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Efficient Recovery of Fluoroquinolone-Susceptible and Fluoroquinolone-Resistant *Escherichia coli* Strains From Frozen Samples

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Abstract

We assessed the rate of recovery of fluoroquinolone-resistant and fluoroquinolone-susceptible *Escherichia coli* isolates from culture of frozen perirectal swab samples compared with the results for culture of the same specimen before freezing. Recovery rates for these 2 classes of *E. coli* were 91% and 83%, respectively. The majority of distinct strains recovered from the initial sample were also recovered from the frozen sample. The strains that were not recovered were typically present only in low numbers in the initial sample. These findings emphasize the utility of frozen surveillance samples.

Given continued significant increases in the prevalence of antimicrobial-resistant organisms,¹ recent efforts have focused on the critical role of gastrointestinal colonization with antimicrobial-resistant pathogens.² The use of surveillance cultures (usually employing perirectal swab samples) is increasingly common, both for infection control initiatives (eg, active surveillance) and as a component of epidemiologic studies.^{3,4} As part of these endeavors, considerable time and effort is dedicated to collecting these specimens, and the ability to freeze collected samples for future investigations is of tremendous potential benefit.^{5,6} However, few data regarding the yield of frozen samples are available. A recent study noted that for certain organisms (eg, vancomycin-resistant *Enterococcus* species, methicillin-resistant *Staphylococcus aureus*, and extended-spectrum β -lactamase-producing *Escherichia coli*), the rate of recovery from frozen specimens (ie, in comparison with the rate before freezing) was from 94% to 98%.⁶ However, to our knowledge, no study has to date focused specifically on fluoroquinolone-susceptible and fluoroquinolone-resistant *E. coli*, and no study of any organism has used molecular epidemiologic methods (eg, pulsed-field gel electrophoresis [PFGE]) to compare the strains obtained initially with those recovered later from the frozen sample.

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The purpose of this study was to assess the rates of recovery of fluoroquinolone-susceptible and fluoroquinolone-resistant *E. coli* isolates from culture of frozen perirectal swab samples, compared with the results for the same swab sample when it was cultured at the time of collection. The isolates obtained initially and those recovered from the frozen sample were also compared using PFGE.

Methods

This study was performed at 2 long-term care facilities (LTCFs) in the University of Pennsylvania Health System: the Penn Center for Rehabilitation and Care (124 beds) and the Philadelphia Veterans Affairs Nursing Home (240 beds). This study was approved by the institutional review boards of the University of Pennsylvania and the Philadelphia Veterans Administration Medical Center.

This study was conducted as part of a larger ongoing investigation in which perirectal swab samples are obtained every 2 weeks to obtain longitudinal data on colonization with fluoroquinolone-resistant and fluoroquinolone-susceptible *E. coli*. All residents of the participating LTCFs were eligible to enroll in the study.

To identify *E. coli* isolates susceptible to fluoroquinolones and isolates demonstrating reduced susceptibility, perirectal swab samples were inoculated onto MacConkey agar, MacConkey agar with 0.25 µg/mL of levofloxacin, and MacConkey agar with 8.0 µg/mL of levofloxacin. *E. coli* isolates with a levofloxacin minimum inhibitory concentration (MIC) of greater than 0.25 µg/mL were considered to be fluoroquinolone resistant.

All perirectal swab samples were stored by vigorously rotating the swabs in tryptic soy broth containing 25% glycerol and then frozen at -80°C. The samples were frozen for a minimum of 3 months. Without thawing, frozen specimens were scraped with sterile sticks and inoculated on agar and assayed using the methods described above.

Recent data suggest that fecal samples containing fluoroquinolone-resistant *E. coli* often harbor multiple strains of the organism and/or fluoroquinolone-susceptible *E. coli*.⁷ For this reason, we sampled up to 12 colonies of both fluoroquinolone-susceptible and fluoroquinolone-resistant *E. coli* (ie, up to 24 colonies total) from each swab culture. This approach was employed both for the initial culture and the frozen-sample culture.

The genetic relatedness of isolates was determined by PFGE.⁸ Chromosomal DNA was digested with *Xba*I and resolved by PFGE with contour-clamped homogeneous electric field electrophoresis (CHEF DR II System; Bio-Rad). All results were interpreted in accordance with established criteria.⁹ Fluoroquinolone-susceptible and fluoroquinolone-resistant strains that demonstrated the same PFGE type were considered different strains. Furthermore, strains were only compared for a single individual (ie, strains from different subjects were not compared).

We compared the initial culture results (ie, those obtained when the swab sample was first collected) with the results obtained from culture of the frozen swab samples. Using the results of the initial culture as the “gold standard,” we calculated the sensitivity and 95% confidence interval (CI) for the identification of fluoroquinolone-resistant *E. coli* in the culture of the frozen sample. This calculation only assessed whether any fluoroquinolone-resistant *E. coli* strain was identified (ie, there was at least 1 strain present) in the initial culture and the culture of the frozen samples, without regard for the specific strains identified by PFGE. Similar calculations were performed for fluoroquinolone-susceptible *E. coli*. Using PFGE, we then compared the specific strains of fluoroquinolone-susceptible and fluoroquinolone-resistant *E.*

coli identified in the initial culture and the culture of the frozen sample for each swab specimen. All statistical calculations were performed using Stata, version 9.0 (Stata).

Results

A total of 19 subjects were enrolled. The median age was 86 years (range, 57-96 years), and 6 (32%) subjects were male. There were 23 swab samples obtained from 19 subjects between March 22, 2006, and April 28, 2006. Four subjects contributed 2 samples each, with the time of sampling separated by at least 2 weeks. All swab samples were then frozen for a period of at least 3 months, after which the samples were cultured again to recover fluoroquinolone-susceptible and fluoroquinolone-resistant *E. coli*.

Of 23 swab samples, 11 yielded fluoroquinolone-resistant *E. coli* on initial culture. Of these 11, there were 10 that also yielded fluoroquinolone-resistant *E. coli* on culture of the frozen sample (sensitivity, 91%; [95% CI, 74%-100%]). For the 1 sample that did not yield fluoroquinolone-resistant *E. coli* when the frozen sample was cultured, the sample had initially yielded only 2 colonies. For 1 sample, fluoroquinolone-resistant *E. coli* was not found in the initial culture but was present in culture of the frozen sample.

A total of 18 swab samples yielded fluoroquinolone-susceptible *E. coli* on initial culture. Of these 18, there were 15 that also yielded fluoroquinolone-susceptible *E. coli* on culture of the frozen sample (sensitivity, 83%; [95% CI, 66%-100%]). For the 3 samples that did not yield fluoroquinolone-susceptible *E. coli* when the frozen sample was cultured, the isolates initially recovered from the sample had only been present in small numbers (these 3 samples yielded 1, 1, and 3 colonies, respectively). There were no samples for which fluoroquinolone-susceptible *E. coli* was found on culture of the frozen sample but not in the initial culture.

In addition to evaluating the overall presence or absence of fluoroquinolone-resistant and fluoroquinolone-susceptible *E. coli*, we also compared specific strains identified by PFGE in the initial and frozen sample. There were 12 specific strains of fluoroquinolone-resistant *E. coli* found in both the initial culture and the culture of the frozen sample. When the strains recovered from the initial sample were compared with strains recovered from the frozen sample, there were 4 strains that were lost and 4 strains that were newly identified in the frozen samples. For the 4 resistant strains that were lost, the median number of colonies per strain in the initial sample was 2.0 (mean, 4.0). In comparison, for the 12 resistant strains that were recovered from the frozen sample, the median number of colonies per strain in the initial sample was 6.5 (mean, 6.8).

Similarly, 17 fluoroquinolone-susceptible *E. coli* strains were recovered from both the initial and the frozen sample. When strains recovered from the initial and frozen samples were compared, there were 5 strains that were lost and 2 strains that were newly identified in the frozen samples. For the 5 susceptible strains that were lost, the median number of colonies per strain in the initial sample was 1.0 (mean 1.6). In comparison, for the 17 susceptible strains that were recovered, the median number of colonies per strain in the initial sample was 10.0 (mean, 8.2).

Discussion

We found that for both fluoroquinolone-resistant and fluoroquinolone-susceptible *E. coli* isolates, the rate of recovery from frozen specimens was excellent. These findings, which are consistent with sensitivities of greater than 90% recently reported for culture of frozen samples containing other antimicrobial-resistant organisms (eg, extended-spectrum β -lactamase-producing Enterobacteriaceae),⁶ support the usefulness of frozen surveillance samples for infection control initiatives and/or epidemiologic studies. Given the considerable effort

involved in surveillance activities, these results suggest that specimens should be frozen to permit future studies of additional or emerging pathogens. Access to such frozen specimens would provide important historical data when new pathogens or resistance mechanisms emerge.

Our recovery rate for fluoroquinolone-resistant and fluoroquinolone-susceptible *E. coli* isolates from frozen samples was not 100%. In those instances in which isolates identified in the initial culture of the sample were not identified in the culture of the frozen sample, the numbers of colonies of the organism in the initial culture had been low. This suggests that organisms initially present in low numbers are less likely to be recovered following freezing, a finding consistent with previous studies.^{5,6}

In addition to focusing on whether an organism could be recovered from frozen samples, we investigated whether particular PFGE-identified strains could be recovered. Although the majority of both fluoroquinolone-susceptible and fluoroquinolone-resistant strains persisted in the frozen sample, there were a number of strains that were not recovered. In most cases, these were strains that were present in a comparatively small number of colonies in the initial sample, again supporting the contention that organisms initially present in low numbers are less easily recovered. In addition, several strains (both fluoroquinolone-susceptible and fluoroquinolone-resistant) were identified in the frozen sample but not in the initial sample. One explanation for this might be that the initial sample contained larger numbers of colonies of one or more predominant strains, thus limiting the ability to detect a strain that was present in smaller numbers. When the frozen sample was cultured, the original predominant strain(s) may have decreased somewhat in number, permitting a less common strain to become detectable.

This study had several potential limitations. Samples from only 19 subjects were included, thus limiting our ability to calculate the recovery rate with a greater degree of precision. Given our focus on fluoroquinolone-susceptible and fluoroquinolone-resistant *E. coli*, studies focusing on strain recovery in other organisms would be valuable.

In summary, recovery rates for both fluoroquinolone-susceptible and fluoroquinolone-resistant *E. coli* isolates from frozen fecal specimens were excellent. These findings emphasize the future utility of stored frozen surveillance samples collected during infection control or epidemiologic research initiatives.

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