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Clinical features and molecular epidemiology of diarrheagenic *Escherichia coli* pathotypes identified by fecal gastrointestinal multiplex nucleic acid amplification in patients with cancer and diarrhea

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ABSTRACT

Diarrheagenic *Escherichia coli* (DEC) pathotypes with differing epidemiology and clinical features, are known causes of disease with worldwide occurrence. At a major cancer center in the U.S., we studied patients with cancer and diarrhea for whom a GI Biofire FilmArray multiplex GI panel (BFM) was performed. An enteropathogen was identified in 382 of 2017 (19%) samples distributed across 311 patients. Of these, 60/311(19%) were positive for DEC. Patients receiving hematopoietic stem cell transplants (HSCT) 29/60 (48%) or with a hematologic malignancy 17/60 (28%) accounted for the majority of DEC cases. Enteropathogenic *E. coli* (EPEC, n = 35 [58%]), enteroaggregative *E. coli* (EAEC, n = 10 [17%]) and Shiga toxin producing *E. coli* (STEC, n = 3 [5%]) were the most common DEC identified and peaked in the summer months. Stool cultures confirmed infections in 6/10 (60%) EAEC (five typical *AggR*$^+$), and EPEC was recovered in 8/35 (22%) samples (all atypical *eaeA*$^+$, *bfp*$^-$). DEC was identified in 22 cases (37%) that developed diarrhea >48 hours after admission suggesting health care acquisition. Chronic infections were found in 2 EPEC and 1 EAEC cases that were tested at 1 month or beyond with shedding that ranged from 58 to >125 days. Two patients that underwent hematopoietic stem cell transplantation carried EAEC strains resistant to multiple antibiotics including fluoroquinolones and expressed extended spectrum beta lactamases. While in some instances BFM results were not verified in culture and could represent false positives, DEC pathotypes, especially EPEC and EAEC, caused chronic infections with antimicrobial-resistant strains in a subset of immunosuppressed cancer patients.

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1. Introduction

Cancer chemotherapy and radiotherapy-induced injury to the mucosa of the alimentary tract can cause epithelial cell loss and inflammation resulting in diarrhea (Shaw and Taylor, 2012). In addition to acute symptoms such as pain, diarrhea in cancer patients can be associated with dehydration, malnutrition, poor drug absorption and in the setting of mucositis and immunosuppression, result in bacterial translocation across the mucosa resulting in bacteremia, sepsis and potentially fatal septic shock. The empiric use of antibiotics for presumed bacterial diarrhea or concomitant infections can further disrupt the composition of the microbiome and place patients at risk for infection with *Clostridium difficile* resulting in increased costs and prolonging hospital length of stay (de Blank et al., 2013; Kim et al., 2013). Importantly, diarrhea is a common reason for delaying or decreasing the intensity of chemotherapy, ultimately leading to increased mortality (Chopra et al., 2010).

Clinicians are frequently confronted with the challenge of determining if diarrhea in a cancer patient is due to infection with an enteropathogen, chemotherapy associated epithelial cell loss, mucositis, hyperosmotic enteral nutrition, radiation injury and in the case of allogeneic hematopoietic stem cell recipients, intestinal graft versus host disease (Cherny, 2008). An important component in this process includes the evaluation for potentially treatable infectious agents. While infections with *C. difficile*, cytomegalovirus, norovirus and other opportunistic agents have been well recognized as enteropathogens in cancer and immunosuppressed patients, the role of diarrheagenic *Escherichia coli* (DEC) has not been previously well studied in this population.

Diarrheagenic *E. coli* pathotypes are classified according to the presence of defined virulence genes and for in some instances, by their adhesion patterns to intestinal epithelial cells (Nataro and Kaper, 1998).

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In the case of enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic (ETEC), Shiga-toxin producing *E. coli* (STEC) and enteroinvasive *E. coli,* there is clear epidemiologic, clinical and molecular evidence to validate their role as enteropathogens (Cohen et al., 2005; Nataro et al., 2006). For other pathotypes such as diffusely adhering *E. coli* (DAEC), the data is less clear in supporting an association with infectious diarrhea. ETEC, EPEC, EAEC are important causes of diarrhea in children of developing countries and ETEC along with EAEC are the most common pathotypes in adult travelers from industrial countries that visit less developed regions of the world (Dupont et al., 2007; Kotloff et al., 2012). STEC in turn has been more frequently identified in industrialized countries in outbreaks linked to processed food and petting zoos (Luna-Gierke et al., 2014; Wasteson, 2001).

The availability of Nucleic Acid Amplification Tests (NAATs) that can simultaneously detect the presence of multiple enteropathogens in stools of patients in a cost efficient manner is increasing our ability to define the epidemiology, clinical implications and outcomes of infectious diarrhea in pediatric and adult populations (Buss et al., 2015). However, these tests have not been validated in cancer and immunosuppressed patients. In this study, we sought to describe the epidemiology and clinical features of infections with DEC as defined by NAATs in cancer patients seen at a large comprehensive cancer center.

2. Materials, methods, and human subjects

We prospectively studied 2017 stools from 311 cancer patients with diarrhea in whom infection with an enteropathogen was suspected and were submitted to the clinical microbiology department between November 2016 and January 2017. Fecal enteropathogens were detected using a multiplex NAAT according to the manufacturer instructions (FilmArray, Biofire Diagnostics, Salt Lake City, UT). The FilmArray for gastrointestinal pathogens simultaneously detects and identifies nucleic acid from multiple viral, bacterial and parasites. For detection of the different DEC, the FilmArray targets the *eae* gene of EPEC which codes for the adhesin intimin. For EAEC the targets include *aggR,* which encodes a virulence regulator and *aatA,* which encodes an outer membrane protein transporter. In the case of ETEC the targets include genes for the heat labile (LT) toxin gene *ltA,* and two heat-stable (ST) toxin variants *st1a* and *st1b*. The *stx1* and *stx2* genes are targeted to identify STEC and the array includes a specific gene for O157:H7 serotype determination. For the identification of EIEC, the targeted gene is *ipaH,* which is also present in *Shigella spp.* Residual stools were transported to the research laboratory and streaked onto MacConkey plates. Following overnight growth at 37 °C, 10 coliform-like single colonies were selected and tested by PCR for the presence of DEC using primers specific for the 5 pathotypes (Boisen et al., 2009; Paredes-Paredes et al., 2011). EAEC strains were further studied for the presence of putative virulence genes with a second multiplex PCR and the aggregative phenotype was verified using HEp-2 adhesion assays (Boisen et al., 2009). Antimicrobial susceptibility on selected strains was determined by micro dilution according to Clinical Laboratory Standard Institute guidelines.

We then reviewed the electronic medical records of patients and retrospectively extracted demographic, clinical and laboratory data on the day in which the first DEC was identified as well as clinical outcomes at various time points thereafter. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Texas MD Anderson Cancer Center (Harris et al., 2009). This study and a waiver of informed consent was reviewed and determined to be exempt of institutional review approval by the IRB committee at the University of Texas MD Anderson Cancer Center.

3. Definitions

Diarrhea possibly due to DEC was defined by the passage of at least 3 unformed stools in a 24 hour period associated with at least one sign of enteric infection (fever, nausea, vomiting, abdominal pain, cramping, tenesmus, or the presence of mucus or blood in stools) and the identification of at least one diarrheagenic pathotype in the Biofire FilmArray GI multiplex PCR. Separate episodes were considered if patients had at least 1 week without diarrheal stools between episodes. A health care associated infection was defined as the onset of diarrhea after 48 hours of admission in a patient in whom an *E. coli* pathotype was identified. Chronic diarrhea was defined as lasting ≥4 weeks. Neutropenia was defined as an absolute neutrophil count (ANC) of <500/mL, severe neutropenia was defined as <100/mL. Persistent neutropenia was defined as <500/mL for >7 days.

3.1. Statistical analysis

Demographic, clinical, laboratory characteristics and outcomes summaries are shown in descriptive statistics. Proportions of subjects with a given outcome were compared by X^2 analysis. Fisher's exact test was used when expected numbers were less than 5 in over 20% of cells. Analysis of variance was used to compare means on linear variables with normal distribution, and Kruskall-Wallis statistical analysis was used to compare the means between multiple groups. To examine the seasonality of DEC infections, we first estimated the number of DEC divided by the number of Biofire FilmArray tests performed on a given month per 1000 tests ran.

4. Results

During the study period, 311 patients submitted 2017 stools for microbiology studies. Of these, 382 (19%) samples were positive for an enteropathogen by GI multiplex NAAT. At least one DEC was identified in 60 of 382 (16%) samples and 60 of 311(19%) patients. Most of the DEC infections were identified in Caucasian patients with hematologic malignancies or following hematopoietic stem cell transplantation and had a high Charlson co-morbidity index at the time of diagnosis (Table 1). As a group, the overall incidence of DEC and their proportion relative to all episodes of pathogen-proven diarrhea was 60/382 (16%). EPEC (35/60, 58%) and EAEC (10/60, 17%) followed by ETEC (2/60, 3%) were the most common pathotypes identified. Only one of the three Shiga-toxin producing *E. coli* (STEC) was O157:H7. Only two enteroinvasive *E. coli* (EIEC) were identified. Mixed DEC was seen in 8 of 60 (13%) cases. The ages, gender distribution, race, the types of underlying malignancies and other co-morbidities were similar for all pathotypes.

The presenting symptoms (Table 2) were also similar regardless of the pathotype identified. The proportions of patients presenting with emesis and bloody diarrhea were similar across groups. Duration of illness prior to presentation could not be ascertained in all cases but when available ranged from 1 to 40 days. In terms of risk factors, recent foreign travel was reported by only 8 (13%) patients and almost all had received immunosuppressive agents (88%) or chemotherapy (62%) within 90 days of DEC diagnosis. Among the solid tumor patients, 23% had significant immunosuppression and 62% had received recent chemotherapy. The majority of patients 37/60 (63%) acquired the infections in the community. However, a significant proportion of patients 22/60 (37%) developed diarrhea more than 2 days after hospitalization for an estimated DEC health care acquired rate of 0.49 per 10,000 patient days, which is significantly lower than the *C. difficile* health care acquired rate of 9 per 10,000 patient days at our institution for the same period of time.

Only 4 patients had leukocytosis at presentation. In contrast, neutropenia with ANC <500 was observed in 13/60 (22%) DEC cases and lymphopenia <1000 was observed in 42/60 (70%) of DEC cases. The proportions of patients with neutropenia and lymphopenia, as well as serum creatinine and albumin levels were similar across all pathotypes. Stool bacterial cultures confirmed infections in 8/35 (22%) of patients with EPEC and in 6/10 (60%) of patients with EAEC. We considered the finding of at least 1 of 10 colonies with virulence determinants to be diagnostic of DEC. However, the semi-quantitative density of positive

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