

Supplementary Appendix

This appendix has been provided by the author to give readers additional information about his work.

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Table S1. Infectivity of Enteric Pathogens Based on Infectious Inoculum in Healthy Adult Volunteers and Potential for Person-to-Person Spread

| Enteric Pathogen | Infectious Dose for Humans Evidence of Infectivity | Communicability – Frequency of Secondary Spread |
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| Low Inoculum Organisms, <100 to 500 viable organisms | | |
| <i>Shigella sonnei</i> , <i>S. flexneri</i> , <i>S. dysenteriae</i> 1 (Shiga bacillus) | Based on volunteer challenge studies | Very Common |
| Shiga toxin-producing E. coli including E. coli O157:H7 strains | Epidemiologic and animal model studies suggest the dose is low | Common |
| Noroviruses | Based on volunteer challenge studies | Very Common |
| Rotaviruses | Based on volunteer challenge studies | Common |
| <i>Giardia</i> | Based on volunteer challenge studies | Common |
| <i>Cryptosporidium</i> | Based on volunteer challenge studies | Common |
| Moderate Dose 1,000 to 100,000 Viable Microorganisms | | |
| <i>Salmonella</i> Enterica (non- typhoid strains) | Unknown (foodborne outbreak studies suggest dose is highly variable and relates to strain; most outbreaks are associated with doses of > 1,000 viable bacteria, some are < 1,000) | Not uncommon |
| <i>Campylobacter jejuni</i> | 800-2 x 10 ⁹ viable bacteria | Rare |

| High Inoculum Organisms, >10 ⁶ Viable Microorganisms | | |
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| Enteroinvasive E. coli (EAEC) | Based on volunteer challenge studies | Rare |
| Enterotoxigenic E. coli (ETEC) | Based on volunteer challenge studies | Rare |
| Enteropathogenic E. coli (EPEC) | Based on volunteer challenge studies | Rare |
| <i>Vibrio cholerae</i> | Based on volunteer challenge studies | Rare |

Table S2. Summary of Key Controversies in Enteric Infections

| Controversy | Background |
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| Diagnosis | |
| Inflammatory markers in stool to help diagnose colonic enteropathogens | Inflammatory pathogens involving the colon produce inflammatory products that can be detected in stool including leukocytes (WBCs), IL-8, lactoferrin or calprotectin. When present a pathogen is more likely identifiable and the finding may have prognostic significance and help identify carriage of an organism with disease due to another cause. Their lack of sensitivity and insufficient study make their value in the workup of acute diarrhea uncertain. |
| Diagnosis of <i>Clostridium difficile</i> associated diarrhea (CDAD) | Available tests lack sensitivity or specificity or they take too long to return; very sensitive assay may fail to discriminate between asymptomatic infection and CDAD; the available tests do not allow detection of more virulent strains of <i>C. difficile</i> . |
| Chemotherapy and Prevention of Enteric Infection | |
| Antimotility drugs in the symptomatic treatment of acute | The agents decrease number of stools without necessarily reducing fecal volumes, may result in worsening invasive |

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| diarrhea | bacterial diarrhea (with this risk reduced by providing concomitant effective antibiotics); they may potentiate hemolytic uremic syndrome in STEC infection; their major value is in international travelers who are on a tight schedule with fixed transportation times; value outside this setting for acute diarrhea is uncertain although they are safe if antimicrobial therapy is given concomitantly |
| Antibiotics for patients with diarrhea due to non-typhoid strains of <i>Salmonella</i> | Antibiotics may prolong <i>Salmonella</i> fecal shedding ¹ and fail to predictably shorten the illness, however, these strains may produce bacteremic disease requiring antimicrobial therapy |
| Antibiotics for patients with Shiga toxin-producing <i>Escherichia coli</i> diarrhea | Some antibiotics induce Shiga toxin-encoding phage and may precipitate HUS |
| Antibiotic treatment of travelers' diarrhea | The illness is self-limiting and many cases are mild; it is unclear when to start antibiotics, with passage of the first unformed stool or with full blown disease; there is a concern that widespread use of antibiotics, particularly with those with value outside the gut, will lead to clinical resistance |
| Prophylactic antibiotics to prevent travelers' diarrhea | The illness is self-limiting and many cases are mild; there is concern that travelers given preventive drugs may not exercise caution about food selection during high-risk travel |

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| | <p>because of false sense of safety; and the efficacy of chemoprophylaxis is not high in seasons or places with low diarrhea rate ²</p> |
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Table S3. Priority Areas for Future Research in Enteric Infections

| Research Question | Comments |
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| Diagnosis | |
| Diagnostics for viral gastroenteritis pathogens | Norovirus diagnostics are needed for routine laboratories including assays for norviruses and viruses of potential interest include astrovirus, enteric adenovirus, sapovirus, bocavirus, polyomavirus, parechovirus, torovirus, and Aichi virus. In one study whole genome sequencing methods for detection of norovirus outbreaks was employed ³ which is not routinely used in outbreak investigation. |
| Diagnostics to define the etiology of currently non-diagnosable cases of acute and persistent diarrhea | New methods are needed to look for established and new pathogens in patients with diarrhea including with or without enrichment: molecular detection and typing methods including deep 16S rRNA mass metagenomic sequencing for novel microbial sequences; DNA microarray technology with various amplification strategies; conventional and real-time reverse transcription-PCR, luminex xMAP technology and flow cytometry methods; probe-independent RNA sequencing approaches identifying pathogen transcriptomics and applications of immunoelectron microscopy |
| Diagnostic biomarkers in understanding pathogenic | Cytokine profiles working with fecal samples and sera and quantitation of lactoferrin or calprotectin to develop indicators |

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| mechanisms to help with diagnosis and to identify therapeutic targets | of diagnosis and prognosis ⁴ |
| Predisposing Host Factors | |
| Host genetics in enteric infection | Defining host genetic factors in enteric infection may help our understanding of susceptibility and provide opportunities for therapy and disease prevention |
| Proton pump inhibitor therapy in the pathogenesis of diarrhea | Whether acid-reducing drugs importantly predispose to enteric infection ⁵ and how health benefits of the drugs can be separated from disease potentiation effects should be studied |
| Post-infectious complications of enteric disease | Host factors and pathogenesis needs to be studied to define the population at risk and to develop therapeutic and prevention strategies with an emphasis on functional bowel disorders which are so common |
| Microbial Studies of Pathogenesis | |
| Diarrheagenic <i>E. coli</i> with inter-pathogen enhancement of virulence | More research is needed on the biology of various <i>E. coli</i> enteric pathogens; the serious outbreak of dysentery and HUS due to <i>E. coli</i> O104:H4 in Germany and France in 2011 due to an enteroaggregative <i>E. coli</i> strain that had acquired Shiga toxin 2-producing phage serves as a warning that we are likely to see super-pathogens containing multiple diarrheagenic <i>E. coli</i> virulence factors with additive or |

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| | synergistic virulence ⁶ |
| Noroviruses and other viruses and <i>C. difficile</i> as causes of morbidity and mortality in immunocompromised solid organ and hematopoietic stem cell transplant patients | Diarrhea is common in this setting with enteric infection and GVHD both being possible; noroviruses have been shown to cause persistent shedding and diarrhea in these patients complicating their therapy and outcome ⁷ |
| Non-typhoid salmonella bacteremia | Studies of antibacterial resistance and virulence factors of salmonella strains and host resistance factors should be carried out to explain the excess bloodstream infections seen in sub-Saharan Africa and in many people worldwide ⁸ |
| Changing virulence of <i>C. difficile</i> | Strain and clade variation of strains and host biomarkers need to be studied to understand why the organisms is associated with increasing frequency and severity of infection ⁹ |
| Therapeutic Considerations | |
| Optimal treatment of CDAD and CDAD recurrence | Current therapy with high rates of reoccurrence probably need longer term therapy initially and in patients with multiple reoccurrences methods need to be studied to re-populate the colon with homeostatic bacterial flora to resist infection |
| Non-antimicrobial drugs targeting disease pathophysiology | Studies of mechanisms of acute diarrhea are needed to look for optimal physiologically-directed therapeutic agents including antisecretory, antimotility and anti-inflammatory drugs; the gut chloride channel blocking antisecretory drug, |

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| | crofelemer, has been shown to improve diarrhea in travelers and diarrhea in HIV infection and the antisecretory enkephalinase inhibitor, racecadotril is being developed for treatment of secretory diarrhea |
| Therapy of STEC and HUS | Azithromycin and rifaximin appear not to induce Shiga toxin-encoding phage and may have some value in disease treatment. Clinical trials in patients with STEC-associated HUS are needed to confirm value of plasma exchange, and therapy with anti-complement humanized monoclonal antibody, eculizumab ¹⁰ |
| Intestinal Microbiota | |
| Intestinal microbiome in health and disease | The study of intestinal flora and human disease needs much greater study focusing on human disease from diarrhea to chronic intestinal disorders to GI cancers; available probiotics have limited value on improving health and research in this area is needed; improved evidence-based selection of anaerobic gram-positive and anaerobic gram-negative bacteria that promote intestinal health and homeostasis is needed; current probiotics do not have important value in promoting intestinal health |
| Chemoprophylaxis and Immunoprophylaxis | |
| Prevention of CDAD and | Vaccine candidates directed toward the organism or toxins A |

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| CDAD recurrences | and B are in development stages; this is a priority for controlling the infection that is showing increasing importance, anti-toxin A and B monoclonal antibodies are in trials aimed at prevention of CDAD recurrences |
| Vaccines for selected forms of infectious diarrhea | Priority enteric pathogens for vaccine development: rotavirus (for developing countries), norovirus (genogroup I and II, especially GII.4), cholera, ETEC, shigella and campylobacter |
| Mechanisms of efficacy of fecal microbial transplantation (FMT) and the development of refined approaches to improve microflora | FMT has had success in the treatment of recurrent CDAD and anecdotal therapy of IBD and IBS appear promising; the components of successful FMT and routes of administration need to be studied focusing on bacteria and other constituents in stool such as organic acids and intestinal alkaline phosphatase |

ETEC = enterotoxigenic *E. coli*; CDAD = *Clostridium difficile*-associated diarrhea; HUS = hemolytic uremic syndrome; IBD = inflammatory bowel disease; IBS = irritable bowel syndrome; STEC = Shiga toxin producing *E. coli*

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