Ampicillin-Resistant Escherichia coli in Gestational Pyelonephritis: Increased Occurrence and Association with the Colonization Factor Dr Adhesin

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The pattern of ampicillin resistance and possible association with virulence factors of 78 Escherichia coli isolates taken from 78 pregnant women with pyelonephritis were evaluated. The current incidence of ampicillin resistance among pyelonephritis isolates (46%) was significantly higher than that reported in 1985 (22%). Resistance was found more frequently during the first (60%) and third (53%) trimesters than during the second trimester (33%). Of all dra+ E. coli isolates, 75% were ampicillin resistant, whereas dra− isolates of O75 serotype E. coli accounted for 87% of ampicillin-resistant strains. The significant increase of ampicillin resistance among gestational pyelonephritis E. coli and the association with the dra gene cluster encoding colonization and invasive capacity may warrant further study involving obstetric and neonate wards, with the latter being at the higher risk for potential problems.

There have been several recent outbreaks associated with multiple antibiotic–resistant Escherichia coli in European hospitals [1–4]. With the advent of the prophylactic use of ampicillin, increasing antibiotic resistance among E. coli, and frequent reports of E. coli–associated complications in neonates, a current epidemiologic surveillance of pregnant women with E. coli pyelonephritis is of importance.

About 20%–40% of pregnant women with untreated bacteriuria develop pyelonephritis [5]. Pyelonephritis in pregnant women has been associated with increased risk of preterm labor, low birth weight, poor child health during early infancy, maternal adult respiratory distress syndrome, and possible renal failure [5, 6]. E. coli is the main etiologic agent in urinary tract infection (UTI) and pyelonephritis [6].

Ampicillin has been the drug of choice for treatment of acute pyelonephritis during pregnancy because of its clinical efficacy and low risk to both the mother and fetus [5, 6]. Because of a current limited penicillin supply, ampicillin is being used for the prevention of colonization with group B streptococci. Extended antepartal use of ampicillin has been implicated in the increased occurrence of ampicillin-resistant E. coli and associated neonatal sepsis and death [7]. Infections with antibiotic-resistant pathogens may be a risk factor for therapeutic failure.

We postulated that, along with anatomical host factors of the pregnant woman, bacterial virulence factors, such as E. coli adhesins, probably are involved in establishing ascending gestational UTI [8, 9]. DNA profiles showed that gestational pyelonephritis E. coli represented nonrandom, genetically related clones [9]. The virulence factors inherent to E. coli that are necessary for the development of gestational pyelonephritis are still under investigation but may include P, type 1, and Dr adhesins [9]. Pyelonephritis with E. coli that bear Dr fimbriae was found to occur more frequently late in gestation [9–12]. Infection with Dr+ E. coli was associated with a risk for recurrent UTI [8]. It was found recently that Dr-fimbriated E. coli can invade epithelial cells of the urogenital tract [11] and can cause chronic interstitial nephritis in mice and lethal uterine infections in pregnant rats [13, 14]. Colonization and invasion are mediated by E. coli Dr adhesin and the epithelial receptor decay accelerating factor (DAF). The primary role of DAF is to protect the mother and, presumably, the semiallogenic fetus from complement attack.

Antibiotic sensitivity of gestational pyelonephritis E. coli has not been evaluated recently. A report from 1984 estimated that 22% of gestational pyelonephritis E. coli were resistant to ampicillin [12]. Because of the increasing frequency of ampicillin resistance among E. coli from extraintestinal infections and more frequent reports of severe E. coli infections among neonates, we evaluated the frequency of antibiotic resistance and virulence factors in pregnant mothers with pyelonephritis.

Materials and Methods

Clinical isolates. E. coli strains were isolated from midstream urine samples of pregnant women who were admitted to John Sealy...
Hospital (University of Texas Medical Branch at Galveston) between 1996 and 1998, with clinical symptoms of acute pyelonephritis, bacteriuria $>10^5$/mL, fever $\geq 38.3^\circ$C, costovertebral pain, and dysuria [2]. This retrospective study included isolates from 78 patients who developed pyelonephritis with E. coli only: 15 of the isolates were from first trimester patients, and 35 and 28 were from second and third trimesters patients, respectively.

O serotyping of E. coli isolates. Pyelonephritis and control E. coli isolates were tested at the E. coli Reference Center (Pennsylvania State University) for the O:H antigens against 188 different O serogroups (173 classified according to World Health Organization guidelines and 15 unclassified) for nephrotoxicogenic (NPG) O serogroups. Serogroups said to belong to common NPG O serogroups were O1, O2, O4, O6, O25, and O75. Untypeable isolates were designated OX. All other serogroups were categorized as non-NPG.

Preparation of DNA samples for polymerase chain reaction (PCR) analysis. E. coli strains were grown overnight on Luria broth agar plates at 37°C. A 0.3-mL suspension of bacterial cells in sterile deionized water was made by using a sterile cotton swab. The samples were boiled at 100°C for 10 min. The lysate was centrifuged at 20,000 g for 5 min in a microcentrifuge at room temperature. The supernatant was collected and was stored at $-20^\circ$C until used for PCR assays.

Detection of dra and pap E. coli isolates by PCR analysis. The afaB gene is highly conserved among the Dr family of adhesins. Primers used for afaB were 5'-GCTGGGACGAACTGATAACTCTTC-3' and 5'-CATCAAGCTTTTGCTGCCGCCG-3' [15]. PCR ready-to-go beads (Pharmacia) were used according to the manufacturer’s protocol for all PCR assays. The amplification reactions were done in Uno-Thermoblock (Biomera), using 30 cycles of 94°C for 45 s (denaturing), 64°C for 30 s (annealing), and 72°C for 45 s (extension). Primers for detection of pap genes were those referred to by Johnson and Brown [16].

Antibiotic sensitivities of E. coli isolates. An automated system (VITEK; BioMérieux) was used to identify urine isolates and to routinely evaluate, according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS), antibiotic sensitivities by MICs. Isolates with MICs $\geq 32$ for ampicillin were classified as resistant, and isolates with MICs $<8.0$ were classified as sensitive. We used the Kirby-Bauer disk diffusion method to test isolates for sensitivities to selected antibiotics that were not routinely tested in the clinical laboratory. Tests were run on Mueller-Hinton agar plates, according to NCCLS guidelines.

Statistical analysis of results. The statistical analysis of observed differences was done using Fisher’s exact test. $P \leq .05$ was considered to be significant.

Results

Frequency of antibiotic resistance among E. coli strains isolated from pregnant women. This retrospective study evaluated the antibiotic sensitivity of 78 E. coli isolates from pregnant women with pyelonephritis. Analysis of antibiotic sensitivities of pyelonephritis E. coli isolates from the pregnant women indicated that ampicillin resistance occurred as many as 46% (table 1). Multiple antibiotic resistance to $\geq 4$ antibiotics was observed in 86% of antibiotic-resistant E. coli isolates. Among the 36 ampicillin-resistant E. coli isolates from pregnant women with pyelonephritis, 87% had combined resistance to mezlocillin, piperacillin, and ticarcillin; 36% showed resistance to $\geq 1$ of the cephalosporins (primarily cephalothin); and 22% also were resistant to trimethoprim/sulfamethoxazole.

<p>| Table 1. Association of ampicillin resistance with colonization factors and with the most frequent O serogroups. |
|----------------------------------|-----------------|-----------------|-----------|</p>
<table>
<thead>
<tr>
<th>Genotype, serogroup</th>
<th>No. of AmR isolates</th>
<th>Percentage of AmR isolates</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>dra +</td>
<td>18/24</td>
<td>75</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>pap +</td>
<td>16/43</td>
<td>37</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Total</td>
<td>36/78</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>dra + pap +</td>
<td>2/11</td>
<td>18</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Total</td>
<td>18/24</td>
<td>75</td>
<td>&lt;.032</td>
</tr>
<tr>
<td>Total</td>
<td>2/15</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>pap +</td>
<td>3/8</td>
<td>37</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>pap +</td>
<td>2/4</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>pap +</td>
<td>1/6</td>
<td>17</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>pap +</td>
<td>2/4</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>pap +</td>
<td>2/6</td>
<td>33</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>pap +</td>
<td>0/1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. All remaining serogroups were tested with regard to adhesins and ampicillin resistance, and associations were not significant ($P > .05$). The frequencies of ampicillin-resistant (AmR) isolates in serogroups O4, O21, and OX were 40% (2/5), 60% (3/5), and 40% (4/10), respectively.

* Comparison between positive and negative dra and pap genotypes for each O serogroup.

Frequency of antibiotic resistance among women with pyelonephritis. The 36 ampicillin-resistant E. coli isolates from pregnant women with pyelonephritis, 87% had combined resistance to mezlocillin, piperacillin, and ticarcillin; 36% showed resistance to $\geq 1$ of the cephalosporins (primarily cephalothin); and 22% also were resistant to trimethoprim/sulfamethoxazole.

Distribution of ampicillin-resistant pyelonephritis isolates among the different trimesters of pregnancy. Gestational isolates were categorized into first (weeks 1–12), second (weeks 13–26), and third (weeks 27–40) trimesters. Among the pyelonephritis strains, frequency of ampicillin resistance was higher during the first (60%) and third (53%) trimesters than during the second trimester (33%; $P < .001$; figure 1D).

Distribution of dra +, ampicillin-resistant isolates during the different trimesters of pregnancy. E. coli isolates were evaluated by PCR analysis for the presence of the Dr operon (coding for Dr family of adhesins). Those isolates that were dra + and pap +, as determined by PCR assay, were categorized as dra + pap +. The presence of colonization factors was associated with gestational age. The dra + isolates occurred more frequently during the third trimester (39%) than during the first (27%) and second (26%) trimesters (figure 1B), which appears to correlate with expected increases in progesterone levels during the third trimester (figure 1C).

We next compared the occurrence of dra + isolates with the frequency of ampicillin resistance and found that 75% of the dra + isolates were resistant to ampicillin ($P < .001$). Even though dra + E. coli were isolated more often than were dra + isolates, the frequency of ampicillin resistance among them was significantly lower (33%).

Association of ampicillin resistance with O serogroup and colonization factors. The results indicated that 52% of the isolates from patients with pyelonephritis belonged to the common
Figure 1. The gestational distribution of ampicillin-resistant (AMR) and -sensitive (AMS; $A$) and $dra^+/H11001$ and $dra^-/H11002$ Escherichia coli (B) isolated from pregnant patients with pyelonephritis and the expected increase of progesterone serum levels during pregnancy (C). The highest no. of AmR E. coli occurred in the first, followed by the third, trimester.

NPG O serogroup. E. coli serogroups that occurred most frequently among the pregnant women with pyelonephritis were O75 (61%) and those with the $dra^+$ genotype (75%) showed the highest frequency of resistance to $\beta$-lactam antibiotics and occurred more frequently in the third trimester of pregnancy.

A comparison of our findings with antibiotic resistance in pregnant patients with pyelonephritis reported by Duff (22%) [12] indicates a dramatic increase of resistance, with some E. coli types reaching 87% (O75, $dra^+$). This finding not only should alert obstetric and gynecologic physicians with respect to treatment and prevention methods for pyelonephritis, but also should alert epidemiologists to monitor for the potential risk of spreading such isolates in hospital, especially in neonate wards.

The increasing incidence of ampicillin-resistant strains of E. coli may result from the frequent use and selective antibiotic pressure in the clinical environment. It is also worth noting that pregnancy is associated with shortened serum half-life and increased total body clearance of $\beta$-lactam antibiotics. This may affect treatment of the pregnant patient and contribute to the selection of antibiotic-resistant strains. Another potential risk for selection of ampicillin-resistant E. coli in pregnant patients may be related to the prophylactic use of ampicillin for group B streptococci that colonize pregnant women [7], a practice that results from the shortage of penicillin and that currently is in common use among US hospitals.

In recent years, clinical outbreaks of E. coli have been associated with multiple antibiotic–resistant strains belonging to specific O serogroups. An outbreak of diarrhea caused by the NPG serogroup E. coli O4, which expressed multiple antibiotic resistance, was recorded in a neonate nursery ward [1]. A cluster of multiple antibiotic–resistant E. coli O78 occurred in Copenhagen [2], and outbreaks of E. coli urosepsis with antibiotic-resistant serogroup O15 occurred in Denmark [3] and England [4]. In our study, 61% of isolates in the most common serogroup, O75, showed multiple antibiotic resistance. Serogroup O15 was the second largest serogroup isolated from several pregnant women with pyelonephritis, among which 42% were ampicillin resistant and predominantly expressed the $pap$ adhesin. Closer clinical follow-up on E. coli O75 and O15 isolates may be necessary; in addition to adhesive and/or invasive properties, these isolates may carry multiple antibiotic resistance. Careful evaluation is needed of the potential risk for outbreaks of E. coli infections in US hospitals, including the risk in obstetric and neonate units.

Gestational age–dependent infection of pregnant women by O75, $dra^+$ E. coli may be explained by the temporary increased expression of tissue receptor DAF (CD55), which is up-regulated by progesterone [10]. Increased density of DAF receptor may allow for increased colonization by Dr$^+$ E. coli, thereby temporarily increasing the risk for infection [10]. Although the observed increase in Dr$^+$ E. coli may be biologically relevant, statistically the trend was not considered significant ($P = .309$). The genetic mechanism of a close association of O75 serogroup and Dr-fimbriated E. coli with ampicillin resistance is currently under investigation [13].
Studies indicate that Dr\textsuperscript{+} *E. coli* can enter and survive in uroepithelial cells and can cause bacteremia and lethal infection in pregnant animals [11]. The antibiotic resistance, combined with invasiveness of Dr\textsuperscript{+} *E. coli*, requires further study with respect to clinical implications and choice of appropriate antimicrobial treatment, since aminoglycosides, such as gentamicin, are known not to kill intracellular bacteria [11].

In summary, studies of antibiotic resistance of uropathogenic *E. coli* may be relevant for both clinical management and pathogenesis of gestational complications [8] and may contribute to exploration of alternative therapeutic approaches. A significant increase of antibiotic resistance among *E. coli* that cause gestational pyelonephritis may warrant a multicenter study, including obstetric and neonatal units, with the latter being at higher risk for potential problems.

References