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Risk Factors for Fecal Colonization with Multiple Distinct Strains of *Escherichia coli* Among Long-Term Care Facility Residents

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Abstract

Of 49 long-term care facility residents, 21 (43%) were colonized with two or more distinct strains of *Escherichia coli*. There were no significant risk factors for colonization with multiple strains of *E. coli*. These results suggest future efforts to efficiently identify diversity of colonizing strains will be challenging.

Keywords

Escherichia coli; colonies; fecal

INTRODUCTION

The important role of gastrointestinal (GI) tract colonization with bacterial pathogens has been increasingly recognized in efforts to elucidate the epidemiology of antimicrobial resistance [1]. The use of surveillance cultures (usually employing a rectal swab approach) is common both as a component of epidemiologic studies as well as infection control initiatives [2]. It is recognized that an individual may be colonized with more than one distinct strain of *Escherichia coli* at any given time [3]. Many different approaches to colony sampling (i.e., the number of colonies from a plate that are evaluated) have been employed [3-7]. Improved ability to predict an individual's likelihood of being colonized with multiple distinct *E. coli* strains (both antibiotic-susceptible and antibiotic-resistant) would improve the efficiency of such colony sampling strategies. Despite this, risk factors for colonization with multiple strains of

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E. coli have not been studied. The goal of the current study was to identify risk factors for colonization with more than one strain of *E. coli*.

METHODS

This study was performed at the Philadelphia Veteran's Administration Medical Center (PVAMC) long term care facility (LTCF). Approval was obtained from the Institutional Review Boards of the University of Pennsylvania and the PVAMC. This study is based on data originally collected as part of a study investigating the epidemiology of GI tract colonization with fluoroquinolone-resistant and fluoroquinolone-susceptible *E. coli* in LTCF residents [8]. As described previously, LTCF residents were recruited for this study between March and July 2002. All residents were considered eligible for inclusion and were enrolled if informed consent could be obtained. For enrolled subjects, one rectal swab was obtained at enrollment.

Swabs were inoculated onto MacConkey agar without antimicrobial additives (nonselective medium). Up to 25 lactose-positive colonies (as available) were arbitrarily selected and inoculated onto nonselective media and MacConkey agar containing 8 μg/ml of ofloxacin. If fewer than 25 colonies were present, all were sampled. If greater than 25 colonies were present, 25 colonies were selected at random. Antimicrobial susceptibilities and species identification were confirmed by automated testing (Vitek, BioMerieux, Hazelwood, MO) [8].

Chromosomal DNA was digested with *Xba*I and resolved by pulsed-field gel electrophoresis (PFGE) using the CHEF DR II System (Bio-Rad, Hercules, CA) to determine genetic relatedness [8]. Strain identity was interpreted according to established criteria [9].

To determine risk factors for colonization with multiple distinct strains of *E. coli*, we conducted a cross sectional study. Cases were defined as those subjects for whom two or more distinct strains of *E. coli* were identified while controls were defined as subjects harboring only one strain of *E. coli*. The computerized VA medical record was reviewed for potential risk factors. Data obtained included age, gender, race, the number of days from LTCF admission to study enrollment, and prior hospitalizations. Presence of a decubitus ulcer, surgical wound and indwelling devices (i.e., tracheostomy, urinary catheter, and feeding tube) were ascertained. Data on co-morbid conditions included renal insufficiency, malignancy, diabetes, congestive heart failure, coronary artery disease, dementia, and depression were also assessed. Finally, pharmacy records were reviewed for all antibiotic prescriptions for one year prior to study entry. Overall antibiotic exposure as well as exposure to specific agents and classes was assessed [10].

Bivariable analyses were performed to determine the association between each variable and the outcome of interest (i.e., colonization with multiple *E. coli* strains). Categorical variables were compared using Fishers exact test while continuous variables were analyzed by the Wilcoxon rank sum test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine both the strength of any association and the precision of the estimate of the effect. Multivariable analyses were considered to further evaluate those variables found to be significantly associated with the outcome on unadjusted analyses. All statistical calculations were performed using STATA version 10.0 (Stata Corp, College Station TX).

RESULTS

A total of 49 LTCF residents were included in the study. The median age of study subjects was 69 years (range, 38-98 years) and two (4.1%) subjects were female. Twenty-nine (59%) subjects were White, 18 (37%) were African-American, one (2%) was Hispanic, and one (2%) was unknown [3].

There were 21 subjects (43%) colonized with multiple strains of *E. coli*. Of these 21 subjects, 11 were colonized with two distinct strains, eight were colonized with three strains, and one subject each was colonized with four strains and five strains, respectively. The median number of colonies sampled per subject was 23 (interquartile range (IQR) = 17-25). The median number of colonies sampled for the 28 subjects with only one strain identified was 22 (IQR= 17-25) while the median number of colonies sampled for the 21 subjects with greater than one strain identified was 23 (IQR = 16-25) [3].

There were no significant differences when comparing the demographic variables and comorbidities of cases and controls (Table). Similarly, prior antibiotic exposure was not significantly different when comparing cases and controls. The primary assessment of antibiotic use included all use in the past year. We then conducted secondary analyses in which we assessed antibiotics in the prior three months, six months, and nine months. The results of these analyses were not substantively different from the primary analysis. As there were no significant risk factors identified on bivariable analyses, multivariable analyses were not conducted.

DISCUSSION

Among 49 LTCF residents, we found nearly half were colonized with multiple strains of *E. coli*. GI tract colonization with multiple strains of *E. coli* has not previously been assessed in the LTCF population. As there is a greater focus on GI tract colonization with both antibiotic-susceptible and antibiotic-resistant bacteria, infection control and research initiatives are increasingly focusing on elucidating the characteristics of colonization. Given limited resources, optimizing the ability to identify the breath of colonizing strains (e.g., to characterize potential person to person spread of strains), must be balanced against the need for efficiency. The resources required to evaluate numerous colonies per swab are substantial and often beyond the scope of research and infection control endeavors. Being able to identify particular patient populations who are more likely to be colonized with multiple strains of *E. coli* might enable these more intense microbiological evaluations to be more specifically targeted. (i.e., to those individuals at greatest risk of colonization with multiple strains). This work represents the first effort, to our knowledge, to identify risk factors for colonization with multiple distinct clones of *E. coli*. However, our findings reveal there are no clear characteristics that help to distinguish those individuals at greatest risk of colonization with multiple colonies. These results suggest that efforts to more efficiently identify diversity of colonizing strains will be challenging.

Our study had several potential limitations. Although we sampled up to 25 colonies per subject, it is likely that sampling an even larger number would provide some incremental increase in the ability to determine breadth of diversity. Whether results of our study, conducted in a VA LTCF, can be generalized to other institutions is unknown.

In summary, we found subjects are often colonized with more than one strain of *E. coli*. Furthermore, no clear characteristics help to distinguish those populations at greater risk of colonization with multiple colonies. Future studies should focus on how patient populations colonized with multiple *E. coli* strains may be most efficiently identified.

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Table 1

Risk Factors for Colonization with Multiple Distinct *E. coli* Strains

Variable	Controls (n=28) n (%)	Cases (n=21) n (%)	OR (95%CI)	P value*
Demographics				
Age †	67	72		0.79
Sex (male)	27 (96%)	20 (95%)	0.74 (0.01, 61.01)	>0.99
Race (African-American)	10/26 (37%)	8 (38%)	1.04 (0.27, 3.95)	>0.99
Prior hospitalization	18 (64%)	13 (62%)	0.90 (0.24, 3.44)	>0.99
LTCF days †‡	430	403		0.72
Comorbidities				
Congestive heart failure	6 (21%)	8 (38%)	2.26 (0.54, 9.73)	0.22
Coronary artery disease	7 (28)	4 (21%)	0.71 (0.13, 3.37)	0.74
Diabetes	10 (36%)	8 (38%)	1.11 (0.29, 4.15)	>0.99
Cancer	6 (21%)	5 (24%)	1.14 (0.23, 5.41)	>0.99
Renal insufficiency	5 (18%)	6 (29%)	1.84 (0.38, 9.03)	0.49
Decubitus ulcer	4 (14%)	4 (19%)	1.41 (0.23, 8.68)	0.71
Feeding tube	3 (11%)	0 (0%)	0 (0, 1.65)	0.25
Surgical wound	1 (4%)	2 (10%)	2.84 (0.14, 173.75)	0.57
Tracheostomy	2 (7%)	1 (5%)	0.65 (0.01, 13.44)	>0.99
Urinary catheter	6 (21%)	4 (19%)	0.86 (0.15, 4.35)	>0.99
Dementia	12 (43%)	5 (24%)	0.41 (0.09, 1.68)	0.23
Depression	5 (18%)	8 (38%)	2.83 (0.64, 13.23)	0.19
Prior Antimicrobial Use				
Use of any antibiotic	20 (71%)	14 (67%)	0.80 (0.20, 3.27)	0.76
Fluoroquinolone use	13 (46%)	11 (52%)	1.26 (0.35, 4.57)	0.78
Gentamicin use	3 (11%)	1 (5%)	0.42 (0.01, 5.75)	0.63
Amoxicillin/clavulanate use	8 (29%)	7 (33%)	1.25 (0.31, 5.02)	0.76
Cephalexin use	5 (18%)	5 (24%)	1.44 (0.28, 7.36)	0.73
Metronidazole use	3 (11%)	5 (24%)	2.60 (0.43, 18.75)	0.26
Vancomycin use	3 (11%)	3 (14%)	1.38 (0.17, 11.53)	>0.99

*Fisher's exact test (categorical variables); Wilcoxon Rank Sum test (continuous variables)

†median

‡Days from LTCF admission until study enrollment