Characterization of Enteropathogenic Escherichia coli (EPEC) in Immunocompromised and Cancer Patients with Suspected Bacterial Diarrhea
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Abstract
Background: Multiplexed nucleic acid amplification tests (NAAT) are becoming widely utilized for the diagnosis of bacterial diarrhea in the US and include probes specific for EPEC. However, these platforms do not differentiate typical EPEC (EPEC, defined as strains carrying eaeA and hpy) which have strong epidemiologic associations with diarrhea from atypical EPEC (aEPEC, defined as carrying eaeA but not hpy) for which the association with diarrhea is less strong. Emerging data suggests that aEPEC subsets carrying efa1/lifA that encodes for adherence factor Lymphocyte inhibitory factor A are associated with diarrheal disease. Furthermore, the role of EPEC and its subtypes as agents of bacterial diarrhea have not been well defined in US cancer patients.

Methods: We sought to characterize EPEC subtypes in a case-control study that included healthy individuals with no diarrhea (HI, N=21), Patients with diarrhea and negative Biofire NAAT for enteropathogens (DN, N=25) and patients with NAAT positive for EPEC (DP, N=54). Quantitative PCR was performed in stools using probes specific for eaeA, efa1/lifA. The qPCR dynamic range was optimized to detect from 5X10^8 to 5X10^12 bacteria/mg stool with a cutoff limit of 35 cycle thresholds (CT) for eaeA and 32 for efa1/lifA. EPEC strains recovered from stool cultures from DP patients were tested for eaeA and hpy, etc and other diarrheagenic E. coli virulence factors.

Results: Demographic characteristics and underlying malignancy were similar between DN and DP groups. DP were more likely to have diarrhea on admission than DN (46/52 (88%) vs. 13/52 (25%); p<0.001). Stools confirmed EPEC in 24/52 (46%) DP of which 23/24 (96%) were aEPEC. Fecal qPCR for eaeA confirmed EPEC in 43/52 (83%) of DP, 0.25 DN and in 3/21 (14%) of HI (p<0.001). DP excreted a higher number of EPEC bacteria/mg of stool than HI (median 2.71 X 10^5 vs. 2.54 bacteria/mg, p=0.001) and only DP excreted efa1/lifA (+) [4/52 DP (27%) vs. 0.25 DN and 0/21 HI; p<0.001]. When compared to DP EPEC eaeA(-), DP EPEC efa1/lifA (+) were more likely to be hematopoietic stem cell transplant (HSCT) recipients [7/14 (50%) vs. 7/38 (18%), p<0.05] and had a higher EPEC eaeA fecal burden (median 2.9 X 10^6 vs. 3.3 X 10^5 bacteria/mg, p=0.05). Co-infections with other pathogens were equally represented in efa1/lifA (-) and efa1/lifA (+) DP subgroups [8/14 (57%) vs. 21/38 (55%)].

Conclusions: Most EPEC in cancer patients with diarrhea are due to aEPEC acquired in the community and in HSCT patients are associated with a higher bacterial burden.

Introduction
• The Global Enteric Multicenter Study has shown that EPEC is associated with diarrhea related mortality in children of developing countries.1
• In contrast, aEPEC which lacks the plasmid carrying hpy, has not been consistently associated with diarrhea. However, recent studies suggest that aEPEC containing alternate virulence factors such as those in pathogenicity island O123 are associated with diarrhea.2
• The pathogenicity island O123 contains efa1/lifA which codes for an adherence factor that has lymphopoietic properties.3
• DEC is frequently identified in stools of patients with cancer and diarrhea but the role of EPEC as an enteric pathogen in this population is unclear.4
• Quantitative molecular diagnostics with qPCR can assist in differentiating entero-pathogen colonization from true infection causing diarrhea.5

Results
Table 1. Clinical presentation and risk factors for EPEC

Discussion
• Most EPEC in cancer patients are acquired in the community.
• Fecal E/copload was found to be significantly higher in PD group than in the ND and asymptomatic controls.
• One QPCR was done to detect eaeA in only 35% of patients with EPEC found by the Biofire NAAT that targets the same gene. Possible explanations include by differences in the CT, PCR conditions, primers used, PCR inhibitors, and sample preparation methods, sample preservation (done on frozen specimens), and transport media used.
• Hematopoietic stem cell transplant patients and EPEC in their stools, shed larger amounts of efa1/lifA

Conclusions
• The frequency of eaeA in healthy controls was low and was not associated with strains that carried efa1/lifA
• Risk factors for finding aEPEC with efa1/lifA in cancer patients with diarrhea include community onset and immunosuppression due to hematopoietic stem cell transplantation
• Results from this pilot study done in cancer and immunocompromised patients, agree with similar studies done in developing countries demonstrating higher EPEC burden when carrying efa1/lifA

References
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