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Duration of Outpatient Fecal Colonization Due to *Escherichia coli* Isolates with Decreased Susceptibility to Fluoroquinolones: Longitudinal Study of Patients Recently Discharged from the Hospital[▽]

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Among 10 subjects colonized with *Escherichia coli* isolates with reduced susceptibility to fluoroquinolones, the median duration of colonization following hospital discharge was 80 days (range, 8 to 172 days). Colonization was longer for isolates demonstrating organic-solvent tolerance than for isolates that were not organic-solvent tolerant (151 versus 29 days, respectively; $P = 0.07$) but was not associated with other resistance mechanisms, demographics, or antibiotic use.

Significant increases in the prevalence of fluoroquinolone (FQ) resistance in *Escherichia coli* isolates have been noted in recent years (6). Approximately 20% of hospitalized patients are colonized with *E. coli* isolates exhibiting reduced susceptibility to FQs (4). Elucidating the duration of colonization with such organisms is vital because the human gastrointestinal (GI) tract serves as a major natural reservoir for *E. coli* and clinical *E. coli* isolates are usually derived from an organism colonizing the GI tract (3). In addition, the GI tract may serve as the primary source from which *E. coli* can spread through the population.

Few studies have addressed the duration of fecal colonization with *E. coli* isolates demonstrating reduced susceptibility to FQs (1, 8, 9, 11). These studies have been limited by focusing on only patients with specific chronic conditions (e.g., cancer or prostatitis) or patients who reside in long-term-care facilities (1, 8, 9, 11), including only subjects receiving FQ therapy (1, 9, 11), and utilizing infrequent fecal sampling (i.e., ≥1-month intervals) (8, 11). Furthermore, the impact of FQ resistance phenotype and/or genotype on duration of colonization has not been studied.

The goal of this study was to identify the duration of fecal colonization due to *E. coli* isolates with reduced susceptibility to FQs among patients recently discharged from the hospital. Furthermore, we sought to identify variables associated with prolonged colonization and characterize changes in the FQ resistance genotype and phenotype of isolates over time.

The study was performed at the Hospital of the University of Pennsylvania, a 625-bed academic tertiary-care medical center. Eligible study subjects were identified from an ongoing prospective study of patients who, while hospitalized, developed new GI tract colonization due to *E. coli* isolates demonstrating reduced susceptibility to FQs (4, 5). From this larger study, a convenience sample of consecutively identified colonized subjects was enrolled. Subjects were sampled using a perirectal swab technique (5), with all in-hospital swabs collected by the same research nurse. Patients whose swabs demonstrated growth of *E. coli* isolates with reduced susceptibility to FQs were then prospectively monitored as outpatients. Following hospital discharge, each subject continued to collect fecal samples every 2 weeks (using a swab to collect a sample of a freshly passed bowel movement). Subjects were asked to send in fecal samples for 6 months after discharge.

At the time of hospital discharge, inpatient medical records of subjects were reviewed to obtain data regarding age, sex, race, and number of hospital days prior to identification of FQ-resistant *E. coli* colonization. During outpatient follow-up, brief telephone interviews were conducted every 2 weeks to ascertain outpatient antimicrobial therapy.

Microbiological methods. All outpatient fecal swab samples were placed into a vial with Cary-Blair medium and then express mailed to the laboratory. To detect *E. coli* isolates with reduced susceptibility to FQs, all patient samples were inoculated to MacConkey agar plates supplemented with levofloxacin at a concentration of 0.125 μg/ml. Plates were streaked for isolation of colonies and incubated at 37°C in atmospheric air supplemented with 5% to 10% CO_2 and were checked for growth at 24 and 48 h. Colonies suspected of being *E. coli* colonies were subcultured and definitively identified using a Vitek 2 system (bioMerieux, Inc.). To determine the MIC of levofloxacin between the concentrations of 0.002 μg/ml and 32 μg/ml, *E. coli* isolates were subsequently tested for susceptibility to levofloxacin by use of the Etest method (4).

The FQ resistance-determining regions of the *gyrA* and *parC*

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TABLE 1. Study subjects[a]

Subject no.	Sample no.	Sample day	MIC(s) (μg/ml)	Mutation(s) in:		OST[d]	PFGE pattern(s)
				gyrA	*parC*		
1	1	0	70.25	A87T	None	Neg	A
	2	28	0.25	A87T	None	Pos	A
	3	41	Neg				
	4	57	Neg				
	5						
	6						
	7						
	8						
	9						
	10						
2	1	0	0.5	S83L	Neg	Neg	B
	2	58	Neg				
	3	71	Neg				
	4	85	Neg				
	5	99	Neg				
	6	119	Neg				
	7	176	Neg				
	8						
	9						
	10						
3	1	0	32	S83L, D87G	S80I	Pos	C
	2	17	Neg				
	3	28	Neg				
	4	44	Neg				
	5	57	32	S83L, D87G	S80I	Pos	C
	6	71	Neg				
	7	85	32	S83L, D87G	S80I		C
	8	100	Neg				
	9	128	0.064	Neg	Neg	Pos	D
	10	156	Neg				
4	1	0	0.19	Neg	Neg	Neg	E
	2	17	Neg				
	3	35	Neg				
	4	45	Neg				
	5	60	Neg				
	6	72	Neg				
	7	92	Neg				
	8	102	0.064	Neg	Neg	Neg	E
	9	140	Neg				
	10	178	Neg				
5	1	0	>32	S83L, D87T	S83L, D87T	Pos	F
	2	17	>32	S83L, D87T	S83L, D87T	Pos	F
	3	29	>32	S83L, D87T	S83L, D87T	Pos	F
	4	38	>32	S83L, D87T	S83L, D87T	Pos	F
	5	58	>32	S83L, D87T	S83L, D87T	Pos	F
	6	71	>32	S83L, D87T	S83L, D87T	Pos	F
	7	85	>32	S83L, D87T	S83L, D87T	Pos	F
	8	112	>32	S83L, D87T	S83L, D87T	Pos	F
	9	139	>32	S83L, D87T	S83L, D87T	Pos	F
	10	167	>32	S83L, D87T	S83L, D87T	Pos	F
6	1	0	32	S83L, D87D	S80I, G84V	Pos	NT
	2	12	32	S83L, D87D	S80I, G84V	Pos	NT
	3	27	32/32[b]	S83L, D87D	S80I, G84V	Neg/neg	NT
	4	41	32	S83L, D87D	S80I, G84V	Pos	NT
	5	56	32	S83L, D87D	S80I, G84V	Neg	NT
	6	70	Neg				
	7	83	32	S83L, D87D	S80I, G84V	Pos	NT
	8	134	Neg				
	9	153	32	S83L, D87D	S80I, G84V	Pos	NT
	10						

Continued on facing page

TABLE 1—*Continued*

Subject no.	Sample no.	Sample day	MIC(s) (μg/ml)	Mutation(s) in:		OST[d]	PFGE pattern(s)
				gyrA	*parC*		
7	1	0	0.25	D87T	Neg	Neg	G
	2	12	0.25	D87T	Neg	Neg	G
	3	26	Neg				
	4	41	Neg				
	5	56	Neg				
	6	70	0.25	D87T	Neg	Neg	G
	7	84	0.25	D87T	Neg	Neg	G
	8	106	0.38	D87T	Neg	Neg	G
	9	132	NA	NA	NA	NA	NA
	10	162	0.19	D87T	Neg	Neg	G
8	1	0	0.19	Neg	Neg	Pos	H
	2	28	Neg				
	3	37	0.38	Neg	Neg	Pos	H
	4	51	0.25	Neg	Neg	Pos	H
	5	65	0.125	Neg	Neg	Pos	H
	6	79	0.125	Neg	Neg	Pos	H
	7	94	0.19	Neg	Neg	Pos	H
	8	107	0.19	Neg	Neg	Pos	H
	9	141	Neg				
	10	177	Neg				
9	1	0	>32	S83L, D87N	S80I	Pos	I
	2	21	>32	S83L, D87N	S80I	Pos	I
	3	34	0.5	S83L	Neg	Neg	J
	4	50	0.5	S83L	Neg	Neg	J
	5	64	>32/0.5[c]	S83L, A87N[c]	S80I[c]	Pos/neg[c]	I/J
	6	92	>32	S83L, D87N	S80I	Pos	I
	7	99	0.5	S83L	Neg	Neg	J
	8	112	0.19	S83L	Neg	Neg	J
	9	146	>32	S83L, D87N	S80I	Pos	I
	10	173	>32	S83L, D87N	Neg	Pos	I
10	1	0	0.38	D87N	Neg	Neg	K
	2	38	0.38	D87N	Neg	Neg	K
	3	53	0.38	D87N	Neg	Neg	K
	4	66	0.38	D87N	Neg	Neg	NA
	5	80	0.38	D87N	Neg	Neg	K
	6	95	0.25	D87N	Neg	Neg	K
	7	127	0.10	D87N	Neg	Neg	K
	8	148	Neg				
	9						
	10						

[a] Sample no. 1 represents the final inpatient sample; sample day refers to the number of days after discharge the sample was obtained. There was no genetic relatedness of isolates across subjects. Neg, negative; Pos, positive; NA, not available; NT, not typeable.
[b] Two strains identified differed only in that one strain had the noted *parC* mutations while the other strain had no *parC* mutations.
[c] The MIC values, *gyrA* and *parC* mutations, and the OST findings were consistent with both previously identified strains in this patient.
[d] OST, organic-solvent tolerance.

genes were amplified and sequenced using previously described primers (13). Both strands of the amplified product were sequenced using an ABI 3730 DNA analyzer with BigDye *Taq* FS Terminator, v. 3.1 (Applied Biosystems, Foster City, CA). Sequence data were analyzed and compared to reference sequences by use of the LASERGENE software package (DNASTAR, Inc., Madison, WI).

Overexpression of the AcrAB efflux pump was measured indirectly by an organic-solvent tolerance assay (13). The appearance of confluent growth in the presence of a hexane: cyclohexane (3:1) mixture was interpreted as positive for AcrAB overexpression. Finally, two sets of primers were used to detect the plasmid-encoded FQ resistance gene *qnr* (2) as described previously (4).

The genetic relatedness of *E. coli* isolates was determined by molecular typing using pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was digested with the XbaI enzyme and separated by PFGE using a CHEF Mapper XA system (Bio-Rad, Hercules, CA). All results were analyzed using Fingerprinting II Informatix software (Bio-Rad, Hercules, CA).

Statistical methods. For each subject, the first outpatient fecal sample that did not reveal *E. coli* isolates with reduced susceptibility to FQs was noted. If the subsequent fecal sample was also negative, colonization was considered terminated. The actual end date of colonization was considered to be the midpoint between the date of the last positive culture and the date of the first negative culture. If the last culture during the follow-up period was positive, this was considered to be the

date of the last positive culture. The unadjusted association between specific variables (i.e., baseline demographics, resistance phenotype, and resistance genotype) and duration of colonization was assessed using the Wilcoxon rank sum test. A two-tailed P value of <0.05 was considered significant. All statistical calculations were performed using Stata, v. 8.0 (Stata Corp., College Station, TX).

This study was reviewed and approved by the University of Pennsylvania Committee on Studies Involving Human Beings.

Ten subjects were enrolled during the study period (Table 1). The median age of subjects was 59 years (range, 21 to 81 years); four (40%) subjects were male, and five (50%) were Caucasian. The median duration of hospitalization was 14 days (range, 4 to 60 days). Following hospital discharge, subjects were monitored for a median of 166 days (range, 57 to 178 days). Two subjects had received an FQ while in the hospital.

The median duration of outpatient colonization with *E. coli* isolates demonstrating reduced susceptibility to FQs (i.e., levofloxacin MIC of $\geq$0.125 μg/ml) was 80 days (range, 8 to 172 days). Four subjects remained colonized on the last sample. There were three subjects who, after having two negative swabs (i.e., meeting criteria for resolution of colonization), subsequently had one or more additional positive swabs. Although most subjects remained colonized with the same PFGE strain throughout the study, some subjects were colonized with several distinct strains, occasionally in the same sample (Table 1). Across study subjects, colonizing isolates were genetically unrelated. Except for isolates from one patient (patient no. 4), isolates with reduced susceptibility to FQs exhibited mutations in *gyrA* or *parC* and/or positive organic-solvent tolerance. The *qnr* gene was not detected in any study isolates.

The median duration of colonization for the five subjects whose initial isolate exhibited organic-solvent tolerance was 151 days. In comparison, the median duration of colonization for subjects whose isolates were not organic-solvent tolerant was 29 days ($P = 0.07$). The initial isolates of four subjects demonstrated greater than two target gene mutations. The median duration of colonization for these subjects was 160 days, compared to 32 days for the six subjects with fewer than two mutations ($P = 0.15$). There was no association between baseline demographics and duration of colonization.

Seven subjects received at least one outpatient course of antibiotics. Of the 11 total courses, 7 occurred in the first month after discharge and were often continuations of therapies initiated in the hospital. Outpatient antibiotics prescribed included amoxicillin, amoxicillin-clavulanate, cotrimoxazole, moxifloxacin, metronidazole, and vancomycin. The median duration of colonization for the seven subjects who received outpatient antibiotics was 124 days, compared to 35 days for the three subjects who did not receive outpatient antibiotics ($P = 0.73$). Of the seven subjects who received antibiotics, four received antibiotics that were not active in vitro against the colonizing isolate. The median duration of colonization for these four subjects was 146.5 days (range, 29 to 172 days). For the three subjects who received antibiotics with in vitro activity against the colonization isolate, the median duration of colonization was 8 days (range, 8 to 151 days). Finally, antibiotic exposures were not temporally related to reappearance of *E. coli* colonization in those subjects who had previously met criteria for resolution of colonization.

We found that fecal colonization with *E. coli* isolates demonstrating reduced susceptibility to FQs may often last up to 6 months or longer. In contrast, past studies have noted maximum lengths of outpatient colonization between 2 weeks and 3 months (1, 9, 11). Possible reasons for the long duration of colonization noted in this study compared to those in past reports include the more frequent fecal sampling, the inclusion of a more general patient population, and the inclusion of isolates with reduced susceptibility to FQs. Of note, we specifically sought to include *E. coli* isolates with reduced susceptibility to FQs because these organisms, while not meeting the clinical threshold for resistance, often harbor one or more FQ resistance mutations (4). Although we noted a prolonged period of colonization, it should be noted that a recent study of patients in a long-term-care center found that the duration of colonization with resistant gram-positive organisms (e.g., methicillin-resistant *Staphylococcus aureus*) was significantly longer than the duration of colonization with resistant gram-negative pathogens (e.g., *Pseudomonas aeruginosa*) (10).

We also noted a borderline significant association between overexpression of efflux and prolonged colonization. Efflux overexpression, by conferring resistance to multiple antimicrobials, may result in an organism that is more difficult to eradicate from the GI tract by use of other antibiotics. Also, the AcrAB efflux pump in *E. coli*, in addition to expelling FQs, extrudes bile salts (12). Since bile salts represent a vital component in the host defense in the GI tract, overexpression of this efflux pump may confer a survival advantage (i.e., prolonged colonization) to the organism (7).

Stepwise changes in FQ resistance mechanisms in *E. coli* in the clinical setting (e.g., in response to antibiotic selective pressure) are believed to occur at the level of the GI tract (9). However, we found that typically a subject would simply lose colonization of the organism altogether without the organism first exhibiting loss of specific resistance phenotypes or genotypes. Whether a colonizing organism was eliminated entirely from the GI tract or simply fell below the level of detection is unknown.

It has been suggested that when an individual is colonized with an *E. coli* isolate with reduced susceptibility to FQs, there is usually one predominant resistant strain (9). However, we found that several subjects were colonized with more than one strain and that detection of these strains often alternated during the course of follow-up. This finding, if confirmed in larger studies, suggests that future studies of colonization with FQ-resistant *E. coli* isolates should sample multiple colonies to determine if multiple resistant clones exist in the same subject.

We also recognize the difficulty inherent in attempting to ascertain the "true" termination of colonization. While there are no accepted criteria, we used two negative samples to indicate "clearance" of the colonizing organism. While two negative samples may indeed represent the termination of colonization, it may also represent sampling error. This is particularly called into question when the same strain reappears at a later date. Furthermore, reintroduction of the same strain from another source (e.g., food supply) is another possible explanation for such a finding. Future studies should consider exploring additional measures to assess longitudinal colonization.

The primary limitation of this study is the small sample size, which diminishes the ability to demonstrate statistically signif-

icant associations. Despite these limitations, the novel findings of this study should serve to generate hypotheses which should be tested further in larger subsequent studies.

We found that prolonged fecal colonization with *E. coli* isolates demonstrating reduced susceptibility to FQs is common and that certain resistance characteristics (e.g., efflux overexpression) may be associated with prolonged colonization. These results underscore the importance of initiatives to prevent fecal colonization with these organisms and suggest that efforts to limit prolonged colonization should explore targeting specific resistance mechanisms.

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