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Temporal Changes in Resistance Mechanisms in Colonizing *Escherichia coli* Isolates with Reduced Susceptibility to Fluoroquinolones

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Abstract

The objective of this study was to characterize the temporal variability of fluoroquinolone resistance mechanisms among *Escherichia coli* colonizing the gastrointestinal tract of hospitalized patients. Patients with new fluoroquinolone-resistant *E. coli* (FQREC) colonization were followed with serial fecal sampling until discharge or death. Genetic mechanism(s) of resistance for all FQREC isolates were characterized, including mutations in *gyrA* and *parC* and efflux pump overexpression. Of 451 subjects, 73 (16.2%) became newly colonized with FQREC. There was significant variability in regard to temporal changes in resistance mechanisms and levofloxacin MICs among isolates from individual patients. Compared to patients with transient colonization, patients with persistent colonization were more likely to have a urinary catheter (P=0.04), diarrhea (P=0.04), and a longer duration of hospitalization (22 and 9.0 mean days, respectively; P=0.01) prior to sampling. Our data demonstrate the significant variability of resistance mechanisms in colonizing *E. coli* isolates among hospitalized patients.

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1. Introduction

The rapid increase in the prevalence of fluoroquinolone-resistant *Escherichia coli* (FQREC) in recent years is of significant public health concern (Lautenbach et al., 2004). The major mechanisms leading to FQ resistance in *E. coli* include 1) mutations in the genes encoding the drug targets DNA gyrase and topoisomerase IV, most commonly in the *gyrA* and *parC* genes in the quinolone resistance-determining region (QRDR), and 2) overproduction of the AcrAB-TolC drug efflux pump (Jacoby, 2005; Li and Nikaido, 2009).

In vitro studies characterizing the emergence of FQ resistance in *E. coli* have demonstrated that selection of resistance occurs in a stepwise fashion, with increasing numbers of mutations leading to correspondingly higher FQ minimum inhibitory concentrations (MICs) (Kern et al., 2000; Chang et al., 2007; Singh et al., 2012). In the clinical setting, studies have also suggested that MICs to FQs in *E. coli* are typically higher in organisms with a greater number of resistance mutations (e.g., in target enzymes or genes mediating efflux) (Komp Lindgren et al., 2003; Lautenbach et al., 2006a; Morgan-Linnell et al., 2009; Moon et al., 2010). However, these studies have focused on isolates derived from clinical infections, whereas FQ resistance likely arises at the level of gastrointestinal tract colonization (Richard et al., 2001; Donskey, 2006).

Characterizing the stepwise accumulation of resistance mutations in colonizing *E. coli* isolates from individual patients is critical to enhanced understanding of the development of FQ resistance in the clinical setting, including informing potential strategies targeting specific resistance mechanisms to limit the emergence of FQREC. Therefore, we conducted this study to characterize the temporal changes in FQ resistance and resistance mutations among adult inpatients with new FQREC gastrointestinal tract colonization. In addition, we compared characteristics of patients who demonstrated transient FQREC colonization (i.e., FQREC colonization demonstrated on only one occasion) versus those with persistent colonization (i.e., multiple FQREC isolates over time).

2. Materials and methods

2.1. Study design and setting

This prospective cohort study was conducted at two hospitals in the University of Pennsylvania Health System (UPHS) in Philadelphia: the Hospital of the University of Pennsylvania (HUP), a 725-bed academic tertiary care medical center, and Penn Presbyterian Medical Center (PPMC), a 344-bed urban community hospital. As previously described (Lautenbach et al., 2006a; Lautenbach et al., 2009), three annual fecal surveillance surveys were performed hospital-wide at the two hospitals during the study years 2002, 2003, and 2004. For the present study, target units were selected from the two hospitals based on high prevalence rates of FQREC characterized by the three surveys (two units at PPMC and four units at HUP). The selected units included general medicine, oncology, rehabilitation, and intensive care units.

Subsequently, each unit was surveyed for a 3-month time period, with all patients admitted to the target units eligible for inclusion in the present study cohort. On the first day of a unit

survey, all patients hospitalized on the unit at 8:00 AM were identified and approached, with subsequent enrollment in the study if informed consent was obtained. For those patients who agreed to participate, fecal samples via a perirectal swab were obtained and submitted to the HUP Clinical Microbiology Laboratory for processing. Patients were followed longitudinally and continued to have fecal samples submitted every 48 to 72 hours (depending on patient availability) until the time of hospital discharge or death. New patients admitted to the unit during the survey period were also eligible to be enrolled in the study. Any patient transferred to another unit of the hospital continued to be followed until the time of hospital discharge or death. At the end of the three months, all patients currently undergoing surveillance continued to be followed until the time of hospital discharge or death. However, no new patients were enrolled during the third month of the survey to allow for complete follow up of all patients already enrolled. Each patient was included as a subject only once, with only the first episode of eligibility included. The study was approved by the institutional review board of the University of Pennsylvania.

2.2 Data collection

Data were abstracted from the Pennsylvania Integrated Clinical and Administrative Research Database (PICARD) (Barton et al., 2005; Lee et al., 2009), which includes demographic, laboratory, pharmacy, and billing information. Information for all patients was collected on the following: baseline demographics, year of the surveillance culture, hospital of admission, transfer from another institution or nursing home, admissions to UPHS in the 30 days prior to sampling, service location at the time of sampling (i.e., medical versus surgical), and number of hospital days prior to sampling. The presence of the following comorbid conditions was documented at the time of the sampling: diabetes mellitus, malignancy, renal insufficiency (creatinine 2.0 mg/dL or the requirement of dialysis), HIV infection, solid organ or hematopoietic stem cell transplant, neutropenia (absolute neutrophil count $<500/mm^3$), significant cardiovascular disease (e.g., severe congestive heart failure), significant respiratory disease (e.g., severe chronic obstructive pulmonary disease, chronic bronchitis), and any surgical procedure performed in the 30 days prior to sampling. Data on the presence of a urinary catheter, central venous catheter, or diarrhea prior to the initial surveillance culture were collected for all patients. Furthermore, data on antimicrobial therapy, chemotherapy, and steroids or other immunosuppressive agents administered during the 30 days prior to fecal sampling was ascertained.

2.3. Microbiological methods

Detection of *E. coli* from fecal samples was performed as described previously (Lautenbach et al., 2006a; Lautenbach et al., 2009). Given the multi-step nature of development of FQ resistance in a given isolate, organisms with MICs in the susceptible but elevated range (e.g., with early single mutations) may be critical in explaining the emergence and dissemination of FQ resistance (Gales et al., 2000; Kern et al., 2000; Chang et al., 2007; Singh et al., 2012). As such, for the present study, low-level FQ resistance (i.e., reduced FQ susceptibility) and high-level FQ resistance were defined as a levofloxacin MIC 0.25 g/mL but <8 g/mL and 8 g/mL, respectively. The QRDR of *gyrA* and *parC* were amplified and sequenced as previously described (Lautenbach et al., 2006a; Lautenbach et al., 2009). Overexpression of AcrAB was measured indirectly by the organic solvent tolerance assay as

previously validated (White et al., 1997; Wang et al., 2001). Two sets of primers were used to detect the plasmid-encoded fluoroquinolone resistance gene *qnr* as previously described (Lautenbach et al., 2006a).The genetic relatedness of *E. coli* isolates was determined by molecular typing using pulsed field gel electrophoresis (PFGE) (Lautenbach et al., 2006a), with all results analyzed using the Fingerprinting II Informatix Software v 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA) and interpreted according to established criteria (Goering and Tenover, 1997).

2.4. Statistical analysis

The incidence of new FQREC colonization during the study period was calculated, with three stages of FQREC colonization identified, as follows: 1) no FQREC colonization (levofloxacin MIC <0.25 μg/mL); 2) low-level FQREC colonization (levofloxacin MIC 0.25 μg/mL but <8.0 μg/mL); and 3) high-level FQREC colonization (levofloxacin MIC ≥8 μg/mL). For each patient with new FQREC colonization, resistance mechanisms (e.g., accumulation of mutations) of the isolates were described. For any patient with more than one FQREC isolate identified over time, all FQREC isolates were similarly characterized. Genetic mechanism(s) of resistance for all FQREC isolates were characterized by focusing specifically on mutations in *gyrA* and *parC*, as well as the presence of OST.

Characteristics of patients with colonization with one FQREC isolate (i.e., transient colonization) versus multiple FQREC isolates (i.e., persistent colonization) during the sampling period were compared, including demographic variables, comorbid conditions, and antimicrobial use in the 30 days prior to initial sampling. Continuous variables were compared using the Student's t-test or Wilcoxon rank-sum test and categorical variables were compared using the χ^2 or Fisher's exact test. Bivariable analyses were then performed to determine the association between patient characteristics and colonization with more than one FQREC isolate during the sampling period. All statistical calculations were performed using commercially available software (STATA v11.0; StataCorp LP, College Station, Texas).

3. Results

During the study period, a total of 1,186 hospitalized patients were approached for enrollment (Figure 1). Of these, 522 (44.0%) provided informed consent and had an initial fecal swab obtained. Notably, there were no significant differences with regard to mean age, race and ethnicity, year of enrollment, and hospital of admission (i.e., HUP versus PPMC) when comparing patients who did and did not enroll in the study.

Of the 522 patients who had an initial sample obtained, 429 (82.2%) were hospitalized at HUP while 93 (17.8%) were hospitalized at PPMC. Subsequently, 516 patients had fecal specimens that revealed *E. coli*, of which 451 (87.4%) were FQ-susceptible. These 451 patients who were initially colonized with FQ-susceptible *E. coli* represented the primary study cohort. These subjects underwent serial surveillance sampling during hospitalization with 73 (16.2%) having a subsequent culture positive for FQREC. Among these unique 73 patients, there were a total of 98 *E. coli* isolates with FQ resistance during the sampling period, as follows: 53 (54.1%) isolates with low-level resistance (levofloxacin MIC 0.25

μg/mL but <8.0 μg/mL) and 45 (45.9%) isolates with high-level resistance (levofloxacin MIC ≥8 μg/mL). Molecular characteristics of these FQREC isolates are shown in Table 1. The median number of *gyrA* mutations for *E. coli* isolates with high-level resistance versus low-level resistance was 2.0 and 1.0, respectively ($P<0.001$). The median number of *parC* mutations for *E. coli* isolates with high-level resistance versus low-level resistance was 1.0 and 0.0, respectively ($P<0.001$). The *qnr* gene was not detected in any study isolate. Finally, isolates with high-level resistance were more likely to be OST-positive compared to isolates with low-level resistance ($P<0.001$).

A total of 14 (19.2%) of the 73 patients with new colonization with FQREC had >1 FQREC culture during the sampling time period. Among these 14 unique patients, there were 39 isolates with FQ resistance (low-level and high-level). Temporal changes in levofloxacin MIC values, resistance mechanisms, as well as clonal relationships among the 14 patients with >1 FQREC isolate during sampling are shown in Table 2. Thirty-seven out of 39 isolates were successfully characterized by PFGE. Comparison of the 37 isolates as a group demonstrated no evidence of clustering during the study period (i.e., indication of an outbreak). Subsequently, the variability in PFGE patterns within sample sets for each patient was assessed, with five patients demonstrating clonally related isolates (C, K, M, Q, V; Table 2).

Characteristics of patients with >1 FQREC isolate during sampling are compared to those with only one FQREC isolate in Table 3. Notably, patients with persistent as opposed to transient colonization with FQREC during the sampling period were more likely to have received cefepime (50.0% and 17.0%, respectively; $P=0.02$) and vancomycin (50.0% and 20.3%, respectively; $P=0.04$) in the 30 days prior to sampling. These patients were also more likely to have had a urinary catheter (78.6% and 44.1%, respectively; $P=0.04$) and diarrhea (28.6% and 6.8%, respectively; $P=0.04$) present prior to the initial culture.

4. Discussion

In this 3-year study, we found that 73 (16.2%) patients became newly colonized with FQREC during hospitalization. Of these 73 patients, 14 (19.2%) had >1 FQREC isolate on serial surveillance cultures. Notably, there was significant variability in regard to temporal changes in both resistance mechanisms, as well as levofloxacin MICs, among isolates from individual patients. There was little evidence for persistent colonization with the same FQREC clone among patients with >1 FQREC isolate on serial cultures.

The present study, to our knowledge, is the first to characterize longitudinal changes in resistance mechanisms in FQREC isolates from the same patient during hospitalization. Specifically, we found that the majority of patients who were newly colonized with FQREC had only one resistant isolate during serial surveillance performed during hospitalization. In addition, all of these patients had surveillance cultures that were positive for FQ-susceptible *E. coli*. It is possible that these 59 patients demonstrated resolution of FQREC colonization, or that they were predominantly colonized with FQ-susceptible strains which dominated on subsequent samplings such that the previous FQREC isolate could not be identified.

In contrast, 14 patients who developed new FQREC colonization had more than one resistant isolate during the sampling period. Compared to patients who had only one FQREC isolate during surveillance, these patients were more likely to have had a urinary catheter or diarrhea, as well as a longer duration of hospitalization, prior to the sampling date. All of these factors, notably instrumentation (e.g., indwelling catheters) may have increased the risk of developing new and persistent gastrointestinal and/or urinary tract colonization with FQREC. Furthermore, patients with more than one FQREC isolate were more likely to have received cefepime and/or vancomycin in the 30 days prior to sampling. It is likely that receipt of these antimicrobial agents reflected greater severity of illness overall in hospitalized patients with new FQREC colonization.

Interestingly, only 4 of these patients (subjects #2, 7, 10, and 12) had isolates that progressed longitudinally from no FQREC colonization to low-level FQREC and ultimately to high-level FQREC. The progression in FQ resistance evidenced in our study usually involved acquisition of an additional *gyrA* mutation and/or a *parC* mutation as opposed to changes in efflux pump overexpression. However, as the low-level and high-level resistant isolates in these 4 individual patients were not clonally related by PFGE, it is unlikely that the temporal increase in levofloxacin resistance was due to colonization with *E. coli* strains with high mutation rates (e.g., allowing for relatively rapid development of full FQ resistance).

Rather than *de novo* mutations accounting for the observed increase in the number of resistance mechanisms, it is likely that patients were colonized with more than one FQREC strain during hospitalization. Indeed, a previous study performed at the same institution (Lautenbach et al., 2006b) demonstrated that several subjects who were recently discharged from the hospital were colonized with more than one FQREC strain during the surveillance period. Along these lines, FQ-susceptible strains were detected between that of FQREC isolates for the majority of the 14 patients in the present study. Furthermore, some patients had more than one *E. coli* strain with high-level FQ resistance as determined by differences in resistance mechanisms and lack of clonality (i.e., subjects #4, #7, and #11). These findings suggest that patients may be colonized with multiple *E. coli* strains with varying levels of FQ resistance, and further studies are needed to identify determinants of subsequent infection with FQREC as opposed to FQ-susceptible strains in multiply-colonized patients.

Finally, 5 (35.7%) of the 14 patients had a clonally-related strain detected on more than one sample during serial surveillance (subjects #2, 5, #6, #8, #10) This may have reflected variability in which colonies were sampled during processing. However, the results suggest that duration of colonization and/or predominance of a particular FQREC clone may significantly vary in an individual patient, and future studies will need to evaluate potential risk factors, including specific resistance mechanisms, for persistence of a particular colonizing FQREC strain in hospitalized patients.

There are potential limitations of our study. Selection bias is a potential concern; however, although only ~45% of eligible subjects were enrolled, participants and nonparticipants were similar in regard to demographic characteristics. Sampling variability may have limited the detection of all colonizing FQREC isolates from a single patient. Finally, the present study

was conducted in a single healthcare system, and these results may not be generalizable to other institutions.

In conclusion, the results of our study highlight the significant variability in resistance mechanisms in colonizing *E. coli* isolates among hospitalized patients. The emergence and persistence of FQ resistance is complex, and future studies are need to evaluate selection pressure for specific resistance mechanisms during hospitalization, as well as risk factors for infection with FQREC strains in multiply-colonized patients.

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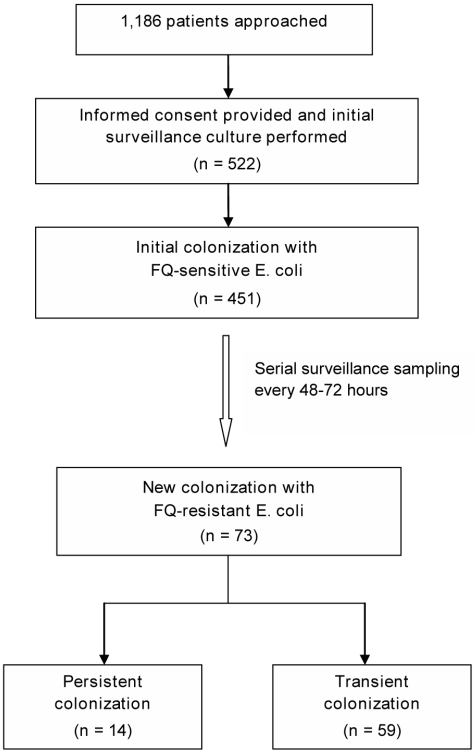

Figure 1.
Study flow diagram.

Table 1

Mechanisms of Resistance in Colonizing *Escherichia coli* Isolates Among Hospitalized Patients

Isolates[a]	**MIC**[b]	***gyrA* mutations n (%)**	**No.*gyrA* mutations Median (IQR)**	***parC* mutations n (%)**	**No.*parC* mutations Median (IQR)**	**OST positive n (%)**	**Total mechanisms of resistance n (%)**
Resistance (n=92)	0.75	72 (78.3%)	1 (1, 2)	37 (40.2%)	0 (0, 1)	35 (38.4%)	**0:**12 (13.2%) **1:**38 (41.8%) **2:** 24 (26.3%) **3:** 17 (18.7%)
High-level resistance[c] (n=43)	32	40 (93.0%)	2 (2, 2)	34 (79.1%)	1 (1, 1)	25 (58.1%)	**0:**0 (0.0%) **1:** 8 (19.0%) **2:** 17 (40.5%) **3:** 17 (40.5%)
Low-level resistance[d] (n=49)	0.38	32 (65.3%)	1 (0, 1)	3 (6.1%)	0 (0, 0)	10 (20.4%)	**0:** 12 (24.5%) **1:** 30 (61.2%) **2:** 7 (14.3%) **3:** 0 (0.0%)

MIC = minimum inhibitory concentration; OST = organic solvent tolerance; IQR = interquartile range.

[a] 6 isolates without information on mutations.

[b] Median levofloxacin MIC by Etest.

[c] Levofloxacin MIC 8 g/mL.

[d] Levofloxacin MIC 0.25 g/mL but <8 g/mL.

Table 2

Temporal Changes in *Escherichia coli* Isolates During Hospitalization

Subject no.	No. isolate(s)	Days from initial sample	MIC[a] (μg/mL)	*gyrA* mutation(s)	*parC* mutation(s)	OST	PFGE pattern(s)[b]
1	3 Susc.	0-5					
	Resistant	7	0.75	Ser83Leu	Neg	Neg	A
	9 Susc.	9-41					
	Resistant	44	0.75	Neg	Neg	Neg	B
	3 Susc.	46-52					
2	8 Susc.	0-19					
	Resistant	21	0.25	Ser83Leu	Neg	Pos	C
	4 Susc.	24-31					
	Resistant	33	0.75	Ser83Leu	Neg	Pos	D
	4 Susc.	35-42					
	Resistant	45	0.25	Asp87Tyr	Neg	Pos	C
	5 Susc.	47-56					
	Resistant	59	32	Ser83Leu;Asp87Gly	Ser80Ile	Pos	E
3	4 Susc.	0-10					
	Resistant	12	0.38	Ser83Leu	Neg	Neg	F
	Resistant	14	0.25	Asp87Tyr	Neg	Neg	G
	6 Susc.	17-28					
4	6 Susc.	0-14					
	Resistant	17	≥ 32	Ser83Leu; Asp87Tyr	Ser80Arg; Glu84Val	Neg	H
	7 Susc.	19-33					
	Resistant	35	≥ 32	Ser83Leu; Asp87Gly	Ser80Ile; Glu84Gly	Pos	I
	7 Susc.	38-52					
	Resistant	54	0.25	Asp87Tyr	Ser80Ile	Neg	J
5	6 Susc.	0-12					
	Resistant	14	≥ 32	Ser83Leu; Asp87Tyr	Ser80Arg; Glu84Val	Pos	K
	1 Susc.	17					
	Resistant	19	≥ 32	Ser83Leu,Asp87Asn	Neg	Pos	K
	4 Susc.	21-31					
	Resistant	33	0.5	Ser83Leu	Neg	Neg	K
	1 Susc.	35					
6	2 Susc.	0-2					
	Resistant	4	≥ 32	Ser83Leu; Asp87Gly	Neg	Pos	L
	Resistant	7	0.25	Asp87Tyr	Neg	Pos	M
	Resistant	9	0.25	Asp87Tyr	Neg	N/A	M
	8 Susc.	11-28					

Subject no.	No. isolate(s)	Days from initial sample	MIC[a] (μg/mL)	*gyrA* mutation(s)	*parC* mutation(s)	OST	PFGE pattern(s)[b]
7	3 Susc.	0-4					
	Resistant	7	1	Neg	Neg	Neg	N
	2 Susc.	9-11					
	Resistant	14	≥ 32	Ser83Leu; Asp87Asn	Ser80Ile	Neg	O
	Resistant	16	≥ 32	Ser83Leu; Asp87Asn	Ser80Ile; Present[c]	Pos	P
	Resistant	18	≥ 32	Ser83Leu,Asp87Tyr	Ser80Ile	Neg	N/A
8	1 Susc.	0					
	Resistant	2	≥ 32	Ser83Leu; Asp87Asn	Ser80Ile	Pos	Q
	4 Susc.	4-11					
	Resistant	14	0.125	N/A	N/A	N/A	R
	Resistant	16	≥ 32	Ser83Leu,Asp87Asn	Present[c]	Pos	Q
	Resistant	18	≥ 32	Ser83Leu; Asp87Asn	Ser80Ile	Pos	Q
9	1 Susc.	0					
	Resistant	2	≥ 32	Ser83Leu,Asp87Asn	Ser80Ile	Neg	S
	1 Susc.	5					
	Resistant	9	0.75	Ser83Leu	Neg	Neg	T
	3 Susc.	12-16					
10	14 Susc.	0-41					
	Resistant	43	0.19	Neg	Neg	Pos	U
	1 Susc.	46					
	Resistant	53	≥ 32	Ser83Leu; Asp87Asn	Neg	Neg	V
	Resistant	55	≥ 32	Ser83Leu; Asp87Asn	Neg	Neg	V
	2 Susc.	60-63					
11	1 Susc.	0					
	Resistant	4	8	Ser83Leu; Asp87Asn	Ser80Ile	Neg	W
	14 Susc.	6-42					
	Resistant	46	≥ 32	Ser83Leu; Asp87Asn	Ser80Ile; Glu84Val	Neg	X
12	2 Susc.	0-3					
	Resistant	6	0.38	Neg	Neg	Neg	Y
	4 Susc.	8-17					
	Resistant	20	0.5	Neg	Neg	Neg	Z
	Susc.	22					
	Resistant	24	≥ 32	Neg	Ser80Ile	Neg	N/A
13	2 Susc.	0-2					
	Resistant	5	0.25	1*gyrA*			AA
	Resistant	7	0.25	1*gyrA*			BB
14	3 Susc.	0-5					

Subject no.	No. isolate(s)	Days from initial sample	MIC[a] (μg/mL)	*gyrA* mutation(s)	*parC* mutation(s)	OST	PFGE pattern(s)[b]
	Resistant	7	0.5	Ser83Leu	Neg	Neg	CC
	4 Susc.	17-21					
	Resistant	26	0.38	Ser83Leu; Asp87Asn	Neg	Pos	DD
	1	Susc. 28					

Susc. = susceptible; MIC = minimum inhibitory concentration; OST = organic solvent tolerance; PFGE = pulsed field gel electrophoresis; Neg = negative; Pos = positive; N/A = not available.

[a] Levofloxacin MIC by Etest.

[b] Isolates with the same designated letter are considered to be clonally related.

[c] A single parC mutation was present, but unable to be further characterized.

Table 3

Characteristics of Hospitalized Patients with Transient vs Persistent Fluoroquinolone-Resistant *E. coli* (FQREC) Gastrointestinal Colonization

Variable	Single FQREC isolate (n=59)[a]	Multiple FQREC isolates (n=14)[a]	*P* value
Age, mean years (SD)	63.5 (18.5)	61.5 (13.3)	0.62
Female sex	31 (52.5)	6 (42.9)	0.56
Non-white race	26 (44.1)	6 (42.9)	>0.99
Duration of hospitalization prior to culture date, mean days (SD)	9.0 (13.8)	22 (33.5)	0.01
PPMC admission	15 (25.4)	2 (14.3)	0.50
Year of culture[b]	36 (61.0)	5 (35.7)	0.13
Admitted from a nursing home	5 (8.5)	0 (0.0)	0.58
Transferred from another hospital	12 (20.3)	2 (14.3)	>0.99
UPHS admit ≤30 days prior to culture date	22 (37.3)	5 (35.7)	>0.99
Surgical service	9 (15.3)	3 (21.4)	0.69
Urinary catheter	26 (44.1)	11 (78.6)	0.04
Mechanical ventilation	13 (22.0)	5 (35.7)	0.31
Central venous catheter	28 (50.0)	11 (78.6)	0.07
Diarrhea present	4 (6.8)	4 (28.6)	0.04
Diabetes mellitus	15 (25.4)	1 (7.1)	0.17
Neutropenia	0 (0.0)	0 (0.0)	….
Cirrhosis	3 (5.1)	1 (7.1)	>0.99
HIV	1 (1.7)	1 (7.1)	0.35
Malignancy	13 (22.0)	5 (35.7)	0.31
Transplant	4 (7.1)	2 (15.4)	0.32
Renal insufficiency	13 (22.0)	0 (0.0)	0.06
Surgical procedure ≤30 days prior to culture date	15 (25.4)	6 (42.9)	0.21
Receipt of immunosuppression ≤30 days prior to culture date	9 (15.3)	1 (7.1)	0.68

Variable	Single FQREC isolate (n=59)[a]	Multiple FQREC isolates (n=14)[a]	*P* value
Receipt of antimicrobial therapy ≤30 days prior to culture date[c]			
Levofloxacin	11 (18.6)	0 (0.0)	0.11
Gentamicin	1 (1.7)	2 (14.3)	0.09
Cefepime	10 (17.0)	7 (50.0)	0.02
Flagyl	8 (13.6)	5 (35.7)	0.11
Vancomycin	12 (20.3)	7 (50.0)	0.04
Piperacillin-tazobactam	0 (0.0)	1 (7.1)	0.19
Any antibiotic	25 (42.4)	10 (71.4)	0.07

SD = standard deviation; PPMC = Penn Presbyterian Medical Center; UPHS = University of Pennsylvania Health System.

[a] Data are presented as numbers (percentages), except where noted.

[b] Reference year 2002.

[c] Only antimicrobial agents with $P<0.20$ are shown.