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# Decreased Prevalence of Virulence Factors among Ciprofloxacin-Resistant Uropathogenic *Escherichia coli* Isolates

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**Ciprofloxacin resistance was identified in 18% and 6% of consecutively collected, clinically significant urinary tract isolates of *Escherichia coli* from inpatients and outpatients, respectively. In comparison to ciprofloxacin-susceptible isolates, there were fewer resistant isolates that expressed beta-hemolysis (outpatient, 9% versus 87%, $P < 0.0001$; inpatient, 4% versus 76%, $P < 0.0001$) and that had a *papEF* genotype, genes encoding P fimbriae (outpatient, 30% versus 70%, $P = 0.0004$; inpatient, 26% versus 70%, $P < 0.0001$).**

*Escherichia coli* is the principle cause of urinary tract infections in both community and hospital settings in North America. Two of the major uropathogenic virulence factors expressed by *E. coli* include a hemolysin protein and the mannose-resistant P fimbriae. Hemolysin assists in the acquisition of iron to regulate the expression of virulence factors (22). P fimbriae are encoded by a "pilus associated with pyelonephritis" *pap* operon which can be carried on one or more mobile genetic elements called pathogenicity-associated islands (PAIs) (22). This P fimbria construction requires both the tip association protein PapE (*papE*) and the tip protein PapF (*papF*) (7, 19), which then produce a receptor that binds to Galα(1-4)Gal receptors on uroepithelial cells (14). This binding event prevents bacterial washout, breaks the mucosal barrier, and initiates the host immune response (2). We realize that there are a variety of other adhesins and virulence factors that are involved in uropathogenesis, and a subset of urinary tract infection isolates carry the Dr/Afa adhesins (3, 10). This study focuses on P fimbriae because (i) they may be the primary adherence factor isolated from uropathogenic isolates of *E. coli* (4, 28); (ii) they trigger the host immune response (2); and (iii) they fulfil Koch-Henle molecular postulates by conferring on an avirulent nonfimbriated strain the ability to induce a host response in the human urinary tract (30). Since a hemolysin and the P fimbriae are often associated on the same PAI (9, 22), we decided to use hemolysis as a phenotypic indicator for the presence of an island of pathogenicity that might carry genes encoding the P fimbriae.

Fluoroquinolones such as ciprofloxacin have been suggested as an effective empirical treatment for uncomplicated urinary tract infections with high levels (>10%) of resistance among uropathogens to trimethoprim-sulfamethoxazole or trimethoprim (29). However, some evidence indicates that increasing ciprofloxacin use may be associated with ciprofloxacin resistance (11). This concern about the development of ciprofloxacin resistance led us to address the prevalence of ciprofloxacin resistance in urinary tract isolates of *E. coli*. Recent evidence also indicates that resistance of *E. coli* to nalidixic acid may be associated with the loss of beta-hemolysis and P fimbria expression (25). The potential for linkage between fluoroquinolone resistance and the expression of virulence factor proteins prompted us to determine (i) the current levels of ciprofloxacin resistance in urinary tract isolates of *E. coli* in Ontario, Canada, and (ii) the prevalence of these virulence factors among fluoroquinolone-resistant and -susceptible isolates.

A private laboratory serving community physicians and clinics in southern Ontario and a hospital laboratory serving both secondary- and tertiary-care hospitals collected all isolates from patients with significant and reportable bacteriuria between November 2003 and February 2004 (24). Only one isolate per patient was included in our sample. Organisms were identified by conventional methodology. Susceptibility to ciprofloxacin and nalidixic acid was tested by broth microdilution using NCCLS criteria (18).

All ciprofloxacin-resistant isolates and randomly selected ciprofloxacin-susceptible isolates were screened for beta-hemolysis by culturing each on 5% sheep blood agar for 24 h at 35°C. In addition, all ciprofloxacin-resistant isolates and randomly selected ciprofloxacin-susceptible isolates were tested for the presence of *papEF*. All nalidixic acid-resistant isolates and a randomly chosen group of nalidixic acid-susceptible isolates were also tested for beta-hemolysis and *papEF*. *papEF* was detected using PCR as previously described (31). Primers for amplification of a 336-bp sequence spanning the *papE* and *papF* genes were designed as previously published, with the exception that bacterial cells were boiled in distilled water and centrifuged for 10 min at 15,000 × *g* at room temperature (15, 31). To verify the validity of negative results from PCRs, genomic bacterial DNA was isolated from each *papEF*-nega-

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TABLE 1. Ciprofloxacin and nalidixic acid resistance in urinary tract isolates of *E. coli* from outpatients and inpatients

| Antibiotic | No. (%) of resistant isolates[a] | |
|---|---|---|
| | Outpatient | Inpatient |
| Nalidixic acid | 42 (11) | 82 (21) |
| Ciprofloxacin | 23 (6) | 68 (18) |

[a] A total of 381 outpatient isolates and 386 inpatient isolates were tested.

tive isolate by using a phenol-chloroform extraction protocol and quantified by using a spectrophotometer as previously described (1) and then reanalyzed by PCR. Negative samples were then spiked with DNA containing *papEF* to show that amplicon production could occur in the presence of a target gene and that each negative result was not due to technical problems. The identity of the amplicon was verified by restriction enzyme analysis with TacI per the manufacturer's protocol (Boehringer Mannheim, Germany). Following TacI digestion of PCR amplicons, the approximately 100-bp and 240-bp fragments were separated by agarose gel electrophoresis and visualized with ethidium bromide. Positive controls for this PCR included the uropathogenic strain *E. coli* CFT073 (22) (a gift from C. Gyles, University of Guelph, Guelph, Ontario, Canada). Nonuropathogenic *E. coli* BL-21 (Novagen, R&D, Madison, WI) was used as a negative control (23).

A total of 497 and 595 isolates were collected from outpatients and inpatients, respectively, over the study period. Among all urinary tract pathogens, *E. coli* was the most common, isolated with a frequency of 77% ($n = 381$) from outpatient isolates and 65% ($n = 386$) from inpatient isolates (18). The MICs of a variety of antibiotics were tested according to NCCLS guidelines (18). For outpatient isolates of *E. coli*, the frequencies of resistance to commonly tested antibiotics were as follows: cefazolin, 14 isolates (4%); cefprozil, 14 isolates (4%); amoxicillin-clavulinic acid, 17 isolates (4%); gentamicin, 5 isolates (1%); nitrofurantoin, 7 isolates (2%); ceftriaxone, 2 isolates (0.5%); and trimethoprim-sulfamethoxazole, 53 isolates (14%). For inpatient isolates of *E. coli*, the frequencies of resistance to commonly tested antibiotics were as follows: cefazolin, 26 isolates (7%); cefprozil, 36 isolates (9%); amoxicillin-clavulinic acid, 29 isolates (8%); gentamicin, 16 isolates (4%); nitrofurantoin, 8 isolates (2%); ceftriaxone, 3 isolates (0.8%); and trimethoprim-sulfamethoxazole, 82 isolates (21%). Resistance to ciprofloxacin and nalidixic acid was detected in 6% and 11%, respectively, of outpatient *E. coli* isolates and 18% and 21%, respectively, of inpatient *E. coli* isolates (Table 1).

Beta-hemolysis was statistically significantly less likely in both outpatient and inpatient fluoroquinolone-resistant strains in this study than in fluoroquinolone-susceptible isolates (Tables 2 and 3). In addition, *papEF* was also statistically less likely in both the outpatient and inpatient fluoroquinolone-resistant strains than in fluoroquinolone-susceptible isolates (Tables 2 and 3). Similar findings were seen with the nalidixic acid-resistant isolates and the nalidixic acid-susceptible isolates. No statistical association was found between specific ciprofloxacin MICs in ciprofloxacin-resistant isolates and beta-hemolysis and *papEF* (data not shown), but the power to show such a difference was limited due to the small sample size.

TABLE 2. Frequency of beta-hemolysis and *papEF* genotype in urinary tract isolates of *E. coli* from outpatients

| Antibiotic susceptibility pattern | No. (%) of isolates with hemolysis | *P* value | No. (%) of isolates with *papEF* genotype[a] | *P* value |
|---|---|---|---|---|
| Nalidixic acid | | | | |
| Resistant | 10 (22) | | 18 (39) | |
| Susceptible | 110 (95) | <0.0001 | 39 (85) | <0.0001 |
| Ciprofloxacin | | | | |
| Resistant | 2 (9) | | 7 (30) | |
| Susceptible | 117 (87) | <0.0001 | 47 (72) | 0.0004 |

[a] *papEF* encode P fimbriae.

This study adds a new dimension to previous work showing that nalidixic acid-resistant uropathogenic *E. coli* isolates have a lower prevalence of beta-hemolysis and *papEF* than susceptible isolates (25). An important element separating this from previous work is that we have focused on ciprofloxacin, which, unlike nalidixic acid (17), is currently utilized in a twice-daily regime for uncomplicated urinary tract infections (26). Since ciprofloxacin is currently used in protocols to treat urinary tract infections, we feel that it is appropriate to develop a stronger understanding of the interaction between ciprofloxacin resistance and changes in virulence factor expression and genotype. Therefore, as ciprofloxacin use in urinary tract infections drives up the frequency of ciprofloxacin-resistant isolates of *E. coli* (11), clinicians may begin to see a modification in the patterns of urinary tract disease accompanied by changes in the expression of specific *E. coli* virulence factors.

Resistance to fluoroquinolones is largely mediated by point mutations in gyrase and topoisomerase (20, 21) but may be due to efflux and plasmid-mediated mechanisms (27). How fluoroquinolone resistance is linked to loss of beta-hemolysis and *papEF* is not obvious. It has been proposed that fluoroquinolone-resistant bacteria may be less fit than susceptible isolates due to decreased efficiency of gyrase and topoisomerase (5, 12). In contrast, the development of resistance to ampicillin has been correlated to the presence of the Dr adhesin, which plays a role in colonization and invasion of the host (6). Since we did not study ampicillin susceptibility, we cannot compare our results with those of Hart et al. (6). Loss of beta-hemolysis may be due to decreases in beta-hemolysin mRNA due to transcription-coupled DNA supercoiling (13). Loss of PAIs in the face of possible inhibition of gyrase or topoisomerase has

TABLE 3. Frequency of beta-hemolysis and *papEF* genotype in urinary tract isolates of *E. coli* from inpatients

| Antibiotic susceptibility pattern | No. (%) of isolates with hemolysis | *P* value | No. (%) of isolates with *papEF* genotype[a] | *P* value |
|---|---|---|---|---|
| Nalidixic acid | | | | |
| Resistant | 7 (8) | | 25 (30) | |
| Susceptible | 95 (81) | <0.0001 | 67 (73) | <0.0001 |
| Ciprofloxacin | | | | |
| Resistant | 3 (4) | | 18 (26) | |
| Susceptible | 99 (76) | <0.0001 | 74 (70) | <0.0001 |

[a] *papEF* encode P fimbriae.

been described previously (S. Soto, J. Mensa, T. Jimenez de Anta, and J. Vila, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 82905, 2003); the driving force behind PAI loss may be a signal to escape a genome which is less fit to replicate.

Although ciprofloxacin-resistant *E. coli* isolates that are not beta-hemolytic and do not have *papEF* should be less able to be uropathogenic, ciprofloxacin resistance itself may be a virulence factor that allows for the survival of a bacterium within the urinary tract of ciprofloxacin-treated patients. In the future, it may be necessary to study the association between ciprofloxacin resistance and the loss of virulence factors, including other toxins (8) and other adhesion mechanisms (3), in *E. coli*. This future work will be enabled by the publication of a large number of primers designed to study these virulence factors using PCR (16, 31).

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