Enteropathogenic *Escherichia coli* Infection in Cancer and Immunosuppressed Patients

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Background. The role of enteropathogenic *Escherichia coli* (EPEC) as a cause of diarrhea in cancer and immunocompromised patients is controversial. Quantitation of fecal bacterial loads has been proposed as a method to differentiate colonized from truly infected patients.

Methods. We studied 77 adult cancer and immunosuppressed patients with diarrhea and EPEC identified in stools by FilmArray, 25 patients with pathogen-negative diarrhea, and 21 healthy adults without diarrhea. Stools were studied by quantitative polymerase chain reaction (qRT-PCR) for EPEC genes *eaeA* and *lifA/efa-1* and strains characterized for virulence factors and adherence to human intestinal enteroids (HIEs).

Results. Patients with EPEC were more likely to have community-acquired diarrhea (odds ratio, 3.82 [95% confidence interval, 1.5–10.0]; $P = .008$) compared with pathogen-negative cases. Although EPEC was identified in 3 of 21 (14%) healthy subjects by qPCR, the bacterial burden was low compared to patients with diarrhea (≤ 55 vs median, 6×10^4 bacteria/mg stool; $P < .001$). Among EPEC patients, the bacterial burden was higher in those who were immunosuppressed (median, 6.7×10^3 vs 55 bacteria/mg; $P < .001$) and those with fecal *lifA/efa-1* (median, 5×10^4 vs 120 bacteria/mg; $P = .015$). Response to antimicrobial therapy was seen in 44 of 48 (92%) patients with EPEC as the sole pathogen. Antimicrobial resistance was common and strains exhibited distinct patterns of adherence with variable cytotoxicity when studied in HIEs. Cancer care was delayed in 13% of patients.

Conclusions. Immunosuppressed cancer patients with EPEC-associated diarrhea carry high burden of EPEC with strains that are resistant to antibiotics, exhibit novel patterns of adherence when studied in HIEs, and interfere with cancer care.

Keywords. diarrhea; enteropathogenic *E. coli*; lymphostatin; intestinal organoids.

Culture-independent methods based on fecal nucleic acid amplification tests (NAATs) have revolutionized the approach to enteric infectious diseases [1], and quantitative NAATs can assist in differentiating colonized from actively infected patients [2, 3]. Excluding *Clostridioides difficile*, enteropathogenic *Escherichia coli* (EPEC) is the most common bacterial pathogen identified by multiplexed NAATs in patients with diarrhea in the United States (US) [2, 4]. While EPEC is considered to be an important cause of pediatric diarrhea in developing nations [5], its role as an enteric pathogen in US adults and cancer patients is less clear [6].

EPEC are defined by the presence of 3 gene clusters. The first cluster includes the locus of enterocyte effacement that encodes for a type III secretory system and associated effector proteins. The second cluster, the EAF plasmid, mediates localized

adherence and differentiates typical EPEC, which are *eaeA*+/*bfpA*+, from atypical EPEC, which are *eaeA*+/*bfpA*-. The third cluster, PA O-122, contains a gene (*lifA/efa-1*) that codes for lymphostatin, a molecule that inhibits lymphocyte proliferation and proinflammatory cytokine production [7].

The association of EPEC with diarrhea and poor outcomes is stronger for typical EPEC than for atypical EPEC [1, 2], with enhanced virulence modulated for both subtypes by the presence of PA O-122 genes [8]. In children, the presence of fecal *lifA/efa-1* correlates with an increased number of fecal *eae* copies [9] and disease severity [10].

Commercially available NAATs do not distinguish atypical from typical EPEC, do not provide quantitative readouts, and do not detect accessory pathogenicity genes or report antimicrobial resistance. We sought to determine the clinical characteristics, bacterial loads, virulence determinants, and antimicrobial susceptibilities of EPEC identified in immunocompromised and cancer patients with diarrhea. We hypothesized that in cancer and immunosuppressed patients with EPEC-associated diarrhea, fecal EPEC bacterial burden, presence of accessory virulence genes, and cytopathogenicity to human intestinal stem cell-derived enterocytes would correlate with symptoms, underlying malignancy, and degree of immunosuppression.

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MATERIALS AND METHODS

Study Subjects

We prospectively studied patients (inpatient and outpatient) with diarrhea seen at the University of Texas MD Anderson Cancer Center (MDACC) who submitted stool specimens to the clinical microbiology laboratory from 2016 to 2019 and were tested for the presence of 22 enteropathogens with a NAAT panel (FilmArray, Biofire Diagnostics, Salt Lake City, Utah). Prior to discarding fecal samples, an aliquot from residual stools was collected for further microbiology studies. From this convenience group, we selected all consecutive patients with EPEC identified by Biofire FilmArray NAAT ($n = 77$) and patients without an enteropathogen identified by NAAT ($n = 25$). As a second control group, and to determine the incidence and EPEC bacterial load present in stools from healthy adults in the community, we studied frozen stools from 21 healthy adult volunteers (aged 18–45 years) and no enteric pathogens identified, without significant medical illness, gastrointestinal symptoms including diarrhea, recent travel, or antibiotic exposure who participated in past studies [11].

Diarrhea was defined by the passage of ≥ 3 unformed stools in a 24-hour period associated with at least 1 sign of enteric infection (fever, nausea, vomiting, abdominal pain, cramping, tenesmus, or the presence of mucus or blood in stools). Diarrhea was defined as community acquired if present on admission and a stool was submitted to the clinical microbiology laboratory within 48 hours of admission. Medical records were reviewed and data were extracted by investigators who were not aware of research results. Electronic data capture forms with prespecified fields for demographic characteristics, clinical symptoms, comorbidities, laboratory findings, and research outcomes were created and stored in REDCap electronic data capture tools hosted at MDACC [12]. This study and a waiver of informed consent was reviewed and determined to be exempt from institutional review approval by the institutional review board committee at MDACC.

Microbiology Studies

For quantitative polymerase chain reaction (qPCR), stools were weighed and DNA was extracted using QIAmp DNA stool mini kit (Qiagen, Germantown, Maryland). The qPCR was performed as previously described using primers specific for *eaeA* and *lifA/efa-1* [10]. For bacterial studies, stools were streaked onto MacConkey plates. EPEC was isolated and tested for antimicrobial susceptibility as described in the [Supplementary Data](#).

Human Intestinal Enteroids

Based on prior infection studies done on duodenal explants that recapitulate the attachment and effacement characteristics of EPEC [13], we selected to study duodenal human intestinal enteroids (HIEs) derived from an intestinal biopsy done on a

healthy individual (subject 109) and propagated as 3D spheroids. Spheroids were suspended in Matrigel (Corning Inc, Corning, New York) and fed with specialized media made by the Enteroid Core at Baylor College of Medicine as previously described [14]. Monolayers were plated from a single cell suspension [15, 16] on Matrigel-coated chambered slides (Greiner Bio One, Monroe, South Carolina) and differentiated for 5 days. Monolayers were assessed for $>95\%$ confluence before infection with bacterial isolates of interest. A commensal *E. coli* strain (HS) was used as a negative control and EPEC E2348/69 was used as a positive control. Adherence and cytotoxicity assays were performed following methods described by Rajan et al [16].

Statistical Analysis

Descriptive statistics were used to present demographic, clinical, laboratory characteristics, and outcomes. Proportions of subjects with a given outcome were compared by χ^2 analysis or Fisher exact test including odds ratios (ORs). The median concentration of EPEC bacteria in stool samples (EPEC per gram of stool) was extrapolated from the number of *eaeA* and *lifA/efa-1* gene copies observed on a standardized qPCR curve spiked with known quantities of EPEC (10 – 10^8 colony-forming units) and was adjusted per milligram of stool. Given that data were not normally distributed, data were log transformed and we compared EPEC bacterial loads between groups by non-parametric methods using the Kruskal-Wallis test. When found to be significantly different, comparisons between subgroups was performed with adjustment for multiple comparison by Dunn method using IBM SPSS Statistics for Windows, version 24.0 (IBM Corporation, Armonk, New York). For enteroid studies, mean adherence and cytopathogenicity were also compared between isolates using Kruskal-Wallis statistical analysis performed using GraphPad Prism version 8.3.1 software for Windows (GraphPad Software, San Diego, California).

RESULTS

The demographic and clinical characteristics of 102 patients with diarrhea are shown in [Table 1](#). To determine if clinical features of patients with EPEC were different from patients without a pathogen identified, we compared 77 patients with EPEC diarrhea to 25 patients with diarrhea and no enteropathogen identified. The ages, type of underlying cancers, ethnicities, symptoms, and recent therapies received were similar. Most patients with EPEC had diarrhea at the time of admission or were tested within the first 48 hours of admission (62/77 [82%]) in contrast to 13 of 25 (52%) patients with no pathogen identified (OR, 3.82 [95% confidence interval {CI}, 1.5–10]; $P = .008$) who were tested later in their hospital stay. Fever and recent travel abroad tended to be more common in the EPEC-positive group, but differences were not statistically significant. Although 29 of 77 (38%) patients had a

Table 1. Clinical and Epidemiologic Characteristics of 102 Consecutive Cancer and Immunocompromised Patients With Diarrhea With or Without Enteropathogenic *Escherichia coli* Identified in Their Stools as Determined by Biofire FilmArray Nucleic Acid Amplification Test

Characteristic	Biofire FilmArray EPEC Negative (n = 25)	Biofire FilmArray EPEC Positive (n = 77)	P Value
Age, y			
<20	1 (4)	1 (1)	NS
20–39	4 (16)	10 (13)	
40–59	9 (36)	29 (38)	
60–79	11 (44)	33 (43)	
≥80	0	4 (5)	
Female sex	10 (40)	24 (31)	
Race			
White	18 (72)	58 (75)	NS
Black	3 (12)	4 (5)	
Other	4 (16)	15 (20)	
Ethnicity			
Hispanic	4 (16)	17 (22)	
Non-Hispanic	21 (84)	55 (71)	NS
Other	0	5 (7)	
Cancer diagnosis			
Solid tumor	10 (40)	36 (47)	
Hematologic	6 (24)	18 (23)	NS
Stem cell transplan- tation	8 (32)	19 (25)	
None	1 (4)	4 (5)	
Charlson score, median (Range)	4 (0–12)	6 (0–14)	NS
Symptoms at presentation			
Fever	3 (12)	23 (30)	.075
Nausea	12 (48)	27 (35)	NS
Vomiting	8 (32)	21 (27)	NS
Abdominal pain	7 (28)	32 (42)	NS
Bloody stools	2 (8)	7 (9)	NS
Duration of illness, d, median (Range)	3 (1–30)	2 (1–30)	NS
Risk factors for diarrhea			
International travel	0	7/57 (12)	.09
Antibiotic use	21 (84)	54 (70)	NS
Immunosuppression	20 (80)	63/76 (83)	NS
Chemotherapy	21 (84)	56 (73)	NS
Community acquired	13 (52)	62 (81)	.008

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: EPEC, enteropathogenic *Escherichia coli*; IQR, interquartile range; NS, non-significant.

second pathogen (Supplementary Table 1), there were no differences in demographic characteristics or clinical signs when 48 patients with EPEC as the single pathogen were compared to the pathogen-negative group.

To test the hypothesis that EPEC burden was larger in cancer and immunocompromised patients than in healthy adults in the community, we measured the amounts of fecal *eaeA* and *lifA/efa-1* using qPCR (Figure 1). We found low levels of *eae* (≤ 55 copies) but no measurable amounts of *lifA/efa-1* in 3 of the 21 (14%) healthy individuals (Figure 1A). In contrast, patients with cancer and EPEC found by BioFire FilmArray were positive for

fecal *eaeA* and *lifA/efa-1* in 70% and 20% of cases, respectively, by qPCR. Similar proportions were found when we analyzed the 48 EPEC cases without other pathogens identified (75% for *eae* and 18% for *lifA/efa-1*). We then compared the bacterial burden according to underlying malignancy. As shown in Figure 1B, and after adjusting for multiple comparisons, the use of immunosuppressants in the past 90 days was associated with a higher EPEC fecal burden (median, 6.7×10^3 vs 10 bacteria/mg of stool; $P = .001$).

To test the hypothesis that the presence of the accessory virulence gene *lifA/efa-1* was associated with more severe disease, we compared demographic characteristics, presenting symptoms, duration of illness, and bacterial burden by qPCR of patients with fecal *lifA/efa-1* gene to those without fecal *lifA/efa-1*. Although the characteristics and clinical presentation were similar, subjects with fecal *lifA/efa-1* excreted higher numbers of EPEC *eaeA* (median, 1.5×10^6 vs 1×10^3 bacteria/mg of stool; $P < .001$) and were more likely to be neutropenic (mean, 2.5×10^3 vs 5.1×10^3 white blood cells/mm³) as shown in Supplementary Table 2.

To determine the presence of EPEC virulence factors and test for antimicrobial susceptibility, stool cultures were performed on 77 patients. Coliforms were observed in 64 (83%) cases; of these, 40 showed growth on subculture and could be studied for the presence of *eaeA* by PCR. At least 1 of 10 colonies tested positive for *eaeA* in 35 (88%) patients and all were negative for *bfpA*, which excluded typical EPEC, and for *Stx1* and *Stx2* excluding Shiga toxin-producing *E. coli* (STEC). As shown in Table 2, the likelihood of isolating EPEC in culture strongly correlated with fecal *eaeA* and *lifA/efa-1* copy numbers. Following the confirmation as *E. coli*, by matrix-assisted laser desorption/ionization–time of flight, we performed broth microdilution susceptibility testing to antimicrobials on 18 isolates. As shown in Table 3, resistance to ampicillin was observed in 28% of isolates and 67% were nonsusceptible to cefuroxime. Resistance to first-line agents such as ciprofloxacin and trimethoprim-sulfamethoxazole was seen in 6% and 17%, respectively.

To determine if the EPEC isolated from patients adhered to epithelial cells and displayed unique phenotypic characteristics imparted by the accessory gene *lifA/efa-1*, we assessed the ability of EPEC strains to adhere to and destroy a monolayer of duodenal HIEs. As shown in Figure 2, we observed considerable heterogeneity in the number of adherent bacteria with no difference in whether strains carried *lifA/efa-1* or not. Two strains stood out with exceptionally higher numbers of adhered bacteria: PCO-1757, recovered a patient with detectable fecal *lifA/efa-1*, and PCO-1782, recovered from a patient without detectable fecal *lifA/efa-1*. No differences were noted when examined according to presenting signs, fecal EPEC bacterial load, or clinical outcomes. We also observed heterogeneity in the size of the bacterial clusters that were attached onto the epithelium (Figure 3). Strains also differed in their adhesion patterns

Figure 1. A, Real-time fecal quantitative polymerase chain reaction (qPCR) for enteropathogenic *Escherichia coli* (EPEC) done in stools from 77 cancer and immuno-suppressed patients with diarrhea and EPEC identified in stools with Biofire FilmArray nucleic acid amplification test, and from 21 healthy controls without diarrhea. Cancer and immunosuppressed patients are categorized according to underlying malignancy. B, Fecal qPCR for EPEC done in 77 cancer patients with EPEC identified in stools with Biofire FilmArray. Patients are categorized according to concomitant treatment received. The number of EPEC per gram of stool was extrapolated from the number of *eeA* gene copies on a standardized curve spiked with known quantities of EPEC adjusted per milligram of stool. Bars denote medians for each subgroup and 95% confidence intervals. Groups were compared using Kruskal-Wallis test and *P* values adjusted by Dunn multiple comparison test. **P* < .05; ***P* ≤ .01; ****P* ≤ .0001. Abbreviations: CFU, colony-forming units; EPEC, enteropathogenic *Escherichia coli*; GVHD, graft-vs-host disease; HSCT, hematopoietic stem cell transplantation.

demonstrating combinations of diffuse, aggregative, network, or localized adherence (Figure 4). Some bacterial isolates were found to be particularly destructive to the duodenal monolayers (Figure 5). To quantify this effect, we calculated the percentage of the monolayer remaining after 6 hours' infection for each of the strains assessed in Figure 2. Interestingly, although most of the strains did not disturb the integrity of the monolayer over the course of infection (and there was little visible evidence of a change in cell shape or structure), some strains consistently lead to the peeling of large portions of the monolayers from the slide.

In contrast to the quantitative fecal burden results, we did not find a correlation between clinical presentation, fecal bacterial load, and the ability of individual strains to attach and degrade the HIE monolayer.

All patients received antibiotics with expected activity against EPEC-associated diarrhea. We analyzed the response to therapy for the 48 patients who had EPEC as the sole pathogen identified. A clinical response was seen in 44 (92%) cases at the end of antibiotic course. Delays in cancer care attributed to diarrhea occurred in 13% of cases.

Table 2. Real-time Fecal Quantitative Polymerase Chain Reaction for Enteropathogenic *Escherichia coli* (EPEC) *eeA* and *lifA/efa-1* Done in Stools From 77 Cancer and Immunosuppressed Patients With Diarrhea and EPEC Identified in Stools With Biofire FilmArray and 25 Patients With Negative Results on Biofire FilmArray

Microbiology	Fecal qPCR for EPEC Genes, Bacteria/mg Stool, Median (IQR)			
	<i>eeA</i>	<i>P</i> Value	<i>lifA/efa-1</i>	<i>P</i> Value
Biofire FilmArray EPEC positive (n = 77)	6×10^4 (4×10^2 – 3×10^5)	<.001	3×10^4 (2×10^3 – 5×10^5)	<.001
Biofire FilmArray EPEC negative (n = 25)	Undetectable		Undetectable	
Coliforms present (n = 64)	7.0×10^3 (21 – 3×10^5)	.006	10 (10–15)	.064
No coliforms (n = 13)	10 (6–931)		10 (10–27)	
EPEC culture positive (n = 35)	3.5×10^4 (100 – 5.6×10^5)	.069		NS
EPEC culture negative (n = 28)	2.7×10^3 (6 – 4.3×10^4)		10	
Mixed pathogens (n = 29)	3.5×10^3 (10 – 1.3×10^5)	NS	10 (10–10)	NS
EPEC single pathogen (n = 48)	2.9×10^3 (14 – 3×10^5)		10 (10–10)	

Results are stratified according to the presence of coliforms in culture, isolation of EPEC in culture, and if EPEC was identified as the sole pathogen or in combination with other pathogens by Biofire FilmArray. The number of EPEC per gram of stool was extrapolated from the number of *eeA* or *lifA/efa-1* copies in a standardized curve with known quantities of EPEC and adjusted per milligram of stool.

Abbreviations: EPEC, enteropathogenic *Escherichia coli*; IQR, interquartile range; NS, not significant; qRT-PCR, quantitative reverse-transcription polymerase chain reaction.

Table 3. Antimicrobial Susceptibility Testing by Broth Microdilution Method on 18 Enteropathogenic *Escherichia coli* Strains Identified in 16 Cancer Patients With Diarrhea

Antibiotic	Breakpoints				Range	S		I		R		Non-S		MIC ₅₀	MIC ₉₀
	S	I	R	Range		No. (%)	No. (%)	No. (%)	No. (%)						
Aminoglycosides															
Amikacin	≤16	32	≥64	≥16	≤8	18 (100)	0	0	0	0	0	0	...	≤8	≤8
Gentamicin	≤4	8	≥16	≥16	≤2 to >16	17 (94)	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	...	≤2	≤2
Tobramycin	≤4	8	≥16	≥16	≤4 to >8	17 (94)	1 (16)	0	0	0	1 (6)	1 (6)	...	≤4	≤4
β-lactam/β-lactamase inhibitors															
Ampicillin	≤8	16	≥32	≥32	≤4 to >32	13 (72)	0	5 (28)	5 (28)	5 (28)	5 (28)	5 (28)	≤4	>32	>32
Ampicillin-sulbactam	≤8/4	16/8	≥32/16	≥128/4	≤4–16	15 (83)	3 (17)	0	0	0	3 (17)	3 (17)	≤4	16	16
Piperacillin-tazobactam	≤16/4	32/4–64/4	≥128/4	≥128/4	≤16	18 (100)	0	0	0	0	0	0	...	≤16	≤16
Piperacillin	≤16	32–64	≥128	≥128	≤16 to >128	13 (72)	1 (6)	4 (22)	4 (22)	5 (28)	5 (28)	5 (28)	≤16	>128	>128
Ticarcillin–clavulanic acid	≤16/2	32/2–64/2	≥128/2	≥128/2	≤16–32	17 (94)	1 (16)	0	0	0	1 (6)	1 (6)	...	≤16	≤16
Carbapenems															
Imipenem	≤4	8	≥16	≥16	≤2	18 (100)	0	0	0	0	0	0	...	≤2	≤2
Meropenem	≤1	2	≥4	≥4	≤1–2	17 (94)	1 (6)	0	0	0	1 (6)	1 (6)	...	≤1	≤1
Cephalosporins															
Cefazolin	≤8	16	≥32	≥32	≤4	18 (100)	0	0	0	0	0	0	...	≤4	≤4
Cefoxitin	≤8	16	≥32	≥32	≤4–16	15 (83)	3 (17)	0	0	0	3 (17)	3 (17)	8	16	16
Cefuroxime	≤8	16	≥32	≥32	≤4–16	6 (33)	12 (67)	0	0	0	12 (67)	12 (67)	8	16	16
Cefotetan	≤16	32	≥64	≥64	≤8	18 (100)	0	0	0	0	0	0	...	≤8	≤8
Ceftriaxone	≤1	2	≥4	≥4	≤1–2	17 (94)	1 (6)	0	0	0	1 (6)	1 (6)	...	≤1	≤1
Cefepime	≤8	16	≥32	≥32	≤4–8	18 (100)	0	0	0	0	0	0	...	≤4	≤4
Cefpodoxime	≤2	4	≥8	≥8	≤2	18 (100)	0	0	0	0	0	0	...	≤2	≤2
Ceftazidime	≤8	16	≥32	≥32	≤1	18 (100)	0	0	0	0	0	0	...	≤1	≤1
Monobactams															
Aztreonam	≤8	16	≥32	≥32	≤8	18 (100)	0	0	0	0	0	0	...	≤8	≤8
Nitrofurantoin derivatives															
Nitrofurantoin	≤32	64	≥128	≥128	≤16–32	18 (100)	0	0	0	0	0	0	...	≤16	≤16
Fluoroquinolones															
Ciprofloxacin	≤2	2	≥4	≥4	≤0.5 to >4	17 (94)	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	...	≤0.5	≤0.5
Gatifloxacin	≤2	4	≥8	≥8	≤1–8	17 (94)	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	...	≤1	≤1
Sulfonamides															
TMP-SMX	<2/38	...	>4/76	>4/76	<0.5 to >4	15 (83)	0	3 (17)	3 (17)	3 (17)	3 (17)	3 (17)	<0.5	>4	>4

Breakpoints as recommended by Clinical and Laboratory Standards Institute M100, 28th edition, 2018.

Abbreviations: I, intermediate sensitivity; MIC₅₀, minimum inhibitory concentration for 50% of isolates; MIC₉₀, minimum inhibitory concentration for 90% of isolates; NS, nonsusceptible (includes S + I); R, resistant to the antibiotics listed; S, sensitive to antibiotics listed; TMP-SMX, trimethoprim-sulfamethoxazole.

Figure 2. Number of enteropathogenic *Escherichia coli* bacteria adhered to human duodenal enteroid monolayers. Monolayers were infected at a multiplicity of infection of 10 bacteria per enteroid cell for 6 hours. Cells were fixed and the number of adhered bacteria counted. Magnification is equal to $\times 100$ (each image is approximately 140 mm²). Whiskers represent minimum and maximum values of 5 replicates per isolate. Nested *t* test revealed no differences in the number of adhered bacteria between *lifA/efa-1* positive (mean, 309.9) and negative (mean, 464.9) strains ($P = .6646$).

DISCUSSION

In this study we show that, when compared to healthy adults with asymptomatic EPEC infection, cancer patients with EPEC-associated diarrhea carry a larger burden of EPEC, particularly when immunosuppressed. We also show that in this patient population, most strains belong to the atypical EPEC subpathotype. We also demonstrate that in vitro, clinical strains derived from patients show heterogeneity in their ability to attach and kill enterocytes and that in HIEs, EPEC displays novel patterns of adherence.

Our findings are consistent with other studies supporting a quantitative approach to differentiate colonized from truly infected patients with enteric bacteria [2, 17]. In our study, patients on immunosuppressive agents carried higher bacterial burdens above those observed in healthy controls or in children with EPEC-associated diarrhea in developing countries [18]. Most of the infections were diagnosed within 48 hours of admission, likely reflecting community acquisition. In our study, 13% of patients with EPEC identified as the single pathogen experienced a delay in cancer care (either postponing or decreasing

Figure 3. Assessment of the adherence properties of enteropathogenic *Escherichia coli* (EPEC) from patients with diarrhea at a major cancer care hospital. Human duodenal enteroid monolayers were infected with EPEC at a multiplicity of infection of 10 for 6 hours, fixed, and the slides then examined for the size of bacterial clusters for each strain assessed in this study. Magnification is equal to $\times 100$ (each image is approximately 140 mm^2). Data presented are an average of 5 replicates per strain.

the intensity of chemotherapy, modifying immunotherapy, radiation, or surgery) due to diarrheal illness, suggesting that EPEC contributes to morbidity in this patient population.

A limitation of this study is that coinfections were common [2]. We did not see differences in EPEC bacterial loads in monoinfected patients when compared to coinfecting patients. However, the number of individual copathogen combinations

was small. We also saw discrepant results between the detection of EPEC by BioFire FilmArray NAAT and fecal qPCR for *eae*, which was positive in only 70% of cases. Furthermore, we recovered EPEC in culture in only 46% of cases. This suggests that FilmArray may be sensitive at very low pathogen concentrations. However, these discrepancies could be explained by different DNA extraction protocols, assay conditions, low burden, sample dilution, or prior antibiotic therapy.

EPEC is commonly found in persons without diarrhea, spurring controversy on its role as a pathogen and confusion among clinicians on the indications for antibiotic treatment, if any. There is evidence that not all EPEC causes diarrheal disease and not all strains are equally pathogenic. For instance, in the case of typical EPEC, specific gene clusters are associated with lethal and severe infection [19]. In field studies, data implicating atypical EPEC are less robust than for typical EPEC as a cause of diarrhea [1]; however, atypical EPEC has been proven to cause diarrhea in volunteer challenge studies [20]. For some atypical EPEC, the presence of accessory genes in PA-O22 confers additional virulence [10] across several clonal groups [21, 22]. Other studies have associated atypical EPEC with prolonged diarrhea [23, 24] and malnutrition [25]. We examined stools and strains for the presence of *lifA/efa-1*, an accessory gene that codes for a protein involved in inhibiting lymphocyte proliferation and enhancing EPEC attachment to the epithelium. We found fecal *lifA/efa-1* in only 20% of cases, and in low copy numbers. However, fecal *lifA/efa-1* was more commonly seen in neutropenic patients. In agreement with other studies in the US, study subjects with fecal *lifA/efa-1* carried a larger burden of EPEC [9].

Current Infectious Diseases Society of America guidelines for the management of bacterial diarrhea limit the use of antibiotic therapy to patients with severe disease and the immunocompromised when *E. coli* other than STEC is identified [26]. In the US, antimicrobial resistance is not performed for EPEC since most laboratories do not routinely isolate and characterize this pathogen. Antimicrobial resistance in atypical EPEC is common in developing countries. Resistance to trimethoprim-sulfamethoxazole is in excess of 50% in Africa with rapidly emerging resistance to fluoroquinolones in Asia. In our study, we found resistance to aminopenicillins, and nonsusceptibility to second- and third-generation cephalosporins. In contrast to studies in Asia, ciprofloxacin resistance was low (6%) despite the common practice of using fluoroquinolone antibiotic prophylaxis in neutropenic patients with hematologic malignancies or during episodes of chemotherapy-induced neutropenia in other types of cancers. In our cohort, 92% of patients were treated with antibiotics with activity predicted to have activity against EPEC and responded clinically.

When examined for adhesion to duodenal HIE, we observed patterns of adherence that differed from the archetypical EPEC localized and diffuse adherence patterns typically seen in transformed HeLa and HEp-2 cell lines [27]. Some strains

Figure 4. Adherence patterns of clinical enteropathogenic *Escherichia coli* (EPEC) isolates on human duodenal monolayers. Monolayers were infected at a multiplicity of infection of 10 bacteria per enteroid cell for 6 hours before fixation and staining. Five images were taken at $\times 100$ magnification across the span of each well (at least 3 wells per strain were imaged). Images were reviewed and the presence of 4 distinct patterns of adherence was noted for each strain (A). An adherence pattern was considered to be a feature of that strain if the pattern appeared more than twice within a well. Diffuse adherence is defined as single bacteria (and clumps of 2–3 bacteria) adhered dispersed across the monolayer. Aggregative adherence includes clusters of <50 bacteria in a distinct single group without bacteria adhered in the surrounding area. In comparison, localized adherence is defined as clusters of >500 connected bacteria. Prototype EPEC strain E2348/69 was found to display all 4 patterns of adherence (B) on human duodenal enteroids including localized adherence with clusters ranging from tens to thousands of bacteria.

demonstrated little adherence and others produced classical focal adherence, whereas others rapidly formed complex bio-film communities with clusters of bacteria of different densities. In this study, we also observed diverse cytopathogenicity. These findings are consistent with prior observations in intestinal explants [13]. In our study done with a limited number of strains, we did not observe a correlation between clinical outcomes, bacterial shedding and HIE adhesion phenotypes, number of attached bacteria, or cell death. This variability most likely stems from genomic differences present in the EPEC strains as well as from host-specific factors. Although some of the mechanisms by which EPEC causes cell death and detachment of the epithelium have been characterized [28–32], EPEC strains are genetically heterogeneous and do not share all of the known putative virulence factors. Similarly, it is also possible that adherence to the intestinal epithelium for some strains is defined by the host and is segment specific with isolates that preferentially adhere to

the duodenum, jejunum, ileum, or colon. Other potential host factors include glycan receptors, blood group antigens, or other host genetic polymorphisms in proteins that encode factors necessary for attachment or are expressed in response to EPEC. The HIE model for EPEC attachment is a first step in understanding the relative contribution that bacterial and intestinal cell specific factors have during the initial stages of infection.

CONCLUSIONS

Our studies suggest that EPEC is an important pathogen in patients with cancer and diarrhea. However, important limitations of this study including the potential for selection bias, high frequency of coinfections, and the potential for diarrhea being due to other causes (mucositis, neutropenic enterocolitis, chemotherapy, etc), as well as the lack of a placebo-controlled randomized trial, preclude recommendations on antimicrobial

Figure 5. Monolayer integrity after infection with *eaeA*⁺ *Escherichia coli* strains used in this study. Enteropathogenic *E. coli* (EPEC) strains were added to confluent duodenal monolayers at a multiplicity of infection of 10 for 6 hours and the percentage monolayer remaining (A) was assessed after fixation and staining using ImageJ (B). Mean for each isolate is shown. Error bars display standard error of the mean. A nested *t* test revealed no difference between *lifA/efa-1* positive and negative strains in their destruction of duodenal monolayers (*P* = .9138).

therapy use. Large prospective studies that examine the impact of all diarrheagenic *E. coli* pathotypes in the context of coinfection and microbiome composition are needed to better understand the epidemiology and impact of diarrheagenic *E. coli* in cancer patients. Although some patients appeared to benefit from the use of antibiotics, randomized, double-blind, placebo-controlled clinical trials with predefined criteria are needed to determine if antibiotic therapy is of benefit in this patient population.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The funders had no role in experimental design, data collection, or interpretation of data.

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