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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 was 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.

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, 2005). 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) (Chang et al., 2007; Kern et al., 2000; 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; Moon et al., 2010; Morgan-Linnell et al., 2009). However, these studies have focused on isolates derived from clinical infections, whereas FQ resistance likely arises at the level of gastrointestinal tract colonization (Donskey, 2006; Richard et al., 2001).

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 the characteristics of patients who demonstrated transient FQREC colonization (i.e., FQREC colonization

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demonstrated on only 1 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 2 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, 2009), 3 annual fecal surveillance surveys were performed hospital-wide at the 2 hospitals during the study years 2002, 2003, and 2004. For the present study, target units were selected from the 2 hospitals based on high prevalence rates of FQREC characterized by the 3 surveys (2 units at PPMC and 4 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 h (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 3 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 were ascertained.

2.3. Microbiological methods

Detection of *E. coli* from fecal samples was performed as described previously (Lautenbach et al., 2006a, 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 (Chang et al., 2007; Gales et al., 2000; Kern et al., 2000; 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 μg/mL but <8 and ≥8 μg/mL, respectively. The QRDR of *gyrA* and *parC* was amplified and sequenced as previously described (Lautenbach et al., 2006a, 2009). Overexpression of AcrAB was measured indirectly by the organic solvent tolerance assay as previously validated (Wang et al., 2001; White et al., 1997). 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, Hercules, CA, USA) 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 3 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 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 1 FQREC isolate identified over time, all FQREC isolates were similarly characterized. Genetic mechanism(s) of resistance for all FQREC isolates was characterized by focusing specifically on mutations in *gyrA* and *parC*, as well as on the presence of OST.

Characteristics of patients with colonization with 1 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 1 FQREC isolate during the sampling period. All statistical calculations were performed using commercially available software (STATA v. 11.0; StataCorp LP, College Station, TX, USA).

3. Results

During the study period, a total of 1186 hospitalized patients were approached for enrollment (Fig. 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*