antibiogram remained significantly more prevalent in the North. Likewise, although for some antibiograms the most striking difference was between the Pacific region itself versus other regions combined, for

### Table 3

Composite antibiograms for 165 *E. coli* pyelonephritis isolates by geographic region

<table>
<thead>
<tr>
<th>Antibiogram</th>
<th>Prevalence of antibiogram, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-susceptible</td>
<td>89 (54)</td>
</tr>
<tr>
<td>amp</td>
<td>13 (8)</td>
</tr>
<tr>
<td>amp/sul</td>
<td>25 (15)</td>
</tr>
<tr>
<td>amp/gent/sul</td>
<td>1 (1)</td>
</tr>
<tr>
<td>amp/sul/tcl</td>
<td>1 (1)</td>
</tr>
<tr>
<td>amp/acr/sul/tcl</td>
<td>1 (1)</td>
</tr>
<tr>
<td>amp/sul/xst/tmp (ASTW)</td>
<td>25 (15)</td>
</tr>
<tr>
<td>sul</td>
<td>2 (1)</td>
</tr>
<tr>
<td>sul/xst/tmp</td>
<td>4 (2)</td>
</tr>
<tr>
<td>tmp</td>
<td>2 (1)</td>
</tr>
<tr>
<td>tmp/xst</td>
<td>1 (1)</td>
</tr>
<tr>
<td>gent/sul</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

### Table 4

Antibiogram comparisons for 165 *E. coli* pyelonephritis isolates by geographic zones in the US

<table>
<thead>
<tr>
<th>Antibiogram (no.)</th>
<th>North vs. South</th>
<th>West vs. East</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-susceptible (89)</td>
<td>27 (46) 62 (58) n.s.</td>
<td>34 (44) 55 (63) 0.02</td>
</tr>
<tr>
<td>amp (13)</td>
<td>8 (14) 5 (5) n.s.</td>
<td>8 (10) 5 (6) n.s.</td>
</tr>
<tr>
<td>amp/sul (25)</td>
<td>4 (7) 21 (20) 0.04</td>
<td>11 (14) 14 (16) n.s.</td>
</tr>
<tr>
<td>amp/sul/xst/tmp (ASTW) (25)</td>
<td>16 (27) 9 (8) 0.003</td>
<td>18 (23) 7 (8) 0.008</td>
</tr>
</tbody>
</table>

*Note:* When the individual regions were compared with all other regions combined, statistically significant differences were seen for the Pacific region versus all other regions for all-susceptible (*P* = 0.005) and ASTW (*P* = 0.001) antibiograms, and for the South Atlantic region versus all others for ASTW (*P* = 0.008).

*a* amp, ampicillin; sul, sulfisoxazole; gent, gentamicin; tcl, ticarcillin/clavulanate; acl, amoxicillin/clavulanate; sxt, trimethoprim/sulfamethoxazole; and tmp, trimethoprim.

*b* Pacific and Northeast/Mid-Atlantic regions combined.

*c* South Central and South Atlantic regions combined.

*d* Pacific and South Central regions combined.

*e* Northeast/Mid-Atlantic and South Atlantic regions combined.

*f* For the overall prevalence of individual antibiograms by region, only the comparison involving amp/sul/xst/tmp (ASTW) was statistically significant (*P* = 0.005).
other antibiograms (e.g., all-susceptible), different stratifications of the population, albeit still along a west-to-east gradient, yielded the most highly statistically significant differences. These findings suggest a heterogeneous regional clustering of individual and combined resistance phenotypes, perhaps via shared resistance elements (plasmids, integrons, etc.), which might involve different combinations of resistant clones of uropathogenic \( E. \ coli \) in different regions of the country. Support for the hypothesis that a heterogeneous distribution of specific resistant clones may underlie the observed geographical variation in resistance prevalence is provided by the example of \( E. \ coli \) clonal group A (CGA). A previous molecular epidemiological analysis involving the present strain collection showed that (TMP/SMZ-resistance-associated) CGA accounted for a greater proportion of isolates from Los Angeles, CA (5/22, 23%), than from all other participating centers combined (7/148, 5%: \( P = 0.01 \)) or from other Pacific region centers (1/27, 4%: \( P = 0.08 \)) [6]. Likewise, CGA was more prevalent within the Pacific region overall (6/49, 12%) than within the remainder of the population (6/121, 5%: \( P = 0.11 \)) [6]. Similar molecular epidemiological approaches could be used in future studies to compare the clonal composition of drug-resistant populations from different locales.

A possible driving force for the observed regional clustering of resistance may be variation in prescribing practices in different areas of the US. Unfortunately, data regarding overall antimicrobial use in humans and agriculture by geographic region within the US are not available. However, recent work does suggest a linear relationship between antimicrobial prescribing and antimicrobial resistance in a number of bacterial species, providing support for the hypothesis that regions of the country with more extensive antimicrobial use may have a greater prevalence of antimicrobial resistance [12,13].

Importantly, the present study provides the first evidence within the US of a geographical gradient of antimicrobial resistance within a defined UTI syndrome, i.e., pyelonephritis of mild-to-moderate severity, and in a defined host population, i.e., women without medical or urological conditions predisposing to UTI. This observation is consistent with previous data from women with undefined symptomatology and host compromise status, factors that could confound the association of geographical region with susceptibility patterns for complicated UTI and antibiotic-resistant \( E. \ coli \) [2,14].

The implications of these findings for providers are twofold. First, the susceptibility data are not confounded by the inclusion of isolates from complicated UTI patients, and hence presumably better reflect the typically young female patients who present with acute uncomplicated pyelonephritis. Thus, they may be more relevant for selection of empirical therapy of acute uncomplicated pyelonephritis than are generic susceptibility data. Second, they demonstrate the need for providers to be aware of local susceptibility patterns, since pattern of resistance may vary considerably by region (i.e., Pacific versus South Atlantic regions, and North versus South) even within a particular clinical syndrome and host population.

Limitations of the study include the modest number of isolates per region and the absence of representatives from certain states within each region, which may limit the generalizability of these conclusions. Additionally, isolates were obtained in the mid-1990s, possibly reducing their current relevance. However, a recent report indicates that the prevalence of TMP/SMZ resistance among \( E. \ coli \) UTI isolates from across the US has remained relatively constant from 1995 to 2001 (14.8–17%), whereas fluoroquinolone resistance has increased only slightly [15]. Thus, our findings probably remain relevant. Although assembling similar high-quality strain sets will present a challenge, confirmation of these findings among more recent isolates and in other clinical syndromes would be desirable.

In summary, we found that among \( E. \ coli \) isolates from women with acute uncomplicated pyelonephritis in the US, a geographic gradient of resistance exists from west-to-east for ampicillin, trimethoprim, and TMP/SMZ, with the greatest differences seen between the Pacific region (high) and the South Atlantic region (low). Although certain composite resistance profiles, or antibiograms, also followed this prevalence gradient, a unique difference for ampicillin/sulfisoxazole resistance was evident between the North and South. Collectively, these findings suggest the presence of diverse localized "resistance epidemics", which might represent the occurrence of different combinations of resistant clones of \( E. \ coli \), or the associated resistance elements, in different geographical regions within the US. As a result, selection of optimal empiric therapy for patients with acute uncomplicated pyelonephritis will require knowledge of local susceptibility patterns.

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