RANDOX

EDUCATIONAL GUIDE

Gastroenteritis

BOSCH vivalytic

Introduction

Gastroenteritis, commonly referred to as stomach flu or stomach bug, is an inflammation of the gastrointestinal tract that presents with symptoms such as diarrhoea, vomiting, abdominal pain, and fever, which affects millions of individuals annually. It is often caused by viral or bacterial infections, and occasionally by parasitic organisms. Among the primary pathogens responsible for gastroenteritis cases, rotavirus and norovirus are two highly contagious viruses that can spread rapidly in various settings, including healthcare facilities, schools, and community gatherings. On the bacterial front, *Clostridium difficile* is a bacterium notorious for causing gastrointestinal complications, especially in individuals who have been on antibiotics.

Timely and accurate detection of these pathogens is crucial for effective patient management, infection control, and prevention of disease spread. This guide will delve into the specifics of each pathogen mentioned above, its modes of transmission, clinical manifestations, and the innovative diagnostic capabilities of the Vivalytic POCT system. The Vivalytic system's advanced technology offers healthcare professionals a rapid and reliable solution to identify these pathogens, aiding in quicker decision-making, improved patient outcomes, and enhanced public health measures.

Gastroenteritis

Gastroenteritis is characterised by the inflammation of the lining of the stomach and intestines. This can result in a wide range of symptoms including severe dehydration and death. The main symptoms of gastroenteritis include vomiting, diarrhoea, abdominal pain, and fever[1]. Acute diarrhoeal disease is generally self-limiting, and symptoms usually last for less than a week, normally improving after 1 to 3 days. Bacterial and protozoal causes of gastroenteritis can potentially mimic symptoms of viral gastroenteritis but often require a different treatment approach and may cause more severe symptoms. The viruses, bacteria, and parasites that cause gastroenteritis can spread from person to person. The transmission method is determined by the infecting pathogen but is most commonly the faecal-oral route, including contaminated food and water. Less common modes of transmission include fomites, vomitus, and airborne methods[1].

Although in healthy adults the disease is self-limiting, it can have significant effects on children and the elderly. These populations are at higher risk of developing severe, symptomatic disease, with the CDC reporting approximately 200,000 deaths of children each year due to gastroenteritis[1].

Rotavirus

Rotavirus infection is the leading cause of severe, dehydrating gastroenteritis in children under 5 years old[2]. In 2016, 258 million infections were recorded, accounting for 129,000 fatalities in children under 5[3]. More recently, in 2019, rotavirus was responsible for 19% of all diarrhoea-related deaths globally[3]. As part of the Reoviridae family, rotaviruses are segmented, double-stranded RNA (dsRNA) viruses. This virus is large and complex, made of 3 concentric protein layers that engulf an 11-segment double-stranded viral genome[2,4]. The genome encodes 6 non-structural proteins (NSP1-NSP6) and 6 viral proteins (VP1, VP2, VP3, VP4, VP6 & VP7) which, in the mature form, facilitate cell entry and various enzymatic functions. The NSPs play a role in genome replication and act as an inhibitor of the natural immune reaction[2].

There are 10 currently identified rotavirus species, denoted A-J. The most common are species A and these are further classified based on genotype[4]. VP4 and VP7 form spikes that protrude through the outer rotavirus capsid and induce a neutralising antibody response.

These VPs are also used to categorise rotaviruses. VP7 is a glycoprotein, often referred to as the 'G-type' protein, while VP4 is a protease-sensitive protein, known as the 'P-type' protein. There are 32 known G genotypes. The most common are G1, G2, G3, G4, G9 and G12. The nomenclature for P-type proteins is slightly more complex. The serotype is referred to by the number following the P, for example, P1. The genotype is denoted in brackets, for example, P[4]. The P genotype is primarily used for classification methods due to challenges in standardising VP4 serotype assays. These categories form the basis of the dual nomenclature associated with rotaviruses[4]. Among the known strains, six species account for over 90% of global rotavirus infections, namely G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8][2].

Structure & Features

Rotaviruses are not only elaborate by name and category, but these viruses display various feats of structural complexity. Firstly, the triple-layer capsid increases the stability of the virus, facilitating faecal-oral transmission and its treacherous journey to the small intestine. Once here, the virus infects nondividing, differentiated enterocytes which express factors which aid in infection or replication[4].

Rotaviruses have 60 spike proteins emanating from their outer capsid made of a complex that aids in initial viral attachment to host receptors. Once cleaved by trypsin, these spike proteins change their conformation, enabling them to attach to other sites on the glycoprotein surface and interact with a variety of co-receptors[4].

Rotavirus replication only occurs in the cytoplasm within viroplasms, found near the nucleus and endoplasmic reticulum (ER). These viroplasms are made of nascent viral proteins and can alter their size and shape to meet the requirements of replication[4]. Once replication is complete, the new double-layered particles containing dsRNA bud off from the viroplasms into the ER and become transiently enveloped[4].

To aid in their function and replication, rotaviruses exploit the calcium ion (Ca^{2+}) signalling system. Rotavirus infection can increase the concentration of intracellular Ca^{2+} 3-fold and the uptake of the calcium isotope $^{45}Ca^{2+}$ as much as 10-fold[4].

Ca^{2+} is used to maintain the integrity of the rotavirus' outer capsid layer and for activation of endogenous RNA polymerase; for protein binding and viroplasms formation; for morphogenesis[4].

$^{45}Ca^{2+}$ is an isotope used to study calcium metabolism and signalling cells. This isotope has a mass number of 45 and a positive charge of 2.

Pathophysiology

Whilst children under 5 are considered high risk, neonates are rarely infected due to the transplacental transfer of maternal antibodies. Concentrations of these antibodies decline in synchrony with the age of maximum susceptibility, between 3 months and 2 years old[4]. Rotavirus can infect healthy adults, but symptomatic infection is relatively uncommon, usually pertaining to a significantly high viral load or an uncommon strain. Rotavirus virulence is multigenic, the table below details the role of the genes involved.

Gene	Description
3	Encodes the capping enzyme, affecting levels of viral RNA replication
4 & 9	Encodes the outer capsid proteins, crucial for initiating an infection
5	Encodes NSP1 which functions as an interferon antagonist
10	Encodes MSP5 which regulates calcium homeostasis, and virus replication and functions as an enterotoxin.

Rotavirus infects the mature enterocytes and enteroendocrine cells of the small intestine through viral protein attachment to the host cell surface. The gastrointestinal symptoms associated with rotavirus gastroenteritis including osmotic or secretory diarrhoea are the result of the hijacking and activation of signalling pathways that induce diarrhoea and vomiting[2]. The full absorptive mechanisms which cause rotavirus-induced diarrhoea are not understood. However, it is thought to involve the loss of infected cells and the impairment of solute symporters. Oral rehydration therapy is normally a sufficient treatment strategy to counteract these symptoms[2].

As rotavirus infects enterocytes, it can cause damage to these important cells and, in some cases, cell death. In infants, this can lead to duodenal mucosa damage which results in atrophy and shortening of the villi, loss of microvilli, and other histopathological changes[2]. Damage or cell death in the intestinal epithelium is attributed to Non-Structural Protein 4 (NSP4) action and viral replication. The mechanisms involved in cell damage are thought to include virus-mediated apoptosis, NSP4-mediated mislocalisation of tight junction proteins and binding of extracellular proteins[2]. The enterotoxin, NSP4, is thought to be involved in many of the pathological mechanisms of rotavirus infection including its binding to the intestinal cells which causes an increase in calcium levels and a subsequent secretion of excess chloride ions and water transport, and ultimately swelling of the cells[2].

Finally, fever, sleepiness, depression and the reduced intake of food and water, are all associated with the increase of pro-inflammatory cytokines characteristic of rotavirus infection. It is known that IL-1β, Tumour Necrosis Factor and IL-6 are involved in these processes during rotavirus infection, but these exact mechanisms remain elusive[2].

Treatment and Vaccination

The treatment for rotavirus infection is focused on symptom management. Hydration and nutritional support are crucial, and are usually accompanied by supportive care for any pain or fever the patient may experience. The highly infectious nature of rotavirus means those infected should remain in isolation, particularly children or the elderly.

Proper hygiene, including regular handwashing, is essential to limit the spread of rotavirus. However, the most effective means to stop transmission of this virus is vaccination. There are two commercially available rotavirus vaccines: Rotarix (RV1), a monovalent live attenuated virus vaccine, and RotaTeq (RV5), a live attenuated pentavalent virus vaccine. While these vaccines have been shown to be effective, there are some caveats: maternal antibodies and host genetics can affect the immunogenicity and these vaccines should not be administered alongside the polio vaccine, as this has also been shown to reduce the immunogenicity RV[1] and RV5[2].

Monovalent vaccines are those which target a single strain or serotype. Therefore, a pentavalent vaccine is one which targets 5 strains or serotypes. Both have advantages. Monovalent vaccines are more specific and tend to require fewer doses. A multivalent vaccine provides broader protection against a virus and is likely to be more effective in regions where multiple strains of a virus are circulating[13].

Norovirus

Noroviruses are the major cause of gastroenteritis, accounting for 90% of viral gastroenteritis outbreaks and around 50% of cases globally. That's around 21 million cases per year in the US alone[1]. Furthermore, in the UK, a recent report showed that from mid-April to mid-May 2024, the number of laboratory-confirmed norovirus cases was significantly higher than the average for the same period in the previous 5 years[5], illustrating the importance of testing to monitor the spread of viral infections.

Noroviruses are extremely infective and infected people shed high viral loads. Like rotaviruses, infections are commonly asymptomatic or self-limiting in healthy adults but can cause severe symptomatic infections in the young, the elderly and the immunocompromised[6]. This non-segmented, positive-stranded RNA virus is consistently producing new strains which result in increasingly severe infections.

Noroviruses are classified as GI to GX. The most common, and those we will discuss, are GI and GII. The main difference between GI and GII noroviruses lies in their genetic diversity and prevalence. GI noroviruses primarily infect humans and have fewer distinct genotypes, while GII noroviruses have a broader host range, including humans and animals, and exhibit higher genetic diversity. GII noroviruses are more common and are associated with larger outbreaks, as they evolve more quickly and give rise to new variants over time. Both groups can cause similar symptoms of gastroenteritis, but GII noroviruses are considered more transmissible and widespread[7].

GII.4, which is responsible for 70-80% of human norovirus infections, is commonly associated with direct person-to-person transmission. Other genotypes are generally associated with other forms of transmission such as food contamination[6].

Pathophysiology

Noroviruses infect individuals differently, and not all norovirus genotypes are equally pathogenic. To carry out its action, the norovirus needs to bind to histo-blood group antigens (HBGAs)[6]. HBGAs are glycans found on the surface of some types of red blood cells and determine ABO blood grouping system and Lewis blood grouping system. Different norovirus genotypes have differing binding specificity between HBGA and VP1 (the viral binding protein) causing differences in individual susceptibility. It has been reported that those with blood type AB or B are less susceptible to some strains of norovirus compared with those with A or O blood type[6].

Host cell tropism (specific cell types infected by the virus) can be studied using virus histochemistry. This is a process in which tissue sections are incubated with whole viruses, or parts of a virus, and stained with specific antibodies. For most norovirus genotypes, tropism has not been determined. However, it has been confirmed that genotypes GII.1 and GII.6 bind to epithelial and goblet cells while GII.4 binds to lamina propria and Brunner's gland cells but not epithelial cells. It has not been confirmed whether this difference is due to differences in strains, hosts, or cell tropism[6].

The factors inferring protection against norovirus infection have not been fully elucidated, therefore it is unknown why some high-risk groups are afflicted with severe and chronic infections. That said, it has been shown that interferon-mediated innate immunity is essential for the clearance of norovirus infection and the reduction of its pathogenicity[6]. Further studies have shown that increases in serum antibodies that inhibit HBGA binding cause a decrease in the rate of infection and infection severity. Norovirus-specific IgA and IgG responses were associated with protection against gastroenteritis, supporting a role for the host immune system in reducing viral load, incubation period and disease severity. Conversely, some bacterial species, like E. *coli* and H. *pylori*, are known to express HBGA-like sugars, providing more binding options for noroviruses, increasing their virulence[6].

Treatment and Management

There is no currently available cure or vaccine for norovirus infection. Like rotavirus, the treatment of norovirus mostly consists of symptom management, rehydration, and electrolyte replacement. There are several vaccines in development, however, the rapid evolution and genetic diversity associated with noroviruses make this process complex[6].

Clostridioides difficile

Clostridioides difficile (C. *difficile*) is a gram-positive bacterium and the most implicated cause of antibiotic-associated diarrhoea. C. *difficile* is a spore-forming obligate anaerobe that produces 2 main toxins, A and B. Although, some strains have been shown to produce only toxin B[8]. It is commonly found in water, air, human and animal faeces, hospital surfaces and soil. Historically, C. *difficile* infection was thought to be a hospital-acquired infection, but recent studies show that 41% of infections are community-acquired[8].

C. *difficile* is a spore-forming obligate anaerobe, meaning it is a type of bacterium that can only survive in the absence of oxygen and must form spores to survive their exit from the gastrointestinal tract. Spore formation is essential for the transmission of this bacterial pathogen. The spores are highly resistant to environmental stresses, such as heat, radiation, and disinfectants, and can survive for long periods outside of the host.

The leading risk factor for C. *difficile* infection is antibiotic use. Antibiotics including penicillin, cephalosporins, fluoroquinolones and more are strongly implicated in C. *difficile* infection. Other risk factors include advanced age, chemotherapy, use of protein pump inhibitors, chronic kidney disease, chronic liver disease and malnutrition[8].

This bacteria infects up to 500'000 Americans every year, 15'000 of whom die due to C. *difficile* infections directly. In addition, more than half of those hospitalised will receive antibiotic therapy, and it is thought that up to 50% of these may be inappropriately prescribed[8] providing a means for the increased risk of C. *difficile* infection in hospitals.

Pathophysiology

C. *difficile* colonises the large intestine. Like the viral origins of gastroenteritis, those with healthy, intact immune systems will likely be asymptomatic carriers and neonates lack to necessary receptors to contract a symptomatic infection. However, antibiotic use can alter the normal microbial flora of the large intestine, leading to an increase in susceptibility to C. *difficile* infection in the individual[8].

Diarrhoea and colitis, the hallmarks of gastroenteritis, are caused by clostridial glycosylation exotoxins, Toxin A (enterotoxin) and Toxin B (cytotoxin). Toxin A harbours a carbohydrate binding site, facilitating intracellular transport of both toxins. Once in the cell, both toxins act to inactivate pathways mediated by Ras Homologous (RHO) proteins leading to damaged colonocytes, colitis and disruption of intracellular tight junctions[8]. C. *difficile* Toxins A and B are known to stimulate the production of chemokines and proinflammatory cytokines, which can recruit neutrophils to sites of inflammation. Both Toxins A and B are involved in the recruitment of neutrophils to sites of inflammation, but only Toxin A is responsible for the direct activation of neutrophils. The specific mechanisms by which these toxins interact with neutrophils and other immune cells are still being studied[9].

Symptoms associated with C. *difficile* infection will depend on several host and pathogenic factors. Common symptoms include watery diarrhoea with mucus or occult blood, anorexia, nausea, vomiting, low-grade fever, and lower abdominal pain. Additionally, toxic megacolon, extreme inflammation, and distention of the colon are commonly associated with severe C. *difficile* infection[8].

C. *difficile* infection may progress to fulminant colitis, characterised by diarrhoea, diffuse abdominal pain, abdominal distention, hypovolemia possibly leading to sepsis, toxic megacolon, and perforated bowel with peritonitis. Fulminant colitis is a rare form of C. *difficile* infection, occurring in only 1-3% of all cases, but it can be life-threatening and requires prompt medical attention. The progression to fulminant colitis is more likely to occur in older adults, immunocompromised individuals, and those with underlying medical conditions. The symptoms of fulminant colitis can be severe and may include fever, hypotension, tachycardia, and/or leucocytosis[10].

Treatment and Management

The first step to treating C. *difficile* infection is to withdraw the use of any inciting antibiotics, isolating the patient and administering an appropriate antibiotic based on the severity of the infection. Vancomycin and fidaxomicin are commonly used to treat C. *difficile* infection. Where the patient suffers from ileus, intravenous administration of metronidazole can be used to fight the infection[8].

Evaluation of Gastroenteritis

Classical evaluation for gastroenteritis comes in the form of a clinical diagnosis, meaning a clinician evaluates the symptoms and makes a diagnosis based on their experience. This means those who appear hydrated and do not display the common risk factors are generally not subject to additional testing for gastroenteritis. Mild leucocytosis may be present in a complete blood count and mild elevation of some inflammatory markers may occur[1]. Dehydration may present as acute kidney injury which can be measured through urea and creatinine concentration or by more contemporary methods such as the Randox Acute Kidney Injury Array[11,12].

A stool sample can be used to detect bacterial causes of gastroenteritis but is only requested in cases where the patient reports bloody stool, high fever, severe abdominal pain, or severe hydration as these symptoms are not normally seen in association with a mild, gastroenteritis infection[1]. Novel methods of detection use PCR technologies to identify and differentiate viral and bacterial infections responsible for gastroenteritis.

Vivalytic

Vivalytic is revolutionising the molecular diagnostic testing market through groundbreaking innovation. This advanced system is the product of a successful partnership between German technology leader Bosch Healthcare Solutions and Randox Laboratories. Bosch Healthcare Solutions has engineered the Vivalytic system, comprising the test cartridge and analyser. As the pioneering partner on the Vivalytic platform, Randox supplies Bosch with the essential biological components for the test cartridges, enabling the detection of various pathogens in samples. Additionally, Randox distributes the Vivalytic analysers and test cartridges, ensuring comprehensive support for this cutting-edge diagnostic solution.

Vivalytic cartridges are compact, technologically advanced molecular diagnostic tests utilising micro-fluidics to enable simple and accurate diagnostic testing. Depending on the test application, the cartridges are powered by various technologies. The system supports both High-Plex and Low-Plex testing. High-Plex tests, utilising Randox's patented Biochip Array Technology, allow for endpoint qualitative PCR, providing multiple test results from a single sample. Low-Plex tests employ a range of detection methods, including real-time qualitative PCR and melting curve analysis. The Vivalytic cartridges feature all necessary reagents on-board and are designed for room temperature storage, minimising preparation time and the risk of contamination.

The Vivalytic workflow is designed to be user-friendly and efficient, consisting of just four steps for optimal simplicity.

1. Scan/Input Sample Code: Begin by scanning or manually entering the sample information into the Vivalytic system.
2. Scan/Input Cartridge Code: Scan the cartridge code into the embedded Vivalytic software to ensure the correct test is being performed.
3. Insert Sample: Add the patient sample into the dedicated slot on the cartridge, close the cover, and insert the cartridge into the Vivalytic analyser.
4. Run Test and Display Results: The analyser will automatically run the test, with the touchscreen display counting down the time remaining to test completion. Once finished, the results will be displayed on the screen.

Multiple Vivalytic systems can be wirelessly connected, allowing simultaneous management, and reporting to a master Vivalytic platform. This intuitive, four-step process ensures a user-friendly experience and efficient diagnostic testing.

Biochip Technology

The Vivalytic system employs Randox's patented Biochip Array Technology, a revolutionary method that enables simultaneous detection of multiple targets from a single patient sample. Each biochip is pre-fabricated with spatially discrete testing regions (DTRs), with each DTR representing an individual test. These regions can be populated with oligonucleotides specific to a pathogen or target of interest, allowing comprehensive multiplex testing.

The Vivalytic system employs two distinct detection methodologies, offering versatility in molecular diagnostics. One detection method is based on a chemiluminescent signal, which is the emission of light without heat as a result of a chemical reaction. An enzyme catalyses this reaction, producing light that is detected and quantified using a Charge-Coupled Device (CCD) camera within the Vivalytic device. This camera simultaneously records light emission from all the DTRs on the biochip, enabling the system to automatically generate a comprehensive result report for multiple targets. This methodology eliminates the need for multiple time-consuming and sample-intensive assays, making it a highly efficient and powerful tool for high-plex testing.

Additionally, the Vivalytic system is capable of real-time PCR, which involves the amplification of nucleic acids and the detection of the amplified product in real-time. Real-time PCR utilises fluorescent dyes or probes that emit a signal proportional to the amount of PCR product formed, providing precise and rapid results for low-plex testing. By integrating both chemiluminescent detection with CCD and real-time PCR, the Vivalytic system offers a versatile and robust solution for a wide range of molecular diagnostic applications.

Qualitative RT-PCR detection of *C. difficile*

The Vivalytic *C. difficile* panel is specifically designed to detect the presence of Clostridioides *difficile* toxin genes tcdA and tcdB from liquid or soft human stool samples to enhance healthcare practices. With its advanced capabilities, this diagnostic tool empowers healthcare providers in their efforts to combat *C. difficile* infections, contributing to more effective management of this challenging condition.

- Turnaround time = > 50 minutes
- Liquid or soft stool sample
- Real-time PCR
- Sample volume = 300µl
- Detectable pathogens = *Clostridioide difficile* toxin genes tcdA/tcdB

Qualitative RT-PCR detection of Norovirus

The Vivalytic Norovirus Panel is specifically designed to detect Norovirus (genogroup I/II) in various clinical specimens, streamlining healthcare practices. This advanced diagnostic tool enables healthcare providers to effectively combat Norovirus infections and implement necessary preventive measures in diverse settings, such as hospitals, long-term care facilities, and schools.

- Turnaround time = > 60 minutes
- Liquid or soft stool sample
- Real-time PCR
- Sample volume = 300µl
- Detectable pathogens = Norovirus genogroups I/II

Qualitative RT-PCR detection of Rota-, Norovirus & C. diff

The Vivalytic Norovirus Panel is specifically designed to detect Norovirus (genogroup I/II) in various clinical specimens, streamlining healthcare practices. This advanced diagnostic tool enables healthcare providers to effectively combat Norovirus infections and implement necessary preventive measures in diverse settings, such as hospitals, long-term care facilities, and schools.

- Turnaround time = > 60 minutes
- Liquid or soft stool sample
- Real-time PCR
- Sample volume = 300µl
- Detectable pathogens = Rotavirus Type A, Norovirus genogroups I/II & Clostridioides *difficile* toxin genes tcdA/tcdB

Conclusions

Gastroenteritis brings with it discomfort and potential health complications. It encompasses a spectrum of symptoms, from diarrhoea and vomiting to abdominal pain and fever, and can be caused by various pathogens, including viruses, bacteria, and parasites.

In this guide we've covered the specifics of three significant culprits: Rotavirus, Norovirus, and *Clostridioides difficile*. Rotaviruses, notorious for affecting young children, operate with a complex structure and a high degree of genetic diversity. Norovirus, a leading cause of gastroenteritis, continuously evolves, making it a formidable adversary. *C. difficile*, often associated with antibiotic use, poses risks to vulnerable individuals.

The pathophysiology of these pathogens, their mechanisms of infection, and the host immune responses have been explored, providing a deeper understanding of the disease processes involved. We also discussed the importance of prompt diagnosis and appropriate management in preventing severe complications. In the era of advanced diagnostics, the Vivalytic POCT system emergess as a vital tool for healthcare professionals. Its rapid and reliable capabilities empower clinicians to detect these pathogens swiftly and accurately. This not only aids in timely patient management but also contributes to infection control and public health measures.

In the battle against gastroenteritis, knowledge is power, and cutting-edge diagnostics are essential. With the insights gained from this guide, healthcare professionals are better equipped to navigate the complexities of gastroenteritis, improve patient outcomes, and safeguard public health.

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