In recent years, multiple advances have been made in the care, diagnosis, and mechanistic understanding of acute gastroenteritis (AGE). In this review we discuss the current state of the art of diagnosis and management, as well as how changes in practice can improve care and decrease costs. We will discuss current research demonstrating the effect of AGE on the microbiome and how that may be linked to secondary effects or long-term changes. We will explore the use of novel technologies to further our capacity to understand how gastrointestinal infections occur and promulgate. Finally, will discuss advances in our understanding of how gastrointestinal infections capacitate other changes such as post-viral motility or other post viral intestinal dysfunction.
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many figures and tables are included in this manuscript?</td>
<td>2</td>
</tr>
<tr>
<td>What is the word count of your manuscript? (word count applies only to main text; it does not include the abstract, what is new/known, references, or legends)</td>
<td>2987</td>
</tr>
</tbody>
</table>
REVISED VERSION REVIEWER COMMENTS

Reviewer 1: My questions have been satisfactorily addressed. However, in re-reading the manuscript, I believe it has now tilted a bit more towards a review with the addition of some cutting edge concepts. As such, major established therapeutic advances for which there is strong evidence should be included. The authors do mention reduced osmolarity ORS. They should add:

1) Children age >6 months in developing countries may benefit from the use of zinc in the treatment of AGE; however, in regions where zinc deficiency is rare, no benefit from the use of zinc is expected.
2) Selected probiotics can be used in children with AGE as an adjunct to ORS to shorten the course of illness.

We very much appreciate the reviewers feedback. We highlighted the use of zinc in regions that are underserved and how it may benefit patients in that setting.

We also agree that probiotics have shown significant promise, and include reference to both a recent review in JPFGN and specific work covering probiotic impact in acute gastroenteritis.

In addition to ORS, the use of Zinc in children in developing countries may have benefit in treatment of AGE\textsuperscript{17}; however, this beneficial effect is linked the presence of zinc deficiency, and in regions where zinc deficiency is rare, no benefit from the use of zinc is expected\textsuperscript{18}. There is some data suggesting possible benefit of certain probiotic strains, with trials demonstrating efficacy for use of *Saccharomyces boulardii*, *Lactobacillus rhamnosus GG*, and *L reuteri DSM 12246*, with findings supporting improvement in days of diarrhea\textsuperscript{19, 20}.

Reviewer 2: The authors have responded to the previous comments.

The figures are not cited or integrated in the text and add very little overall to the review. Figure 1 is a low quality illustration and given that this topic is not a focus in the review and also only added following review, would seem an odd choice for a figure. Similarly for Figure 2 where the illustration doesn’t add anything and the micrograph is low resolution fluorescence image.

We appreciate the reviews recommendations. We have included a more direct citation of Figure 1 in the review article to highlight it and show the mechanisms of the antidiarrheal drugs. We have removed Figure 2 from the review article.
Editor Comments:
Please address Reviewer 1's comments, making these additions to round out the review of established therapies.
Figure 1 can remain, but please make reference to the figure in an appropriate place in the manuscript. Recommend removing figure 2A and 2B and figure legends.

We greatly appreciate the Editor's comments and we have incorporated Figure 1 to be more clearly incorporated into the review article. Figure 2 and legend have been removed.

INITIAL REVIEWER COMMENTS:
Reviewer 1: The manuscript by Arostegui and Wallach describes the current state and recent advances in the diagnosis, treatment and understanding of AGE. Several points are made in review:

1. In the introduction there is a statement about the causes of AGE but the authors do not differentiate between LMIC and high income countries where the data are different. For example in the Global Enteric Multicenter Study (GEMS) (Lancet. 2016 Sep 24;388(10051):1291-301) the following were the most important causes of diarrhea: Shigella spp, rotavirus, adenovirus 40/41, ST-ETEC, Cryptosporidium spp, and Campylobacter spp. Rotavirus may be the single most common viral cause of diarrhea.

We very much appreciate the reviewer’s feedback, and have adjusted our language to make clear that there is regional variation based on prevalence and efficacy of rotaviral vaccination, and that pathogens causing AGE vary heavily between developed and LMIC nations. Altered language below.

Many studies suggest that the most common cause of AGE globally remains Rotavirus despite comprehensive vaccination efforts, but Norovirus is rising and may have overtaken Rotavirus in the developed world [4-6]. Bacterial AGE remains relatively rare in developed nations, representing less than 10% of AGE in hospitalized children[7]. Low-middle income countries (LMIC) have higher prevalence of non-viral
AGE, with the most common causes consisting of Shigella, rotavirus, adenovirus 40/41, ST-ETEC, cryptosporidium, and campylobacter [8].

2. The statements about the benefits if PCR pathogen panels are misleading. First in the article cited (reference #20) there was a mixed group of pediatric and adult inpatients at a single center. However, as the authors imply, hospitalization for AGE in the US is now uncommon. The cost data is not exclusively from pediatrics and in fact there was NO reduction in stay in pediatric patients who had a PCR panel. I am not sure if the other data can be extrapolated to pediatrics as it is not broken out in the article. Certainly the cost data is an over-reach. And it is inappropriate to generalize about cost reductions occurring from the use of a GI PCR panel in hospitalized adult patients. I would propose that is as likely to cause a cost increase in outpatient pediatric medicine because of relative infrequency of ordering stool studies now (increased testing) and the identification of non-pathogen results with concomitant treatment. For example, the identification of EPEC, the most common organism identified in the GI PCR panel (reference 20), may be highly misleading. The pathogenicity of these organisms (vs their potential to be pathogens) is unproven as the specific genetic sequences that are associated with virulence are still uncertain. Moreover, the infecting dose is unclear and identification of organisms with pathogenic potential in low numbers by PCR may misrepresent causality. They may not have sufficient numbers to cause disease. In many studies, the control groups have high levels of EPEC as well. Which ones cause disease?

We very much appreciate this prompt to be more clear in our language. We have reframed it to make clear that PCR testing does not change baseline recommendations for testing in AGE (which is that it should be rare) and caveat the value of these tests. We do however note that there is some literature reflecting systematic benefits of these tests in adult and pediatric patients.

Novel language: Diagnostic workup is typically not indicated in AGE. Traditional tests such as stool cultures or antigen testing are time consuming and in adult patients have been shown to generate increased costs and testing[31]. However, advances in polymerase chain reaction (PCR) based testing have provided a rapid (can return in <1 hr) and sensitive assay, with some studies demonstrating almost a 10-fold increase in pathogen identification over culture[31]. When used appropriately, PCR testing has substantial capacity to improve antibiotic stewardship, with work in adult patients demonstrating that positive identification of viral strains decreased use of empiric antibiotics, which are widely prevalent in adult primary care [32] [33].
3. Similarly the summary paragraph over concludes the benefits of PCR panels. While the utility of these tests is currently debated (speed and breadth vs over-sensitivity), there is likely utility in this approach for immunocompromised hosts, the critically ill, or individuals with prolonged disease that is refractory to treatment. However, these qualitative PCR-based tests do not distinguish between low-level enteropathogen detection of unclear relevance and infections that are more clearly clinically important. I cannot accept the conclusions offered which are generalized from hospitalized adults. We should not all use this for children presenting with uncomplicated AGE.

We again appreciate the reviewer for noting our need to be more clear in these statements. We had not intended to suggest routine use of PCR panels in pediatric AGE patients, and appreciate the note that this paragraph had implied that. To clarify our language we have made the following changes:

However, this higher sensitivity also raises a concern in use of the PCR panel, in that it may be overly sensitive and identify organisms or remnant genetic material that are not contributing to current pathology.

While overdiagnosis remains a concern which should limit its use, some evidence in hospitalized adult patients also suggests a role for its use in significantly morbid AGE. Recent work in adult patients has demonstrated that using a GI PCR pathogen panel decreased duration of hospitalization by 0.5 days [31], although there was not a significant benefit in pediatrics. In aggregate, health care costs diminished by $293.61/patient in the group testing with GI PCR [31]. Stool PCR testing also allows for appropriate use and faster removal of patient isolation precautions and reduction of costs associated with isolation [35].

4. The comments about the import ance of single-cell transcriptomics allowing us to define infection control policies are overstated. Finding SARS-CoV-2 in stool is sufficient to be concerned about fecal oral transmission with transcriptomic data:

We appreciate the feedback and have removed the following statement.

REMOVED->The evidence provided in this study shows a possible fecal-oral transmission of SARS-CoV-2 and the same principles can be used to discover new disease process [29]

Reviewer 2:
1) Would consider the title - there is little here that is specifically new about management - other than reviewing of the largely ignored European and International guidelines.

We very much appreciate this feedback. We have adjusted the title as follows:

*The Cutting Edge of Gastroenteritis: Advances in Understanding of Enteric Infection*

2) In terms of symptomatic treatments there is are many studies about various strategies for anti-secretory therapies in pre-clinical or phase 1 testing including chloride channel inhibitors, calcium receptor agonists, NHE3 agonists, natural products as well as FDA approved anti-secretory drugs (Crofelemer). Although these are either not approved or not widely used clinically, it would seem that this whole area should have at least brief mention. Although the focus of the review is on other advances such as the microbiome, to completely ignore this evolving area seems an oversight.

We again appreciate this point. We had initially considered adding such a section and you have highlighted the reasons why we should have. Below is the section we have added discussing novel therapies.

---

**Established and Novel Therapeutic Agents for Diarrhea**

Classically, US providers have mainly provided supportive care, with relatively rare use of medication for symptomatic management. However, medications exist which can improve symptoms and recovery time, although some are not available in the United States *(Table 1)*. Loperamide, an opioid receptor agonist which slows intestinal motility, is not recommended in pediatrics due to side effects and concern for intestinal perforation[23]. Racecadotril, an alternative, safe, and more tolerable anti-diarrheal therapy is recommended in ESPGHAN guidelines, but is not unavailable in the US[2][24]. Racecadotril inhibits endorphin-metabolizing enzyme neutral endopeptidase (NEP, or Enkephalinase), decreasing luminal chloride secretion[2]. This mechanism avoids loperamide associated cramping effects, risk of perforation, and retention of infectious agent [2]. Diosmectite has also shown efficacy in decreasing secretions[25].

Multiple concepts are currently under investigation or show promise for treatment of AGE, although as of now AGE specific clinical data is limited and pediatric data essentially nonexistent. Crofelemer, a CFTR and CaCC channel binding agent, is the sole novel FDA approved agent [26], currently approved for use with anti-retroviral therapy in HIV/AIDS and under study for chemotherapy induced diarrhea. This drug has likely efficacy in AGE, targeting the common viral/bacterial diarrheal mechanism of upregulation of cAMP and cGMP driving chloride channel activation and chloride secretion [27]. Multiple other agents with potential efficacy have been identified in basic science contexts, although clinical data remains sparse *(Table 2)*. NHE3 agonists have been shown to block secretion in cholera toxin
mediated murine models [28]. Clotrimazole has been shown to block cAMP mediated chloride channels and Ca+ mediated K+ channels, demonstrating decreased secretion in murine models [29]. Multiple other anti-secretory agents have been identified in vitro or in animal studies, with further investigation pending.

Herbal remedies have also demonstrated some possible utility, with the most promising agent being Shikonin, a compound found in Lithospermum erythrorhizon and used in traditional Chinese medicine. Shikonin has been found to be inhibitor of TMEM16A calcium gated chloride channel activity, and decreases both volume of diarrhea and motility in murine rotavirus models [30].

3) "possibly secondary to reports of long QT induced by 32-64mg IV doses used in chemotherapy patients, an effect which has not been reported with typical 2-8 mg dosage" Other than the review are there data /citations to support this - the level of evidence should be included if not - i.e opinion or anecdotal.

Thank you for noting that this was not adequately supported. We have added in additional language and citations to support this. There have now been multiple well performed trials looking at QT elevation using recommended ondansetron Zofran ranges, and even studies showing an elevation do not show clinically significant changes. This is in keeping with current clinical practice which does not indicate an EKG prior to use of ondansetron. Updated language:

Ondansetron, a highly safe and efficacious therapy, has been increasing in usage in the United States [17] but classically is used at relatively low rates [18], possibly secondary to reports of long QT induced by 32-64mg IV doses used in chemotherapy patients, This effect has been interrogated multiple times in pediatrics, demonstrating either a very low or no effect of QT prolongation at typical dosages of 2-8 mg dosage in otherwise healthy children [19] [20].

4) "By using single-cell transcriptomic analysis we can uncover which cell lines are susceptible to a pathogen and can guide us to help prevent spread of disease, such as implementing appropriate infection control policies. " - this sentence doesn't make sense -or its unclear how single cell level data relates to infection control policies. Would be more specific or clarify the statement.

Thank you for pointing out that this frame needed clarification. Updated language below.

These studies give us insight into how a pathogen causes disruption and entry and therefore provide us with information on possible therapeutic interventions, and will help support future understanding of emergent pathogens.
Many studies suggest that the most common cause of AGE globally remains Rotavirus despite comprehensive vaccination efforts, but Norovirus is rising and may have overtaken Rotavirus in the developed world [4-6]. Bacterial AGE remains relatively rare in developed nations, representing less than 10% of AGE in hospitalized children[7]. Low-middle income countries (LMIC) have higher prevalence of non-viral AGE, with the most common causes consisting of Shigella, rotavirus, adenovirus 40/41, ST-ETEC, cryptosporidium, and campylobacter [8].

PCR: However, in cases where pathogen testing is warranted by guidelines, advances in diagnostic technology have demonstrated a impact to both individual and systematic management. Traditional tests such as stool cultures or antigen testing are time consuming and in adult patients have been shown to generate increased costs and testing[23]. Novel polymerase chain reaction (PCR) based testing is both rapid (can return in <1 hr) and offers an improvement in sensitivity over traditional culture or immunoassay, with some studies demonstrating almost a 10-fold increase in
pathogen identification over culture[23]. In a prospective study looking at children hospitalized with Traveler’s diarrhea, found that by using PCR compared to stools cultures, enteropathogenic bacteria was found more often [26]. By identifying pathogenic bacteria earlier on, can begin treatment if warranted, in a timely manner [26]. However, this higher sensitivity also raises a concern in use of the PCR panel, in that it may be overly sensitive and identify organisms or remnant genetic material that are not contributing to current pathology. This concern is partially alleviated via data interrogating the effect of the PCR panel on system level practice.
Title: The Cutting Edge of Gastroenteritis: Advances in Understanding of Enteric Infection

Authors: Dalia Arostegui MD, Thomas Wallach MD

1SUNY Downstate Dept of Pediatrics, Division of Pediatric Gastroenterology.

Corresponding Author: Thomas Wallach MD
450 Clarkson Ave, MSC 49. Brooklyn, NY, 11203
Fax: (718)270-1985
Phone: (909)374-4350
Email: Thomas.wallach@downstate.edu

Sources of Support: No relevant grants or industry support.

Conflicts of Interest and Sources of Funding: No conflict of interest to report. No external funding.

Word Count: 3096

Figures: 1

Tables: 1
Author Contributions: TW ideated and submitted proposal for review article. DA and TW identified and reviewed source literature. DA wrote review, TW edited and provided guidance and support for interpretation.

Keywords: Acute Gastroenteritis, Enterotype, Microbiome
Abstract: In recent years, multiple advances have been made in the care, diagnosis, and mechanistic understanding of acute gastroenteritis (AGE). In this review we discuss the current state of the art of diagnosis and management, as well as how changes in practice can improve care and decrease costs. We will discuss current research demonstrating the effect of AGE on the microbiome and how that may be linked to secondary effects or long-term changes. We will explore the use of novel technologies to further our capacity to understand how gastrointestinal infections occur and promulgate. Finally, will discuss advances in our understanding of how gastrointestinal infections capacitate other changes such as post-viral motility or other post viral intestinal dysfunction.

Introduction

Acute gastroenteritis (AGE) is a global health problem with an incidence of over 2 billion cases per year. AGE remains the 3rd leading cause of pediatric mortality globally, with over 2 million pediatric deaths a year. In developed countries, AGE, is considered a mild disease, however it remains a major health burden with high numbers of hospitalizations and a significant financial cost. Retrospective analysis has noted that the United States spent $3.88 billion on direct care of AGE from 2001-2006. In this review we discuss state of the art in management, pathophysiology, and sequelae of AGE, including alterations to microbiota, use of innovative technologies to further our understanding on how gastrointestinal infections occur, how post-infectious dysfunction may occur, and potential ways to diminish hospitalizations and costs.

Acute gastroenteritis (AGE) is typically defined as loose or liquid stools and/or an increase in the frequency of evacuations, with 2 more stools than is normal for the patient. An acute diarrheal episode typically lasts less than 7 days and no more than 14 days. Many studies suggest that the most common cause of AGE globally remains Rotavirus despite comprehensive vaccination efforts, but Norovirus is rising and may have overtaken Rotavirus in the developed world. Bacterial AGE remains relatively rare in developed nations, representing less than 10% of AGE in hospitalized children. Low-middle income countries (LMIC) have higher prevalence of non-viral AGE, with the most common causes consisting of Shigella, rotavirus, adenovirus 40/41, ST-ETEC, cryptosporidium, and campylobacter.

Current Guidelines for and Advances in the Management of AGE

While there are no active US guidelines for the management in pediatrics, both the World Health Organization and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) maintain active evidence based guidelines. Acute gastroenteritis generally does not require a diagnostic work up. Differentiating between bacterial or viral pathogens can be difficult based on clinical findings, and typically irrelevant as antibiotic therapy of bacterial AGE is rarely helpful barring specific severe presentations (high-grade fever (>40 degrees Celsius), overt blood in stool, CNS findings, or previous health condition with associated immunodeficiency (such a oncologic or inflammatory processes)).
ESPGHAN recommendations for AGE are rarely followed in the United States, despite data demonstrating substantial (45%) decrease in hospitalizations for adult patients managed systematically. ESPGHAN guidelines focus first on rehydration with an oral replacement solution (ORS) containing glucose and sodium, using the sodium/glucose co-transporter to facilitate water absorption. A reduced osmolarity solution has been found to be more effective than full strength ORS therapy demonstrating reduced stool output, reduced vomiting, and reduced need for intravenous (IV) therapy. ORS has also been found to be equally efficacious as IV rehydration. Dietary changes such as the BRAT diet are unnecessary and may be counterproductive, as are concerns about lactose. A Cochrane analysis also noted that rapid return to normal diet should occur after 4-6 hours of oral rehydration, with benefit to duration of diarrheal symptoms.

In addition to ORS, the use of Zinc in children in developing countries may have benefit in treatment of AGE; however, this beneficial effect is linked to the presence of zinc deficiency, and in regions where zinc deficiency is rare, no benefit from the use of zinc is expected. There is some data suggesting possible benefit of certain probiotic strains, with trials demonstrating efficacy for use of Saccharomyces bouliardii, Lactobacillus rhamnosus GG, and L reuteri DSM 12246, with findings supporting improvement in days of diarrhea.

ORS and return to regular diet can be challenging with nausea and vomiting as primary symptoms. Guidelines support use of Ondansetron, a 5HT3 serotonin antagonist, to improve oral tolerance. Ondansetron, a highly safe and efficacious therapy, has been increasing in usage in the United States but classically is used at relatively low rates, possibly secondary to reports of long QT induced by 32-64mg IV doses used in chemotherapy patients. This effect has been interrogated multiple times in pediatrics, demonstrating either a very low or no effect of QT prolongation at typical dosages of 2-8 mg dosage in otherwise healthy children. Other providers have cited concerns that Ondansetron may mask more serious conditions, which recent studies have demonstrated does not occur. A recent meta-analysis by Tomask, Ziolkowska et al demonstrated both efficacy in AGE as well as diminishing rates of hospitalization and need for IV therapy. This review also concluded that there is no need for routine ECG and electrolyte screening in patients without known risk factors for arrhythmias.

**Established and Novel Therapeutic Agents for Diarrhea**

Classically, US providers have mainly provided supportive care, with relatively rare use of medication for symptomatic management. However, a variety of medications exist with many different mechanisms (Figure 1), which can improve symptoms and recovery time, although some are not available in the United States. Loperamide, an opioid receptor agonist which slows intestinal motility, is not recommended in pediatrics due to side effects and concern for intestinal perforation. Racecadotril, an alternative, safe, and more tolerable anti-diarrheal therapy is recommended in ESPGHAN guidelines, but is not available in the US. Racecadotril inhibits endorphin-metabolizing enzyme neutral endopeptidase (NEP, or Enkephalinase), decreasing luminal chloride secretion.
Racodotril inhibits endorphin-metabolizing enzyme neutral endopeptidase (NEP, or Enkephalinase), decreasing luminal chloride secretion\(^2\). This mechanism avoids loperamide associated cramping effects, risk of perforation, and retention of infectious agent \(^2\). Diosmectite has also shown efficacy in decreasing secretions\(^2\). Diosmectite has also shown efficacy in decreasing secretions\(^2\).

Multiple concepts are currently under investigation or show promise for treatment of AGE, although as of now AGE specific clinical data is limited and pediatric data essentially nonexistent. Crofelemer, a CFTR and CaCC channel binding agent, is the sole novel FDA approved agent \(^3\), currently approved for use with anti-retroviral therapy in HIV/AIDS and under study for chemotherapy induced diarrhea. This drug has likely efficacy in AGE, targeting the common viral/bacterial diarrheal mechanism of upregulation of cAMP and cGMP driving chloride channel activation and chloride secretion \(^3\). Multiple other agents with potential efficacy have been identified in basic science contexts, although clinical data remains sparse (Table 12). NHE3 agonists have been shown to block secretion in cholera toxin mediated murine models \(^3\). Clotrimazole has been shown to block cAMP mediated chloride channels and Ca\(^+\) mediated K\(^+\) channels, demonstrating decreased secretion in murine models \(^3\). Multiple other anti-secretory agents have been identified in vitro or in animal studies, with further investigation pending.

Herbal remedies have also demonstrated some possible utility, with the most promising agent being Shikonin, a compound found in Lithospermum erythrorhizon and used in traditional Chinese medicine. Shikonin has been found to be inhibitor of TMEM16A calcium gated chloride channel activity, and decreases both volume of diarrhea and motility in murine rotavirus models \(^3\).

**Impact of Novel Diagnostics on Management and Burden of AGE**

Diagnostic workup is typically not indicated in AGE. Traditional tests such as stool cultures or antigen testing are time consuming and in adult patients have been shown to generate increased costs and testing\(^3\). However, advances in polymerase chain reaction (PCR) based testing have provided a rapid (can return in <1 hr) and sensitive assay, with some studies demonstrating almost a 10-fold increase in pathogen identification over culture\(^3\). When used appropriately, PCR testing has substantial capacity to improve antibiotic stewardship, with work in adult patients demonstrating that positive identification of viral strains decreased use of empiric antibiotics, which are widely prevalent in adult primary care \(^3\). A prospective study of hospitalized children, compared PCR testing and stool culture, noting increased sensitivity of PCR to pathogenic bacteria \(^3\). Earlier identification helps identify useful therapy or guide against antimicrobial use, ensuring timely and accurate management in higher acuity cases \(^3\). However, this higher sensitivity also raises a concern in use of the PCR panel, in that it may be overly sensitive and identify organisms or remnant genetic material that are not contributing to current pathology.

While overdiagnosis remains a concern which should limit its use, some evidence in hospitalized adult patients also suggests a role for its use in significantly morbid AGE. Recent work in adult patients has demonstrated that using a GI PCR pathogen panel decreased duration of hospitalization by 0.5 days \(^3\), although there was not a significant benefit in pediatrics. In aggregate, health care costs diminished by $293.61/patient in the
group testing with GI PCR \(^{35}\). Stool PCR testing also allows for appropriate use and faster removal of patient isolation precautions and reduction of costs associated with isolation \(^{39}\).

### AGE, Genomic Science, and the Microbiome

Advances in bioscience have revolutionized our understanding of the impact of AGE on intestinal microbial populations and the connection with post-infectious syndromes. A study conducted by Nelson et al used 16s rRNA pyrosequencing to assess microbial diversity in patients with Norovirus infections. The study demonstrated substantial loss of microbial diversity with increased amount of Proteobacteria \(^{40}\). Work by Chen et al recapitulated this finding in pediatric patients, and further demonstrated that post-infectious decreased microbial diversity was not specific to Norovirus \(^{41}\).

Integrating metabolomics, 16s, and computational biology methodology have allowed for more clear characterization of not only specific species, but how they interact to create effects and may contribute to post-infectious syndromes. These constellations of bacterial species have been termed Enterotypes \(^{42}\). Rodriguez et al examined the fecal microbiome in 475 patients with gastroenteritis using 16S rRNA sequencing. The study combined a sequencing approach with computational biology focused on examining associations between bacterial taxa prevalence. Bacteroides and Faecalibacterium predominant enterotypes occurred in healthy controls, and an aberrant enterotype Escherichia Shigella dominant type which occurs after gastrointestinal infection \(^{43}\). Using hierarchical cluster analysis the presence of Escherichia-Shigella was associated with changes in stool color and consistency, specifically colors that were not brown and stools being more liquid \(^{43}\). Importantly, Rodriguez et al found that the presence of Escherichia-Shigella enterotype was not related to specific causative agents of infection, but rather a possible consequence of any intestinal infection. This enterotype was absent in healthy subjects. It was also notable that patients with viral infections showed predominance of Bacteroides and in cases with bacterial infections there was Fusobacterium and Enterobacter predominance \(^{43}\).

Predictive metagenomics demonstrated that Escherichia-Shigella enterotype has a downstream cascade of inflammatory changes to the gut. Using computational biology methods known as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), and nearest-sequenced-taxon index (NSTI) investigators found that downstream pathogenic pathways exhibited markers of bacterial invasion of epithelial cells, alteration of drug metabolism-cytochrome p450, lipopolysaccharide biosynthesis proteins, the RIG-I-like receptor signaling pathway, and glycan biosynthesis and metabolism \(^{43}\). This has tremendous possible significance, implying a pathogenic microbial state occurring after AGE which may drive many post-infectious syndromes or inflammatory pathways. This insight into the post-infectious consequences of AGE may help identify future interventions, improving outcomes substantially with therapeutic agents such as pre- or probiotics \(^{44}\).

Single cell RNA Sequencing (scRNA seq) has been utilized to characterize tissues vulnerable to specific infections. This technique allows for a high-resolution understanding of cell subset behavior in specific tissue types and creates usable databases for downstream study. In a topical finding, a study by Zhang, Kang et al. used 5 datasets of lung, esophagus, stomach, ileum, and colon to identify potential route of entry of a pathogen with single-cell
transcription. The cell types in each database were identified by specific markers, namely ACE2 and TMPRSS2. Specifically, it was found that SARS-CoV-2 enters a cell via ACE2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), which are found in alveolar type 2 cells in lung tissue, upper epithelial gland cells from esophagus, and enterocytes in the ileum and colon. These studies give us insight into how a pathogen causes disruption and entry, providing information on possible therapeutic and prophylactic interventions as a model for further study of emergent pathogens.

AGE and Neurogastroenterology

The Enteric Nervous System (ENS) and Enteric associated neurons (EANs) have a role in maintenance of intestinal homeostasis and barrier function and intestinal motility. Inflammation in the intestine can impact these neurons, contributing to post-infectious symptoms such as pain or abnormal motility. Balemans et al showed that patients with post infectious irritable bowel syndrome have long term changes to the ENS, in which enteric neurons become hyperresponsive resulting in increased secretion into the intestinal lumen and symptoms of diarrhea. Investigators used capsaicin to trigger both human and murine neurons, and a fluorescent calcium indicator to look at changing neuronal activity, demonstrating substantially increased sensitivity in the post-AGE group. This change appears to be mediated by transient receptor potential vanilloid 1 (TRPV1) signaling as demonstrated by increased TPRV1 signaling transcripts in exposed neurons and inhibition of the effect when co-exposed with TRPV1 inhibitors. This may explain the mechanism by which IBS-D follows intestinal insult such as acute gastroenteritis, and provides possible future therapeutic targets.

The immune system is involved in regulation of the enteric nervous system, and can have both positive and deleterious effects in AGE. Muscularis Macrophages (MM) are permanent residents of the gut, interacting with the enteric nervous system (ENS) via secretion of bone morphogenetic protein 2 (BMP2), which regulates ENS signaling driving smooth muscle contraction and peristalsis. These immune cells are neuroprotective, operating as an analogue of glial cells in the brain and providing protective and trophic stimulus to the ENS. The ENS secretes CSF1, a factor driving macrophage maturation, supporting the presence of MMs. Both of these processes are also regulated by interactions with the microbiome. Murine work has demonstrated loss of intrinsic Enteric Associated Neurons (iEANs) during intestinal infection. MMs respond to infection by up-regulating neuroprotective transcriptional programs, a process that is increased by β adrenergic signaling. Matheis et al were able to expand on this, demonstrating this effect as a response to infection in a murine model. Their work also showed a recuperative effect of microbial alteration in the aftermath, as well as the beta-agonist driven neuroprotective effect of muscularis macrophages.

Enteroids as a Novel Model System for AGE

LGR5+ intestinal stem cells can be harvested via biopsy or from murine intestines, and in proper culture conditions will grow into enteroids, miniature guts recapitulating the epithelial compartment and structure of the intestine. Enteroids models allow for close examination of the pathophysiology of AGE, ranging from mechanism of infection to impact
of specific toxins. Using an enteroid platform, Engevik et al were able to evaluate the effects of C. difficile toxin A and B to bind to host receptors, validating and clarifying previous findings. In this study they injected human intestinal organoids with C. difficile and noted that exposed enteroids had decreased levels of NHE3 (Na+/H+ exchanger 3) mRNA compared with healthy subjects. This decrease in NHE3 results in an altered intestinal microbiota in the intestine of mice. This finding confirms and mechanistically supports previous studies demonstrating Toxin B’s capacity to reduce expression of the sodium/hydrogen exchanger, resulting in increased diarrhea, and provides a possible in vitro format for testing therapeutic interventions targeting this process. Human intestinal enteroids (HIEs) were used to evaluate the effect of C. difficile toxins on the cytoskeleton by using fluorescent labeled actin. Mucin, specifically MUC2, was found to protect small intestinal epithelium by inhibiting C. difficile toxin interaction with the cell surface.

Zhang et al studied the effects of Salmonella on enteroids, demonstrating a decrease in epithelial tight junction proteins after exposure. Using a murine enteroid model they characterized Salmonella induced disruption in the epithelial tight junctions, likely mediated by significant increase in pro-inflammatory NF-κB and its downstream inflammatory products IL-2, IL-4, IL-6, TNF-alpha, and IFN-γ. The resulting inflammatory state results in impaired tight junctional function and increased paracellular influx and easier bacterial invasion. Foulke-Abel et al used human enteroids, PCR, and classic immunoblot and immunofluorescence techniques to accurately assess function and location of specific ion transporters. They found that Na+/HCO3- cotransporter 1 is needed for HCO3- secretion in the duodenum, identifying a possible target for treatment of secretory diarrheas or infectious diarrheas triggering secretion via upregulation of cAMP or cGMP.

Enteroids are useful for in-depth basic science investigation of the pathophysiology of AGE, and as an ethically compliant testing platform for prophylactic or therapeutic interventions. In an immediately practical finding, Constantini et al, tested the effects of chlorine and alcohols in inactivation effects on Norovirus, and found that chlorine was more efficacious as an inactivation treatment. The ability to use a higher fidelity in vitro construct of human cells has revolutionized cell level investigation of pathology, and will likely generate substantial further yields in our understanding and treatment of AGE and its sequelae, in particular as multi-compartment enteroid technology improves and allows for inclusion of neuronal and immune tissue.

Summary

Acute Gastroenteritis (AGE) is an entity with a substantial morbidity and economic burden in the United States, as well as the capacity to create long term changes in the function of the intestine. While the disease is not highly mortal in the US, it remains highly morbid, and typically under or incorrectly treated. Focusing on immediate oral rehydration with anti-emetic support, rapid return to regular diet, will allow for improvements to patient care, more rapid recovery, and decreased healthcare spending on these conditions. It is possible that the advent of PCR testing may also lead to improvements in these domains. Recent basic science and translational findings have drawn a clearer picture of how these infections can create chronic and previously difficult to treat illness, such as IBS and motility abnormalities. Studies exploring the microbiome after an episode of AGE have given us
insight into how an infectious agent can change the microbiome, creating less diversity or increasing the abundance of particular bacterial taxa. Metagenomic studies have classified and grouped bacterial taxa into clusters with meaningful functional changes and downstream symptoms. Innovative use of intestinal organoids/enteroids allows models of intestinal epithelial function to help understand the pathophysiology of infections in the gastrointestinal tract, allowing for a rapid pace of discovery and a testing platform for therapeutics. These findings are additionally relevant as we advance our understanding of neurogastroenterology, in particular changes to the ENS after episodes of AGE. Recent work identifying mechanisms underlying ENS functional changes after infection gives us insight into post-infectious pathology and not only how AGE can create secondary illness, but how we can possibly treat it in the future. This review on AGE provides a summary of the basic science that allow us to understand how disease process take place and how this may affect the microbiome after an infection.


22. Gupta VK, Paul S, Dutta C. *Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity*. Frontiers in microbiology 2017;8:1162; 1162-1162.


Fischbach, W., Andersen, V., Eberlin, M., et al., A Comprehensive Comparison of the Efficacy and Tolerability of Racemodotril with Other Treatments of Acute Diarrhea in Adults. Front Med (Lausanne), 2016; 3: p. 44.


Intestinal Enteroids as Model to Evaluate Virus Inactivation.

Table 1: AGE Therapeutics. The table reviews relevant therapeutic agents for acute gastroenteritis, noting mechanism, dose (and origin of dose), as well as availability in the United States.

Figure 1. Antidiarrheal Drug Mechanisms. Diosmectite acts by inhibiting pathogen attachment to the mucus and epithelial layer. NH3 agonists prevent up regulation of cAMP dependent Na+ channels. Clotrimazole blocks cAMP and Ca 2+ mediated K+ channels. Crofelemer inhibits CFTR and CaCC channels, which thereby prevent Cl- secretion. Shikonin blocks chloride channel activity as well as basolateral K+ channel activity. Racecadotril inhibits Enkaphalinase, preventing degradation of Enkaphalin which prevents chloride secretion.

Figure 2. Intestinal Epithelial Organoids. (A) Depicts a model of organoids with villus and crypt areas. (B) Confocal immunofluorescence image of a murine intestinal epithelial organoid with actin labeled blue and e-cadherin labeled in red.
Abstract: In recent years, multiple advances have been made in the care, diagnosis, and mechanistic understanding of acute gastroenteritis (AGE). In this review we discuss the current state of the art of diagnosis and management, as well as how changes in practice can improve care and decrease costs. We will discuss current research demonstrating the effect of AGE on the microbiome and how that may be linked to secondary effects or long-term changes. We will explore the use of novel technologies to further our capacity to understand how gastrointestinal infections occur and promulgate. Finally, will discuss advances in our understanding of how gastrointestinal infections capacitate other changes such as post-viral motility or other post viral intestinal dysfunction.

Introduction

Acute gastroenteritis (AGE) is a global health problem with an incidence of over 2 billion cases per year. AGE remains the 3rd leading cause of pediatric mortality globally, with over 2 million pediatric deaths a year. In developed countries, AGE, is considered a mild disease, however it remains a major health burden with high numbers of hospitalizations and a significant financial cost. Retrospective analysis has noted that the United States spent $3.88 billion on direct care of AGE from 2001-2006. In this review we discuss state of the art in management, pathophysiology, and sequelae of AGE, including alterations to microbiota, use of innovative technologies to further our understanding on how gastrointestinal infections occur, how post-infectious dysfunction may occur, and potential ways to diminish hospitalizations and costs.

Acute gastroenteritis (AGE) is typically defined as loose or liquid stools and/or an increase in the frequency of evacuations, with 2 more stools than is normal for the patient. An acute diarrheal episode typically lasts less than 7 days and no more than 14 days. Many studies suggest that the most common cause of AGE globally remains Rotavirus despite comprehensive vaccination efforts, but Norovirus is rising and may have overtaken Rotavirus in the developed world. Bacterial AGE remains relatively rare in developed nations, representing less than 10% of AGE in hospitalized children. Low-middle income countries (LMIC) have higher prevalence of non-viral AGE, with the most common causes consisting of Shigella, rotavirus, adenovirus 40/41, ST-ETEC, cryptosporidium, and campylobacter.

Current Guidelines for and Advances in the Management of AGE

While there are no active US guidelines for the management in pediatrics, both the World Health Organization and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) maintain active evidence based guidelines. Acute gastroenteritis generally does not require a diagnostic work up. Differentiating between bacterial or viral pathogens can be difficult based on clinical findings, and typically irrelevant as antibiotic therapy of bacterial AGE is rarely helpful barring specific severe presentations (high-grade fever (>40 degrees Celsius), overt blood in stool, CNS findings, or previous health condition with associated immunodeficiency (such a oncologic or inflammatory processes).

ESPGHAN recommendations for AGE are rarely followed in the United States, despite data demonstrating substantial (45%) decrease in hospitalizations for adult patients managed
systematically. ESPGHAN guidelines focus first on rehydration with an oral replacement solution (ORS) containing glucose and sodium, using the sodium/glucose co-transporter to facilitate water absorption. A reduced osmolarity solution has been found to be more effective than full strength ORS therapy demonstrating reduced stool output, reduced vomiting, and reduced need for intravenous (IV) therapy. ORS has also been found to be equally efficacious as IV rehydration. Dietary changes such as the BRAT diet are unnecessary and may be counterproductive, as are concerns about lactose. A Cochrane analysis also noted that rapid return to normal diet should occur after 4-6 hours of oral rehydration, with benefit to duration of diarrheal symptoms. In addition to ORS, the use of Zinc in children in developing countries may have benefit in treatment of AGE; however, this beneficial effect is linked the presence of zinc deficiency, and in regions where zinc deficiency is rare, no benefit from the use of zinc is expected. There is some data suggesting possible benefit of certain probiotic strains, with trials demonstrating efficacy for use of Saccharomyces boulardii, Lactobacillus rhamnosus GG, and L reuteri DSM 12246, with findings supporting improvement in days of diarrhea.

ORS and return to regular diet can be challenging with nausea and vomiting as primary symptoms. Guidelines support use of Ondansetron, a 5HT3 serotonin antagonist, to improve oral tolerance. Ondansetron, a highly safe and efficacious therapy, has been increasing in usage in the United States but classically is used at relatively low rates, possibly secondary to reports of long QT induced by 32-64mg IV doses used in chemotherapy patients. This effect has been interrogated multiple times in pediatrics, demonstrating either a very low or no effect of QT prolongation at typical dosages of 2-8 mg dosage in otherwise healthy children. Other providers have cited concerns that Ondansetron may mask more serious conditions, which recent studies have demonstrated does not occur. A recent meta-analysis by Tomasik, Ziółkowska et al. demonstrated both efficacy in AGE as well as diminishing rates of hospitalization and need for IV therapy. This review also concluded that there is no need for routine ECG and electrolyte screening in patients without known risk factors for arrhythmias.

Established and Novel Therapeutic Agents for Diarrhea

Classically, US providers have mainly provided supportive care, with relatively rare use of medication for symptomatic management. However, a variety of medications exist with many different mechanisms, which can improve symptoms and recovery time, although some are not available in the United States. Loperamide, an opioid receptor agonist which slows intestinal motility, is not recommended in pediatrics due to side effects and concern for intestinal perforation. Racecadotril, an alternative, safe, and more tolerable anti-diarrheal therapy is recommended in ESPGHAN guidelines, but is not available in the US. Racecadotril inhibits endorphin-metabolizing enzyme neutral endopeptidase (NEP, or Enkephalinase), decreasing luminal chloride secretion. This mechanism avoids loperamide associated cramping effects, risk of perforation, and retention of infectious agent. Diosmectite has also shown efficacy in decreasing secretions.

Multiple concepts are currently under investigation or show promise for treatment of AGE, although as of now AGE specific clinical data is limited and pediatric data essentially nonexistent. Crofelemer, a CFTR and CaCC channel binding agent, is the sole novel FDA
approved agent, currently approved for use with anti-retroviral therapy in HIV/AIDS and under study for chemotherapy induced diarrhea. This drug has likely efficacy in AGE, targeting the common viral/bacterial diarrheal mechanism of upregulation of cAMP and cGMP driving chloride channel activation and chloride secretion. Multiple other agents with potential efficacy have been identified in basic science contexts, although clinical data remains sparse (Table 1). NHE3 agonists have been shown to block secretion in cholera toxin mediated murine models. Clotrimazole has been shown to block cAMP mediated chloride channels and Ca+ mediated K+ channels, demonstrating decreased secretion in murine models. Multiple other anti-secretory agents have been identified in vitro or in animal studies, with further investigation pending.

Herbal remedies have also demonstrated some possible utility, with the most promising agent being Shikonin, a compound found in Lithospermum erythrorhizon and used in traditional Chinese medicine. Shikonin has been found to be inhibitor of TMEM16A calcium gated chloride channel activity, and decreases both volume of diarrhea and motility in murine rotavirus models.

Impact of Novel Diagnostics on Management and Burden of AGE

Diagnostic workup is typically not indicated in AGE. Traditional tests such as stool cultures or antigen testing are time consuming and in adult patients have been shown to generate increased costs and testing. However, advances in polymerase chain reaction (PCR) based testing have provided a rapid (can return in <1 hr) and sensitive assay, with some studies demonstrating almost a 10-fold increase in pathogen identification over culture. When used appropriately, PCR testing has substantial capacity to improve antibiotic stewardship, with work in adult patients demonstrating that positive identification of viral strains decreased use of empiric antibiotics, which are widely prevalent in adult primary care.

A prospective study of hospitalized children, compared PCR testing and stool culture, noting increased sensitivity of PCR to pathogenic bacteria. Earlier identification helps identify useful therapy or guide against antimicrobial use, ensuring timely and accurate management in higher acuity cases. However, this higher sensitivity also raises a concern in use of the PCR panel, in that it may be overly sensitive and identify organisms or remnant genetic material that are not contributing to current pathology.

While overdiagnosis remains a concern which should limit its use, some evidence in hospitalized adult patients also suggests a role for its use in significantly morbid AGE. Recent work in adult patients has demonstrated that using a GI PCR pathogen panel decreased duration of hospitalization by 0.5 days, although there was not a significant benefit in pediatrics. In aggregate, health care costs diminished by $293.61/patient in the group testing with GI PCR. Stool PCR testing also allows for appropriate use and faster removal of patient isolation precautions and reduction of costs associated with isolation.

AGE, Genomic Science, and the Microbiome

Advances in bioscience have revolutionized our understanding of the impact of AGE on intestinal microbial populations and the connection with post-infectious syndromes. A study conducted by Nelson et al used 16s rRNA pyrosequencing to assess microbial diversity in patients with Norovirus infections. The study demonstrated substantial loss of
microbial diversity with increased amount of Proteobacteria. Work by Chen et al recapitulated this finding in pediatric patients, and further demonstrated that post-infectious decreased microbial diversity was not specific to Norovirus.

Integrating metabolomics, 16s, and computational biology methodology have allowed for more clear characterization of not only specific species, but how they interact to create effects and may contribute to post-infectious syndromes. These constellations of bacterial species have been termed Enterotypes. Rodriguez et al examined the fecal microbiome in 475 patients with gastroenteritis using 16S rRNA sequencing. The study combined a sequencing approach with computational biology focused on examining associations between bacterial taxa prevalence. Bacteroides and Faecalibacterium dominant enterotypes occurred in healthy controls, and an aberrant enterotype Escherichia Shigella dominant type which occurs after gastrointestinal infection. Using hierarchical cluster analysis the presence of Escherichia-Shigella was associated with changes in stool color and consistency, specifically colors that were not brown and stools being more liquid. Importantly, Rodriguez et al found that the presence of Escherichia-Shigella enterotype was not related to specific causative agents of infection, but rather a possible consequence of any intestinal infection. This enterotype was absent in healthy subjects. It was also notable that patients with viral infections showed predominance of Bacteroides and in cases with bacterial infections there was Fusobacterium and Enterobacter predominance.

Predictive metagenomics demonstrated that Escherichia-Shigella enterotype has a downstream cascade of inflammatory changes to the gut. Using computational biology methods known as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), and nearest-sequenced-taxon index (NSTI) investigators found that downstream pathogenic pathways exhibited markers of bacterial invasion of epithelial cells, alteration of drug metabolism-cytochrome p450, lipopolysaccharide biosynthesis proteins, the RIG-I-like receptor signaling pathway, and glycan biosynthesis and metabolism. This has tremendous possible significance, implying a pathogenic microbial state occurring after AGE which may drive many post-infectious syndromes or inflammatory pathways. This insight into the post-infectious consequences of AGE may help identify future interventions, improving outcomes substantially with therapeutic agents such as pre- or probiotics.

Single cell RNA Sequencing (scRNA seq) has been utilized to characterize tissues vulnerable to specific infections. This technique allows for a high-resolution understanding of cell subset behavior in specific tissue types and creates usable databases for downstream study. In a topical finding, a study by Zhang, Kang et al. used 5 datasets of lung, esophagus, stomach, ileum, and colon to identify potential route of entry of a pathogen with single-cell transcription. The cell types in each database were identified by specific markers, namely ACE2 and TMPRSS2. Specifically, it was found that SAR-CoV-2 enters a cell via ACE II (ACE2) and transmembrane serine protease 2 (TMPRSS2), which are found in alveolar type 2 cells in lung tissue, upper epithelial gland cells from esophagus, and enterocytes in the ileum and colon. These studies give us insight into how a pathogen causes disruption and entry, providing information on possible therapeutic and prophylactic interventions as a model for further study of emergent pathogens.
AGE and Neurogastroenterology

The Enteric Nervous System (ENS) and Enteric associated neurons (EANs) have a role in maintenance of intestinal homeostasis and barrier function and intestinal motility. Inflammation in the intestine can impact these neurons, contributing to post-infectious symptoms such as pain or abnormal motility. Balemans et al showed that patients with post infectious irritable bowel syndrome have long term changes to the ENS, in which enteric neurons become hyperresponsive resulting in increased secretion into the intestinal lumen and symptoms of diarrhea. Investigators used capsaicin to trigger both human and murine neurons, and a fluorescent calcium indicator to look at changing neuronal activity, demonstrating substantially increased sensitivity in the post-AGE group. This change appears to be mediated by transient receptor potential vanilloid 1 (TRPV1) signaling as demonstrated by increased TPRV1 signaling transcripts in exposed neurons and inhibition of the effect when co-exposed with TRPV1 inhibitors. This may explain the mechanism by which IBS-D follows intestinal insult such as acute gastroenteritis, and provides possible future therapeutic targets.

The immune system is involved in regulation of the enteric nervous system, and can have both positive and deleterious effects in AGE. Muscularis Macrophages (MM) are permanent residents of the gut, interacting with the enteric nervous system (ENS) via secretion of bone morphogenetic protein 2 (BMP2), which regulates ENS signaling driving smooth muscle contraction and peristalsis. These immune cells are neuroprotective, operating as an analogue of glial cells in the brain and providing protective and trophic stimulus to the ENS. The ENS secretes CSF1, a factor driving macrophage maturation, supporting the presence of MMs. Both of these processes are also regulated by interactions with the microbiome. Murine work has demonstrated loss of intrinsic Enteric Associated Neurons (iEANs) during intestinal infection. MMs respond to infection by up-regulating neuroprotective transcriptional programs, a process that is increased by β adrenergic signaling. Matheis et al were able to expand on this, demonstrating this effect as a response to infection in a murine model. Their work also showed a recuperative effect of microbial alteration in the aftermath, as well as the beta-agonist driven neuroprotective effect of muscularis macrophages.

Enteroids as a Novel Model System for AGE

LGR5+ intestinal stem cells can be harvested via biopsy or from murine intestines, and in proper culture conditions will grow into enteroids, miniature guts recapitulating the epithelial compartment and structure of the intestine. Enteroids models allow for close examination of the pathophysiology of AGE, ranging from mechanism of infection to impact of specific toxins. Using an enteroid platform, Engevik et al were able to evaluate the effects of C Difficile toxin A and B to bind to host receptors, validating and clarifying previous findings. In this study they injected human intestinal organoids with C.difficile and noted that exposed enteroids had decreased levels of NHE3 (Na+/H+ exchanger 3 ) mRNA compared with healthy subjects. This decrease in NHE3 results in an altered intestinal microbiota in the intestine of mice. This finding confirms and mechanistically supports previous studies demonstrating Toxin B’s capacity to reduce expression of the sodium/hydrogen exchanger, resulting in increased diarrhea, and provides a possible in vitro format for testing therapeutic interventions targeting this process. Human intestinal
Enteroids (HIEs) were used to evaluate the effect of C. difficile toxins on the cytoskeleton by using fluorescent labeled actin. Mucin, specifically MUC2, was found to protect small intestinal epithelium by inhibiting C. difficile toxin interaction with the cell surface.

Zhang et al studied the effects of Salmonella on enteroids, demonstrating a decrease in epithelial tight junction proteins after exposure. Using a murine enteroid model they characterized Salmonella induced disruption in the epithelial tight junctions, likely mediated by significant increase in pro-inflammatory NF-κB and its downstream inflammatory products IL-2, IL-4, IL-6, TNF-alpha, and IFN-γ. The resulting inflammatory state results in impaired tight junctional function and increased paracellular influx and easier bacterial invasion. Foulke-Abel et al used human enteroids, PCR, and classic immunoblot and immunofluorescence techniques to accurately assess function and location of specific ion transporters. They found that Na+/HCO3- cotransporter 1 is needed for HCO3- secretion in the duodenum, identifying a possible target for treatment of secretory diarrheas or infectious diarrheas triggering secretion via upregulation of cAMP or cGMP.

Enteroids are useful for in-depth basic science investigation of the pathophysiology of AGE, and as an ethically compliant testing platform for prophylactic or therapeutic interventions. In an immediately practical finding, Constantini et al, tested the effects of chlorine and alcohols in inactivation effects on Norovirus, and found that chlorine was more efficacious as an inactivation treatment. The ability to use a higher fidelity in vitro construct of human cells has revolutionized cell level investigation of pathology, and will likely generate substantial further yields in our understanding and treatment of AGE and its sequelae, in particular as multi-compartment enteroid technology improves and allows for inclusion of neuronal and immune tissue.

**Summary**

Acute Gastroenteritis (AGE) is an entity with a substantial morbidity and economic burden in the United States, as well as the capacity to create long term changes in the function of the intestine. While the disease is not highly mortal in the US, it remains highly morbid, and typically under or incorrectly treated. Focusing on immediate oral rehydration with antimetic support, rapid return to regular diet, will allow for improvements to patient care, more rapid recovery, and decreased healthcare spending on these conditions. It is possible that the advent of PCR testing may also lead to improvements in these domains. Recent basic science and translational findings have drawn a clearer picture of how these infections can create chronic and previously difficult to treat illness, such as IBS and motility abnormalities. Studies exploring the microbiome after an episode of AGE have given us insight into how an infectious agent can change the microbiome, creating less diversity or increasing the abundance of particular bacterial taxa. Metagenomic studies have classified and grouped bacterial taxa into clusters with meaningful functional changes and downstream symptoms. Innovative use of intestinal organoids/enteroids allows models of intestinal epithelial function to help understand the pathophysiology of infections in the gastrointestinal tract, allowing for a rapid pace of discovery and a testing platform for therapeutics. These findings are additionally relevant as we advance our understanding of neurogastroenterology, in particular changes to the ENS after episodes of AGE. Recent work identifying mechanisms underlying ENS functional changes after infection gives us insight.
into post-infectious pathology and not only how AGE can create secondary illness, but how we can possibly treat it in the future. This review on AGE provides a summary of the guidelines for management, how and when to look for a causative agent, and innovations in basic science that allow us to understand how disease process take place and how this may affect the microbiome after an infection.

32. Zachos NC, van Rossum DB, Murtazina R, et al. 4 A Peptide Mimicking the NHE3 C-Terminus Stimulates Basal and Blocks Ca2+ and cAMP Inhibition of NHE3 Activity, and Prevents Cholera Toxin-Induced Mouse Jejunal Fluid Accumulation: Potential Role as Drug Therapy for Diarrhea. Gastroenterology 2012;142:S-1.


Table 1: AGE Therapeutics. The table reviews relevant therapeutic agents for acute gastroenteritis, noting mechanism, dose (and origin of dose), as well as availability in the United States.

Figure 1. Antidiarrheal Drug Mechanisms. Diosmectite acts by inhibiting pathogen attachment to the mucus and epithelial layer. NH3 agonists prevent up regulation of cAMP dependent Na+ channels. Clotrimazole blocks cAMP and Ca 2+ mediated K+ channels. Crofelemer inhibits CFTR and CaCC channels, which thereby prevent Cl-secretion. Shikonin blocks chloride channel activity as well as basolateral K+ channel activity. Racecadotril inhibits Enkaphalinase, preventing degradation of Enkaphalin which prevents chloride secretion.
<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Mechanism of action/Drug effect</th>
<th>Dosage (PO)</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odansetron</td>
<td>5-HT3 receptor antagonist&lt;br&gt;Antiemetic agent</td>
<td>8-15kg: 2mgx1&lt;br&gt;15 and ≤30kg: 4mg x1&lt;br&gt; &gt;30kg: 8mg x1 (Harriet Lane)</td>
<td>Available in US</td>
</tr>
<tr>
<td>Racecadotril</td>
<td>Enkephalinase inhibitor&lt;br&gt;Anti-secretory&lt;br&gt;Antidiarrheal agent</td>
<td>1.5 mg/kg three TID for children&lt;br&gt;100 mg TID for adults (Guarino et al, 2013)</td>
<td>Not available in US</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Opioid-receptor agonist&lt;br&gt;Anti-motility agent&lt;br&gt;Antidiarrheal agent</td>
<td>Contraindicated in patients less than 2 years&lt;br&gt;9-11yr (&gt;27-43kg): 2mg PO TID</td>
<td>Available in US</td>
</tr>
<tr>
<td>Diosmectite</td>
<td>Natural aluminomagnesium silicate clay with absorbent properties&lt;br&gt;Antidiarrheal agent</td>
<td>3g in each sachet&lt;br&gt;1-12months old- 6g/day (2 sachets)&lt;br&gt;13-36 months and older- 12g/day (4 sachets) (Guarino et al 2009)</td>
<td>Available in US</td>
</tr>
<tr>
<td>Crofelemer</td>
<td>Binds to CFTR and CaCC inhibiting chloride secretion&lt;br&gt;Anti-secretory</td>
<td>125 mg PO BID (Gao et al 2017)</td>
<td>Available in US</td>
</tr>
<tr>
<td>NHE3 agonists</td>
<td>Anti-secretory</td>
<td>N/A</td>
<td>Experimental</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>Anti-secretory by inhibiting cAMP chloride channels and Ca 2+ mediated potassium channels</td>
<td>N/A</td>
<td>Experimental</td>
</tr>
<tr>
<td>Shikonin</td>
<td>Anti-secretory by inhibiting chloride channel activity</td>
<td>N/A</td>
<td>Experimental</td>
</tr>
</tbody>
</table>