MICROBIAL SOURCE TRACKING OF FAECAL BACTERIAL PATHOGENS IN OGBA RIVER, BENIN CITY, NIGERIA.

BY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by Miracle kelechukwu **EZEANOCHIE** with Matriculation number **LSC2209688** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, under the supervision of **Dr. (Mrs.) R . ADAMS.**

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DR. (MRS.) R. ADAMS **DATE**

(Project Supervisor)

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PROF. E.O. IGBINOSA **DATE**

(Head of department)

DEDICATION

This project work is dedicated to GOD ALMIGHTY, for his wisdom, guidance and provision all through the duration of my study in the University of Benin.

ACKNOWLEDGEMENT

My utmost gratitude goes to the giver of life and to the one who loves and cares for me unconditionally (GOD ALMIGHTY), my father in heaven. A very big thank you to my supervisor, DR. (MRS.) R. ADAMS for her patience, supportive ideas and effective correction. I am forever grateful for the guidance and support throughout the project work.

I specially want to thank my HOD, Prof. E. O. IGBINOSA and my course advisers Mr. G. O. Oribhabor and Dr. (MRS.) O. B. Agbonwaneten, as well as my lecturers for their good works, which ensured I come this far.

I also want to appreciate the sacrificial effort of my parents Mr & Mrs G. C. Ezeanochie and my siblings especially my elder brother V. C. Ezeanochie for their unflinching and effective support, morally, financially, physically, spiritually and otherwise. May God continue to bless you. I also want to thank everyone who has been a source of strength and support throughout this academic journey.

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ABSTRACT

This study investigated the microbial source tracking of faecal bacterial pathogens in Ogba River, Benin City, Nigeria. Water samples collected from different locations were analyzed using standard cultural, morphological, Gram staining, and biochemical techniques. The isolates identified were *Escherichia coli, Salmonella spp., Shigella spp.,* and *Staphylococcus aureus. E. coli* and other Gram-negative rods indicated fecal contamination while *S. aureus* reflected anthropogenic input from human activities. Biochemical results confirmed the pathogenic potential of the isolates. The detection of these organisms demonstrates that Ogba River is contaminated with both human and animal wastes, posing serious public health risks. The result highlights the effectiveness of microbial source tracking in identifying contamination origins and emphasizes the need for regular water quality monitoring, improved sanitation, and proper waste management to prevent disease outbreaks and protect community health.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

One of the most important natural resources is water, which is necessary for both biological survival and economic growth. Globally, and especially in developing nations, the quality of freshwater sources has been greatly impacted by growing urbanization, population growth, and human activity (WHO, 2017). The discharge of untreated or inadequately treated wastewater has led to microbial pollution, which is posing a growing threat to rivers, which are frequently the main sources of water for drinking, household tasks, and agriculture (Adewumi *et al.*, 2014; Edokpayi *et al.*, 2015).

Faecal contamination is one of the most alarming types of pollution in freshwater ecosystems because it introduces harmful microorganisms like *Salmonella* spp., *Shigella* spp., *Vibrio cholerae,* and *Escherichia coli* into aquatic environments. These microbes can cause gastroenteritis, cholera, typhoid fever, and dysentery, among other serious waterborne illnesses (Ashbolt, 2004; Harwood *et al*., 2014). Over 2 billion people worldwide, according to estimates from the World Health Organization (WHO), drink water that has been tainted by human waste, which leads to high rates of morbidity and mortality, particularly in low-income areas (WHO, 2017).

The situation in Nigeria is especially concerning. According to Olelemi *et a*l. (2020), runoff from animal farms, open defecation, and the direct discharge of household sewage all have a significant influence on rivers that pass through urban areas. For example, the Ogba River flows through densely populated and industrial areas in Benin City, Edo State. The river provides water to the nearby communities, fisheries and irrigation, among other purposes. To evaluate the risks to public health, however, a rapid scientific investigation is required due to the growing reports of microbial contamination.

One effective method for locating the sources of faecal pollution in water bodies is Microbial Source Tracking (MST) (Scott *et al*., 2002). Determining whether fecal contamination comes from environmental, animal, or human sources is helpful. Although there are sophisticated molecular MST techniques, their application in developing nations is restricted by their expense

and technical requirements. For field-based environmental monitoring in particular, culture-based MST is still a viable option (Fricker, 2003; Byappanahalli *et al.*, 2012).

The Ogba River's fecal bacterial pathogens are identified and measured in this study using culture-based microbiological techniques. With this strategy, it seeks to offer data that is supported by evidence to direct water safety initiatives and environmental health policies in the area.

1.2 Statement of the Problem

Faecal pollution in urban Nigeria is posing a growing threat to the quality of surface water bodies, including rivers. There is still a dearth of localized data on microbial contamination of rivers such as the Ogba, particularly when employing practical and economical techniques, despite the growing concern. It is challenging to identify sources of contamination or to put into practice efficient public health strategies when routine monitoring and microbial source tracking are lacking.

Communities around the Ogba River depend significantly on the water, frequently untreated, for household and recreational purposes. This river's pathogenic bacteria can cause outbreaks of waterborne illnesses and other major health hazards. There is little information explicitly addressing culture-based microbial source tracking in the Ogba River, despite earlier research reporting broad pollution trends in Nigerian rivers.

This study fills this knowledge gap by employing culture-based methods to systematically identify faecal bacterial pathogens, producing useful and actionable data for the region's water safety management.

1.3 Objectives of the Study

This study's main goal is to use culture-based techniques to track the microbial sources of bacterial pathogens found in the feces of the Ogba River.

The particular goals are to:

i. Use culture-based microbiological techniques to identify and measure the bacterial pathogens found in the feces of the Ogba River.

ii. Ascertain how the microbial contamination varies geographically along the river's path.

iii. Assess the possible hazards to public health posed by the identified bacterial pathogens.

iv. Provide mitigation and control measures to enhance the river's microbial quality.

1.4 Research Questions

i. Which bacterial pathogens are most common in the Ogba River's feces?

ii. How does the microbial load change at various river sampling locations?

iii. Which animal or human is most likely the source of the faecal contaminants found?

iv. How does the Ogba River's microbial contamination affect public health?

1.5 Significance of the Study

It is impossible to overstate the significance of clean water for ecosystem stability and human consumption. In developing nations with inadequate public sanitation systems, such as Nigeria, rivers are susceptible to microbial contamination from various sources. MST offers a means of comprehending the dynamics and causes of this pollution.

Despite the development of molecular techniques for MST, routine monitoring in Nigeria is not feasible due to their high cost and technical complexity. On the other hand, culture-based detection techniques provide a reproducible, cost-effective and efficient way to find microbial pathogens in environmental samples (Rompré *et al.*, 2002; APHA, 2017).

Therefore, this study is both necessary and timely because it will;

i. Give localized information about the Ogba River's microbial water quality.

ii. Provide information about areas of the river where contamination is most prevalent.

iii. Participate in the creation of evidence-based public health initiatives and water safety regulations.

iv. Equip local stakeholders with information about best practices and microbial risks.

1.6 Scope of the Study

The Ogba River in Benin City, Nigeria, is the subject of the study. It uses microbiological techniques based on culture to identify and measure bacterial pathogens in feces. Only certain locations along the river, especially those most impacted by human activity, are sampled. Molecular MST techniques and viral and protozoan pathogens are not included in the scope. Additionally, the physical and chemical characteristics of water are not the main focus of this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Microbiological Contamination and Water Pollution

Water bodies are vital to ecological systems and human survival, but they are continuously under strain from pollution, especially from faecal contamination. Uncontrolled waste disposal and a

lack of centralized wastewater treatment frequently cause fecal matter to contaminate rivers and other surface waters in developing countries.

In environmental microbiology, microbial source tracking (MST) is an essential technique, especially when determining the origins of faecal contamination in water bodies. In developing nations like Nigeria, where rivers like the Ogba are vital water sources for residential, agricultural, and recreational purposes, MST is particularly important in protecting public health. Determining whether microbial contaminants in water come from animal or human feces is the main goal of MST. The literature on microbial pollution in surface waters, culture-based detection methods, common pathogens, the health effects of faecal contamination, and the use of MST in locating pollution sources is reviewed in this chapter. It also emphasizes the shortcomings of existing approaches and how this study closes a gap in the literature on culture-based MST in Nigerian rivers.

2.2 Microbial Pollution of Surface Waters

Anthropogenic activities like the release of untreated sewage, agricultural runoff, industrial effluents, and urban waste make surface waters extremely vulnerable to microbial pollution. The health of the general public and the environment is seriously threatened by these activities, which introduce a wide variety of pathogenic microorganisms into water bodies (WHO, 2017). Edokpayi *et al.* (2015) state that discriminatory waste disposal practices and inadequate sanitation infrastructure are the main causes of microbial contamination of rivers in sub-Saharan Africa.

The quality of Nigeria's surface waters has deteriorated due to urbanization and a lack of proper sanitation infrastructure. Stormwater runoff and untreated wastewater are common in rivers like the Ogba that traverse heavily populated and industrialized areas. Studies by Igbinosa and Okoh (2009) and Olalemi *et al.* (2020) have verified that several Nigerian rivers contain high concentrations of pathogenic bacteria and fecal coliforms. These results highlight how urgently ongoing monitoring and intervention are needed.

2.3 Sources of Faecal Pollution

There are two types of faecal pollution in surface waters: point and non-point.

i. Direct sewage discharge, industrial effluents, and hospital waste are examples of point sources; stormwater, agricultural runoff, landfill leachate and animal.

ii. Wildlife feces, as well as defecation in open fields are examples of non-point sources (Harwood *et al.,* 2014). Nearby water bodies are frequently contaminated in rural and informal settlements due to a lack of adequate sanitation facilities.

According to Opara *et al.* (2011), rivers like the Otamiri are used for waste disposal, bathing, and washing in many Nigerian communities, which makes them extremely vulnerable to faecal pollution. Reports from other sub-Saharan nations dealing with comparable regulatory and infrastructure issues are in line with these findings.

2.4 Faecal Contamination in Aquatic Systems

Faecal contamination is one of the biggest problems with managing water quality. Numerous sources, such as untreated sewage, stormwater runoff, agricultural practices, and open defecation, commonly contribute to the fecal matter pollution of rivers, lakes, and streams (Cabral, 2010). Improper waste disposal is the main cause of increased microbial loads in rivers in urban and periurban areas. High concentrations of *Escherichia coli* and other enteric bacteria were introduced into South African water bodies by effluent discharges from informal settlements and wastewater treatment facilities, according to Adefisoye and Okoh (2016).

Numerous rivers in Nigeria have been found to have high faecal loads as a result of uncontrolled human activity. For example, Olalemi *et al.* (2020) discovered that faecal coliform counts in southwest Nigerian rivers were higher than the allowable limits for drinkable water. This was mainly because of the direct discharge of market effluents, agricultural runoff, and household waste. These results demonstrate how critical it is to monitor surface water microbiologically in developing areas.

2.5 Common Faecal Indicator Bacteria (FIB)

MST seeks to identify the source of faecal contamination, be it wildlife, livestock, or humans. Identification of host-associated bacterial species is a prerequisite for culture-based MST techniques. The indicator organisms, for instance, are employed as stand-ins to identify faecal

contamination in water. Although their presence suggests potential faecal matter contamination and, thus, the presence of pathogens, they are not always harmful (APHA, 2017).

Key indicators include:

i. Total coliforms: Represent general microbial contamination.

ii. Faecal coliforms(especially *E. coli*): Specific to faecal origin and more reliable indicators of pathogenic contamination (Rompréet *et al.,* 2002).

iii. Enterococci: More persistent in saltwater and are better indicators in marine and brackish environments (Byappanahalli *et al.,* 2012).

Culture-based methods are used to identify and measure these indicators, and they are still frequently employed because of their affordability and ease of use.

Faecal indicator bacteria are non-pathogenic organisms that indicate the possible presence of pathogenic organisms and contamination with faecal material. *Clostridium perfringens*, *Enterococcus* species, and *E. coli* are the most commonly used indicators. E. coli is the best indicator for freshwater systems, whereas Enterococcus species are better suited for marine settings, according to the United States Environmental Protection Agency (USEPA, 2012).

E. coli counts are essential to evaluating the quality of surface waters because they are a good indicator of recent faecal pollution. Detecting these bacteria with selective and differential culture media is comparatively simple. But in aquatic settings, the survival rate of indicator organisms varies depending on salinity, temperature, and sunlight (Noble *et al.*, 2003; Harwood *et al.,* 2014).

2.5.1 Total coliforms

Total coliforms are rod-related, oxidase-negative, Gram-negative bacteria that are frequently found in soil, surface water, and vegetation. Since their presence may indicate contamination by potentially hazardous pathogens, they are employed as indicator organisms to evaluate the microbiological quality of drinking water. While total coliforms are generally not harmful, their presence in treated water systems suggests a water treatment breakdown or potential distribution

system breach (World Health Organization, 2017). These microorganisms, which belong to the genera *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella,* can ferment lactose at 35–37°C for 48 hours while producing gas and acid (U.S. Environmental Protection Agency, 2006). In order to better evaluate the health risk, additional testing for fecal coliforms and Escherichia coli is frequently prompted when total coliforms are detected in a water sample. One of the most important aspects of maintaining safe drinking water and safeguarding public health is monitoring total coliforms (World Health Organization, 2017).

2.5.2. Fecal coliforms

A subset of total coliform bacteria, fecal coliforms are found only in the digestive tracts of warm-blooded animals, such as humans. Escherichia coli (E. coli), the most well-known fecal coliform, is a crucial marker of recent faecal contamination in water systems (World Health Organization, 2017). Only E. *coli, Klebsiella oxytoca* and *Klebsiella pneumoniae* produced positive test results in environmentally contaminated waters (Cabral *et al.,* 2006). Faecal coliforms, as opposed to total coliforms, are more closely linked to the presence of pathogens, including bacteria, viruses, and protozoa, which can result in illnesses like cholera, typhoid, and diarrhea (U.S. Environmental Protection Agency, 2006).Their presence in water, particularly drinking water, raises the possibility of contracting a waterborne disease and necessitates prompt remedial measures like source protection or system disinfection (WHO, 2017).

Faecal coliforms are usually recognized by their capacity to ferment lactose at high temperatures (44.5°C) in less than a day, resulting in the production of acid and gas. Their presence in water more obviously indicates faecal pollution, which can originate from stormwater runoff, animal waste, or sewage leaks, as they are typically not found in soil or vegetation (EPA, 2006).

2.5.3 Enterococci

Enterococci are ovoid, catalase-negative, Gram-positive, non-sporeforming bacteria. Cells can be found alone, in pairs, or in brief chains. Most species grow best at temperatures between 35 and 37°C. Some will thrive at 10°C and 42–45°C. Although complex nutrients are necessary for growth, they are typically abundant on widely used bacteriological media. Cells possess β-glucosidase, hydrolyze esculin, and are resistant to 40% bile, 0.4% azide, and 6.5% sodium

chloride. Although facultative anaerobic, the enterococci favor anaerobic environments (Svec *et al.,* 2009).

In the 1980s, the genus was distinguished from *Streptococcus*. Groups of enterococci are comparatively distinct. Members of these groups share phenotypic traits, making it challenging to distinguish between different species. According to Byappanahalli *et al.* (2012), *Enterococcus faecalis* and *Enterococcus faecium* are the two most commonly isolated species in environmental monitoring. Their ability to survive in harsh environments and their strong correlation with the presence of gastrointestinal pathogens make them popular faecal indicator bacteria (FIB), particularly in marine and recreational water monitoring programs. *E. faecalis* is one of the species that belong to the *E. faecalis* group. *E. avium* is one of the species in the *E. avium* group. *E. faecium, E. durans*, and *E. hirae* are all members of the *E. faecium* group. *E. gallinarum* is a member of the *E. gallinarum* group.

The majority of species are found in the intestinal flora of birds, reptiles, mammals, and other creatures. Although *E. faecium* may predominate in specific circumstances, *E. faecalis* is the predominant species in the human digestive tract. The intestinal flora of poultry is dominated by *E. cecorum, E. durans, E. faecalis, E. faecium, and E. hirae.* A growing number of nosocomial and other infections, primarily from wound and urinary tract infections, bacteremias, and endocarditis, have been linked to enterococci.

Even though enterococci are thought to be a transient component of plant microflora, under ideal circumstances, cells can multiply on their surfaces. Plants have been used to isolate *E. casseliflavus, E. faecalis, E. faecium, E. hirae, E. mundtii,* and *E. sulfureus*. In general, flowers are where they are isolated more frequently than buds or leaves.

Many foods, particularly those that come from animals, like meat, milk and milk products, and fermented sausages, naturally contain enterococci. Although they frequently contribute to the ripening and development of certain cheeses' aromas, enterococci are typically regarded as secondary contaminants of food. Despite the fact that soil is not an enterococci's natural habitat, rain can carry the bacteria's cells there (Wilson, 2005).

Enterococci do not naturally inhabit environmental waters, and their presence in these environments is thought to be caused by faecal pollution. *E. avium, E. cecorum, E. columbae,*

and *E. gallinarum* are less frequent species in environmental waters than *E. durans, E. faecalis, E. faecium,* and *E. hirae*. However, *E. casseliflavus* has been found in Finland's pristine waters. Enterococci are particularly helpful as indicators in marine and coastal environments because, in contrast to total and faecal coliforms, they are more resilient to environmental stressors and can endure longer in saltwater and harsh conditions (U.S. Environmental Protection Agency, 2012). Using culture-based or molecular techniques, *Enterococcus faecalis and Enterococcus faecium* are the species most frequently observed in water.

In 1999, Pinto *et al.* reported isolating *E. casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum,* and *E. hirae* from environmental samples (compost, sewage effluent, harbor sediments, brackish water, and swimming pool water). Except for harbor sediments, *E. durans, E. faecium,* and *E. hirae* were isolated from every source. Only compost and swimming pool water were used to isolate E. raffinosus. The great majority of enterococcal strains were *E. faecalis* and *E. faecium.* Health authorities can evaluate the safety of recreational waters and issue advisories or closures when contamination surpasses predetermined limits by keeping an eye on enterococci levels. High levels of enterococci are associated with a higher risk of skin infections, eye/ear infections, and gastrointestinal disorders, especially in children and swimmers (EPA, 2012; CDC, 2023).

Membrane filtration and molecular techniques like qPCR are frequently used to measure enterococci. Regulatory organizations like the EPA have set recreational water quality standards based on enterococci levels to inform beach advisories and public health decisions (EPA, 2012).

2.6 Pathogenic Faecal Bacteria in Rivers

While indicator organisms offer valuable information about the quality of water, precise risk assessment depends on the identification of particular pathogens. *Salmonella* species, *Shigella* species, *Vibrio cholerae*, pathogenic *E. coli* (e.g., EHEC, ETEC) and Campylobacter species are among the waterborne pathogenic bacteria (Cabral, 2010). Waterborne illnesses like cholera, gastroenteritis, bacillary dysentery, and typhoid fever are caused by these pathogens.

According to a study by Obi et al. (2002), enteric pathogens that were genetically similar to clinical isolates from patients with diarrhea were found in water samples from the Venda region of South Africa. Likewise, Igbinosa and Okoh (2009) documented the existence of Shigella and

Salmonella species that are resistant to multiple drugs in Nigerian rivers, highlighting the dangers that untreated wastewater discharges pose to public health.

i. Escherichia coli (E. coli): Shows the possibility of other enteric pathogens and recent faecal contamination (Rompré *et al.*, 2002).

ii. Salmonella spp.: Causes gastroenteritis and typhoid fever (Ashbolt, 2004).

iii. Shigella spp.: Often found in waters tainted by sewage, these bacteria cause dysentery.

iv. Vibrio cholerae: Linked to outbreaks of cholera in places with inadequate sanitation (WHO, 2017).

v. Indicators of faecal pollution, particularly in marine environments, are Enterococcus species (Harwood *et al.,* 2014).

2.6.1 Salmonellosis

2.6.1.1 The Genus Salmonella

In 1900, Lignières designated the genus *Salmonella.* Castellani's description of a technique for absorbing antisera in 1902 marked the beginning of antigenic analysis. White published the first antigenic scheme for *Salmonella i*n 1926, and Kauffmann went on to develop it in two seminal works published in 1966 and 1978. By 1988, there were roughly 2,250 distinct serovars in the Kauffmann-White antigenic scheme (Popoff *et al.,* 2005).

After many years of use, the idea of "one serovar, one species" is no longer acceptable. There has been discussion regarding the taxonomy and nomenclature of the genus *Salmonella* ever since Le Minor and Popoff's 1987 paper suggested modifications. A 2005 publication by the International Committee on the Systematics of Prokaryotes resolved the matter. The first letter of each serovar's name must be capitalized in accordance with the rules of bacterial nomenclature, and the names are not italicized (Tindall *et al.,* 2005). The two primary species of Salmonella are *Salmonella enterica* and *Salmonella bongori.* Based on antigenic differences, *S. enterica* is further subdivided into six subspecies and more than 2,600 serovars (Grimont and Weill, 2007).

The most commonly isolated serovar from humans worldwide is *S. enterica* subsp. *enterica serovar enteritidis*. Other serovars, however, may predominate locally. Outbreaks of salmonellosis occurred in Tunisia in 1997, 1999, 2002, and 2004 during the 1994–2004 period. *Serovar Mbandaka* caused a salmonellosis outbreak in 1997. Three outbreaks of salmonellosis were reported from hospitals in three different regions in 1999. *Mbandaka, Livingstone, and Typhi Vi+* were the serotypes linked to each outbreak. The same hospital that reported an outbreak of serovar Typhi Vi+ in 1999 also reported an *S. enterica subsp. enterica serovar Livingstone* infection in 2002, but in a different unit. In Tunisia, the *Livingstone serovar* surged to the top of the human infection list that year. There was a second outbreak of *serovar Typhi Vi+* in 2004. A fermented juice derived from palm trees was the source of isolation (Ben Aissa *et al.,* 2007).

2.6.1.2 Characterization of the Diseases

Salmonellosis can be classified as either typhoidal or non-typhoidal due to the presence of human pathogenic Salmonellae.

1. Typhoid and Paratyphoid Fever (Enteric Fevers)

*Salmonella enterica serovars Typh*i (S. Typhi) and *Paratyphi* A, B, and C are the cause. According to Parry *et al.* (2002), the illnesses are known as typhoid fever (caused by *S. Typhi)* and paratyphoid fever (caused by *S. Paratyphi*). They are characterized by a high, prolonged fever, weakness, abdominal pain, constipation or diarrhea, hepatosplenomegaly, and occasionally rose-colored spots on the chest. Contaminated food or water can spread the infection through the fecal-oral route, and the incubation period lasts 7–14 days.

If left untreated, complications include encephalopathy, bleeding, intestinal perforation, and death.

When treated with the right antibiotics, the mortality rate is less than 1%, but it can reach 10% to 30% if left untreated (Crump *et al.,* 2004).

2. Non-Typhoidal Salmonellosis (NTS)

Typhimurium, Enteritidis, Newport, and other bacteria are the cause. The illness is known as invasive non-typhoidal salmonellosis (iNTS) or *Salmonella gastroenteritis*. The symptoms include fever, nausea, vomiting, diarrhea, and cramping in the abdomen. Usually self-limiting in healthy people. Additionally, the incubation period lasts between 6 and 72 hours.

Transmission occurs through contact with infected animals and the consumption of tainted eggs, poultry, dairy products, or produce. Additionally, complications include meningitis, sepsis, and bacteremia, particularly in immunocompromised individuals (Majowicz *et al.,* 2010; Kariuki and Gordon, 2015).

Individuals at risk include children under five, the elderly, people living with HIV, and people suffering from malnourishment or malaria in developing nations. In healthy people, the death rate is usually low (<1%), but in sub-Saharan Africa, it can reach 25% for NTS cases (Feasey *et al.,* 2012).

Table 2.1: Key Differences of Typhoid fever and Non- Typhoidal salmonellosis :

Feature	**Typhoid Fever**	**Non-Typhoidal salmonellosis**
Main Serovars	***S. Typhi, S. Paratyphi***	***S. Typhimurium, S. Enteritidis***
Host Range	**Human-specific**	**Broad (humans and animals)**
Symptoms	**Systemic, prolonged fever**	**Acute gastroenteritis**
Severity	**Potentially fatal**	**Usually self-limiting**

Treatment	**Antibiotics**	**Often supportive care only**
Vaccine Available?	**Yes (*S. Typhi*)**	**No (under development)**

2.6.1.3 Ecology of Salmonellae and the Cycle of Salmonellosis

The intestinal tracts of both humans and animals are Salmonella's primary habitat. Since humans, pets, farm animals, and wildlife all excrete *salmonellae,* they are always present in environmental samples. According to Arvanitidou *et al.* (2005), the primary sources of these pathogens in natural waters are stormwater runoff, agricultural pollution, and municipal sewage. In the natural environment, *salmonellae* do not appear to multiply much, but if the pH, humidity, and temperature are right, they can live for weeks in soil and water.

Under ideal circumstances, *salmonella* species can survive in the environment for long stretches of time. Research indicates that they live:

i. In water for weeks to months, particularly when protected by organic materials.

ii. On plant surfaces and in soil, where they have the ability to adhere and create biofilms (Brandl, 2006).

iii. They can survive for months on dry surfaces such as processing equipment and animal feed (Podolak *et al.*, 2010). These ecological characteristics allow *Salmonella* to function as an environmental contaminant and an enteric pathogen, facilitating frequent transmission through food, water, and contact with feces or animals that are infected.

Most of the *salmonellae* that are isolated from environmental sources are either Paratyphi or non-Typhi serovars. The most frequent serotypes isolated from food in a study conducted in Tunisia between 1994 and 2004 were *S. enterica subsp. enterica serovars Anatum, Enteritidis,* and

Corvallis. The vast majority of the strains were isolated from fruits, vegetables, poultry, red meat, and milk and dairy products. Seventy-three percent of the isolates from environmental sources came from tap water. The most prevalent serovars were *Corvallis, Enteritidis,* and *Anatum.* A comparative study conducted in the northern Greek rivers of the Aliakmon and Axios over a one-year period, from May 2002 to April 2003, was reported by Arvanitidou *et al.* (2005). From the water samples, 29 different species of *Salmonella* were found. Many of the *Salmonella* serovars that were isolated were from non-human animals, including Senftenberg, Virchow, Hadar, Infantis, and Mbantaka, which were frequently isolated from poultry farms.

In contrast to cholera, *salmonellae i*nfections in humans can persist in the gut without causing symptoms. The bacteria can remain in infected humans for extended periods of time. After being clinically cured of typhoid fever, about 5% of patients continue to carry the virus for months or even years. These individuals are the primary source of bacteria in the environment and may harbor the bacteria in their guts for an extended period of time.

Human populations, the environment, and animal reservoirs all interact in a complicated way during the Salmonella transmission cycle:

1. Animal Reservoirs

Poultry, pigs, cattle, rodents, reptiles, and wild birds are the main reservoirs. Salmonella is frequently excreted by these animals, contaminating the environment (Foley *et al.,* 2011).

2. Environmental Contamination

The following are contaminated by fecal shedding: groundwater and surface water, Manure or runoff from the soil, crops from irrigation or handling, food processing tools, and animal feed

3. Human Exposure

Infections in humans are mostly caused by eating food that has been tainted (e.g., undercooked meat, eggs, dairy, vegetables), consuming or utilizing tainted water and Contact with animals or feces that are infected (pet reptiles, for example)

4. Disease and Shedding

In addition to developing typhoid fever or gastroenteritis, infected humans may also become asymptomatic carriers and release bacteria into sewage systems, which further contributes to the environmental cycle (Crump *et al.,* 2004).

5. Back to the Environment

The *Salmonella* cycle is perpetuated by contaminated human and animal waste reentering water systems, agricultural fields, and food supply chains.

Shellfish may be a part of the environmental salmonellosis cycle. If appropriate germicides are not applied during sewage processing*, salmonellae* can survive sewage treatments. Edible shellfish (oysters, mussels) may become contaminated if sewage plant effluent enters a coastal area. As they filter multiple liters of water every hour, shellfish concentrate bacteria. Human consumption of these seafoods, whether raw or only partially cooked, can result in salmonellosis or typhoid fever. The use of strain markers, such as phage typing, has provided evidence of such a cycle (Popoff *et al., 2005).*

2.6.2 Shigellosis or Bacillary Dysentery

2.6.2.1 The Genus Shigella

Shigella belong to the family *Enterobacteriaceae* and are rod-shaped, gram-negative, non-motile, and non-sporeforming. Sugars are fermented in cells without producing gas. Adonitol, myo-inositol, and salicin do not undergo fermentation. Cells don't make H_2S or use citrate, malonate, and acetate as their only carbon sources. Decarboxylation of lysine does not occur. Cells are both catalase-positive and oxidase-negative. Taxonomy is based on the somatic O antigens of the genus, which have a complex antigenic pattern (WHO, 2008). According to their serological characteristics, *Shigella* can be divided into four species (or groups);

i. *Shigella dysenteriae* – Group A

ii. *Shigella flexneri* – Group B

iii. *Shigella boydii* – Group C

iv. *Shigella sonnei* – Group D

The most frequent cause of endemic shigellosis in developing countries is *S. flexneri*, whereas *S. sonnei* is more common in industrialized countries (Livio *et al.*, 2014). *Shigella* is one of the leading causes of diarrheal illness in the world, particularly in young children. Over 100,000 deaths are attributed to *Shigella* each year, primarily in low-income areas with inadequate sanitation, according to the Global Burden of Disease Study (Troeger *et al.,* 2018). The need for new vaccines and public health initiatives is being highlighted by the rise in antibiotic resistance, which makes treatment more challenging, particularly in *S. dysenteriae type 1* and *S. sonnei.*

Among these, *S. flexneri* is the most common cause of endemic shigellosis in developing

2.6.2.2 Characterization of the Disease

Shigellosis, sometimes referred to as bacillary dysentery, is the main illness brought on by the genus *Shigella.* It is a serious intestinal infection that is typified by frequent passage of bloody, mucus-filled stools and colon inflammation. Children under five are most likely to contract shigellosis, especially in environments with inadequate sanitation and hygiene (Troeger *et al.,* 2018).

The incubation period lasts between one and four days. Fever, anorexia, fatigue, and malaise are typically the first symptoms of the illness. Patients frequently have cramping in their abdomens and small, bloody stools that can occasionally be extremely purulent. When diarrhea turns into dysentery 12 to 36 hours later, the feces lose volume (no more than 30 mL of fluid per kg per day) and contain blood, mucus, and pus (Emch *et al.,* 2008).

The first step in the pathogenesis of shigellosis is penetration of the colonic mucosa, despite the complexity of its molecular basis. Degeneration of the epithelium and acute inflammatory colitis in the lamina propria are the hallmarks of the ensuing *Shigella* infection focus. The mucosa eventually becomes desquamated and ulcerated, allowing blood, inflammatory substances, and mucus to seep into the intestinal lumen. The colon's ability to absorb water is hindered in these circumstances, and the ileocecal flow determines the stool's volume. This will cause the patient to have frequent, thin, dysenteric stools.

Before *Shigella* can enter an epithelial cell, it must first attach itself to its target cell. According to Todar (2009), the bacteria typically enters the body through an endosome, which it then lyses to enter the cytoplasm, where it multiplies.

2.6.2.3 Etiologic Agents and Disease Forms

The type of infection determines how severe shigellosis is;

i. *Shigella dysenteriae* type 1 : Produces the most severe form and is frequently linked to epidemic outbreaks and complications like hemolytic uremic syndrome (HUS) because of the production of Shiga toxin (Sansonetti, 2001).

ii. Moderate to mild illness is caused by *Shigella flexneri* and *S. sonnei.* While *S. flexneri* is more common in developing countries, *S. sonnei* is more common in industrialized nations (Livio *et al.,* 2014).

iii. *Shigella boydii* : Found throughout South and Southeast Asia, but less common.

2.6.2.4 Clinical Features

Tenesmus (painful straining to pass stool), an incubation period of one to three days, and an abrupt onset of fever and abdominal cramps are among the symptoms. Typical low-volume with potential blood, pus, and mucus, feeling sick and throwing up continues for a while. Without treatment, it typically lasts 4–7 days, though symptoms may persist longer in more severe cases. The complications include dehydration and electrolyte imbalance. hemolytic uremic syndrome (HUS), which is primarily brought on by *S. dysenteriae type 1*, rectal prolapse, seizures in children (caused by neurotoxic effects or a high fever), and malnourishment in recurrent infections, especially in children. It spreads through contaminated food, water, hands, or surfaces through the fecal–oral route. To cause disease, only 10–100 organisms are required.

2.6.2.5 Virulence Factors

High concentrations of a cytotoxic Shiga toxin are produced by *S. dysenteriae serotype 1*. Much less of this toxin is produced by *S. flexneri* and *S. sonnei.* Shiga toxin cleaves the N-glycosidic bond at adenine 4324 in 28S rRNA, binding to Galotl-4Galp (galabiose) glycolipid receptors and

preventing the synthesis of mammalian proteins. The mechanism of toxicity is the same as that of ricin, a plant toxin that is produced by Ricinus communis. Additionally, *Shigella* releases an inflammatory response-inducing LPS endotoxin (O antigens).

Shigella 180–230 kb plasmids encode genes that are necessary for virulence, specifically adhesin production, which helps bacteria adhere to the surface of target epithelial cells; invasion plasmid antigens (Ipa), which directly contribute to *Shigella* invasion; transport or processing functions that guarantee the proper surface expression of the Ipa proteins; induction of bacterial endocytic uptake and disruption of endocytic vacuoles; and regulation of plasmid-encoded virulence genes (Todar, 2009).

During evolution, *Shigella* separated from *E. coli.* A significant shift in pathogenesis was made possible by the acquisition and evolution of the pathogenicity island, which encodes every gene needed for cell invasion and phagolysosomal lysis.

Here are Key Virulence Factors

1. Type III Secretion System (T3SS)

i. A device that resembles a needle and is used to directly inject effector proteins into host cells.

ii. Genes on the virulence plasmid (e.g., mxi, spa) encode this.

iii. Allows the bacterium to evade immune detection, alter host signaling, and infiltrate epithelial cells.

iv. It is vital for intracellular survival and dissemination (Ashida *et al.,* 2015).

2. Ipa Proteins (Invasion Plasmid Antigens)

i. Include IpaA, IpaB, IpaC, and IpaD.

ii. Injecting host cells through the T3SS:

iii. In order to let bacteria in, IpaB and IpaC cause membrane ruffling.

iv. The T3SS is assembled at the host membrane with the aid of IpaD.

v. To help bacteria move, IpaA destabilizes the host cytoskeleton.

vi. It is essential for host cell invasion and endosome escape (Sansonetti *et al*., 2001).

3. Shiga Toxin (Stx) – S. dysenteriae Type 1

i. The strong AB5 exotoxin damages 28S rRNA, which prevents the synthesis of proteins.

ii. Results in hemolytic uremic syndrome (HUS), bloody diarrhea, and cell death (Melton-Celsa, 2014).

iii. Neither *S. flexneri, S. sonnei,* nor *S. boydii* produce it.

4. Actin-Based Motility Proteins (IcsA/VirG)

i. The bacterium is propelled through the cytoplasm and into neighboring cells by IcsA, which recruits host actin.

ii. Makes it possible for *Shigella* to move laterally between cells without being noticed by the extracellular immune system.

5. Lipopolysaccharide (LPS)

i. An important part of the outer membrane.

ii. Prevents killing mediated by host complement.

iii. Induces severe inflammatory reactions that lead to damage to colonic tissue (Phalipon and Sansonetti, 2007).

6. Effector Proteins (e.g., Osp proteins)

Modulate host immune responses;

i. Inflammatory signaling (such as MAPK pathways) is suppressed by OspF and OspG.

ii. OspE aids the bacterium's ability to stick to host cells and withstand detachment (Kim *et al.*, 2009).

7. Acid Resistance Systems

i. *Shigella* uses acid resistance mechanisms like glutamate decarboxylase to survive in the acidic gastric environment.

ii. Improves stomach survival and colon delivery.

2.6.2.6 Risk Factors

Numerous studies have found protective factors and risk factors for the incidence and mortality of shigellosis. Shigellosis remains widespread among the underprivileged populations in the tropics, frequently among displaced populations after natural disasters and political upheavals, despite slow improvements in the water supply. *Shigella dysenteriae serotype 1* was found to be most prevalent in Guatemala in males aged 15–44, the elderly, and young children. Children under five years old in Sierra Leone had a higher attack rate than the general population. Shigellosis was more prevalent in rural Bangladesh in people 60 years of age or older and in children ages 1-2. Shigellosis mortality was highest in severely malnourished individuals of all ages, in children under 2 who were not receiving breast milk, and in all children under 1 in Dhaka, Bangladesh. According to a three-year study conducted in Matlab, Bangladesh, between 1992 and 1994, children under the age of two had the highest incidence of *S. dysenteriae serotype 1* and *S. flexneri*, followed by children between the ages of two and five. While the risk areas for *S. flexneri* remained constant over time, the locations of S. *dysenteriae serotype 1* risk fluctuated. The areas most at risk for *S. dysenteriae serotype 1* were those close to bazaars with lots of unsanitary restrooms. In areas under flood control, *S. flexner*i was most prevalent. It was determined that while *S. flexneri* was more associated with the environment, *S. dysenteriae serotype 1* risk was more associated with sanitation and hygiene (Emch *et al.,* 2008).

Shigella is a highly contagious bacterial infection that is mainly spread by the fecal–oral pathway. Different environmental, societal, and individual risk factors contribute to infection, especially in areas with inadequate infrastructure for sanitation and hygiene.

1. Poor Sanitation and Hygiene

The spread of *Shigella* is largely caused by inadequate sanitary facilities and inadequate hand hygiene. When feces contaminate food, water, or surfaces, the bacteria can spread quickly.

Risk is elevated in;

i. Places without handwashing stations and sanitary restrooms.

ii. Areas where people practice open defecation.

iii. Societies lacking access to potable water.

2. Young Age

Particularly in developing nations, children under five are the most impacted demographic. They are more susceptible due to their frequent hand-to-mouth behaviors and immature immune systems (Troeger *et al.,* 2018).

3. Overcrowded Living Conditions

Shigellosis outbreaks are common in internally displaced persons (IDP) shelters, prisons, childcare facilities, refugee camps, and crowding all contribute to the spread of the disease and restrict access to sanitary facilities (Gu *et al.,* 2012).

4. Contaminated Food and Water

A direct risk factor is drinking water or eating food tainted with human waste. Unwashed vegetables, street food prepared by unclean hands, and untreated water from tanks, rivers, or wells are common sources.

5. Lack of Hand Hygiene

One important contributing factor is not regularly washing your hands with soap, particularly after urinating and before eating or cooking (WHO, 2005).

6. Endemic Areas and Travel

Travelers are more likely to become infected when they visit low-income nations with inadequate public health facilities, especially if they consume local food or water (Riddle *et al., 2016).*

7. Immunocompromised Individuals

More severe and protracted forms of shigellosis may occur in people with compromised immune systems (for example, as a result of HIV/AIDS, malnourishment, or chronic illness).

8. Antimicrobial Resistance

Patients may suffer from longer illness and complications in regions where *Shigella* has become resistant to widely used antibiotics, particularly if treatment is postponed (Livio *et al.,* 2014).

2.6.2.7 Shigellosis through the World

Globally, and especially in low- and middle-income nations, shigellosis caused by bacteria of the genus *Shigella* remains a leading cause of diarrheal illness. Children under the age of five are disproportionately affected, and outbreaks usually take place in areas with inadequate sanitation, high population density, and restricted access to potable water.

An estimated 164.7 million *Shigella* episodes occur annually worldwide, with 163.2 million of those cases occurring in developing nations, of which 1.1 million are fatal. Sixty-one percent of all shigellosis-related deaths occur in children under the age of five (Kotloff *et al.,* 2013).

The distribution of *Shigella* species is not consistent worldwide. *Shigella dysenteriae* is typically found in regions of Asia, Africa, and South America that are heavily populated. Expansions of the epidemic are typically caused by infections. Serotype 1 is known for its virulence and capacity to cause devastating epidemics. India, Malaysia, and Guatemala are where it is most prevalent. Serotype 2 is more common in Nigeria and Yemen. In regions where endemic shigellosis is prevalent, *S. flexner*i is typically found. Only in the Indian subcontinent, where it was initially discovered, does *S. boydii* occur infrequently. Typically, *S. sonnei* is found in Western developed nations like the USA and France (German *et al.,* 2003).

2.6.2.7.1 Important epidemics reported in the last decades

The important epidemics reported in the last decades were;

i. In Central America in 1970, when 13,000 people perished and 112,000 were impacted.

ii. Five thousand people in Texas, USA, contracted the disease in 1985 after eating tainted lettuce.

iii. Several European nations, including Norway, Sweden, and the United Kingdom, reported domestic cases of S. sonnei infection in May and June 1994. Epidemiological data implicated imported iceberg lettuce as the spread agent.

iv. In Paris in 1996, where 153 patients were reported.

v. According to the Global Burden of Disease Study, *Shigella* caused more than 212,000 deaths worldwide in 2016 (Troeger *et al.,* 2018).

Table 2.2: Regional Trends and Prevalence

REGION	KEY NOTES
Sub-Saharan Africa	**High child mortality; *S. flexneri* is the dominant species**
South Asia	**High incidence, particularly in Bangladesh, Pakistan and India**
Southeast Asia	**increasing antibiotic resistance and frequent outbreaks**
Latin America	**Sanitation and water improvements have decreased cases, but there are still areas of risk**
High-income nations	**Incidence is lower, but outbreaks happen in childcare facilities and among men who have sex with men (MSM) (dominant S.**

sonnei)

2.6.2.7.2 Species Distribution

i. *S. flexneri*: Found mostly in developing countries.

ii. *S. sonnei* is most prevalent in developed countries (Livio *et al.*, 2014).

iii. *Type 1 S. dysenteriae*: Currently uncommon but linked to high mortality rates, this pathogen causes severe, epidemic dysentery

2.6.2.8 Ecology of Shigellae

The *Enterobacteriaceae* family of facultative intracellular Gram-negative bacteria includes *Shigella species*. Even though *Shigella* and *Escherichia coli* are closely related, *Shigella* has evolved to operate as a highly adapted human pathogen in the human gastrointestinal tract, a more specialized ecological niche.

1. Environmental Survival

Long-term environmental survival is difficult for *Shigella*, which is also susceptible to drying, high temperatures, and ultraviolet light. On the other hand, it can spread for days in moist environments, like tainted food or water (Sack *et al.,* 2004). The only natural host and reservoir is humans; animal reservoirs are not necessary.

2. Reservoir and Habitat

i. Primary reservoir: the intestines of humans

ii. Carrier state: For weeks following their recovery, people may continue to secrete the bacteria, thereby aiding in its spread (WHO, 2005).

Poor water quality, sanitation, and hygiene (WASH) conditions are the main places where transmission takes place.

2.6.2.9 Cycle of Shigellosis (Transmission Cycle)

The fecal–oral route of infection and the way *Shigella* spreads in human populations are highlighted by the shigellosis transmission cycle.

1. Excretion

Shigella, even in small amounts (as few as 10–100 organisms can cause disease), is excreted by infected people.

2. Environmental Contamination

Feces can contaminate food, hands, surfaces, fomites, and water sources like rivers and wells. This is particularly prevalent in places with open defecation and inadequate infrastructure for clean water and sanitation.

3. Transmission

Contaminated food or water, person-to-person contact (particularly in crowded settings like daycare centers or refugee camps), and inadequate hand hygiene practices are the three main ways that infection spreads to a new host.

4. Colonization and Invasion

After consumption, *Shigella* survives stomach acid, uses its Type III Secretion System (T3SS) to invade colon epithelial cells, and spreads intracellularly, causing severe inflammation and tissue damage that results in fever, diarrhea, and cramping in the abdomen.

5. Shedding

Within one to three days of exposure, infected people start excreting bacteria in their stool, which feeds the cycle.

2.6.2.10 Factors Supporting Shigella Ecology and Persistence

i. Minimal level of infection

ii. Carriers who don't show any symptoms

iii. Resistance to antibiotics, which increases the spread of infection and prolongs its duration

iv. Inadequate immunity (people can get infected again and again)

2.6.3 Cholera

2.6.3.1 The Genus Vibrio

Gram-negative rods with a single polar flagellum that are small and curved are called *vibrio. Vibrios* are facultative anaerobes that can metabolize both fermentatively and respiratively. Sodium is an essential element for the majority of species and promotes their growth. Most species convert nitrate to nitrite and are oxidase-positive. Certain species' cells (*V. cholerae, V. parahaemolyticus,* and *V. vulnificus*) have structures called pili (fimbriae) that are made of the protein TcpA. One important factor influencing in vivo colonization is TcpA formation, which is co-regulated with the expression of cholera toxin (Farmer *et al.*, 2005).

2.6.3.2 Major Human Pathogens in the Vibrio Genus

1. *Vibrio cholerae*

i. Causes the severe diarrheal illness known as cholera.

ii. Epidemic strains include serogroups O1 and O139.

iii. Spread through tainted food and water.

2. *Vibrio parahaemolyticus*

i. Causes gastroenteritis; raw or undercooked seafood is frequently implicated.

ii. Linked to outbreaks in coastal regions.

3. *Vibrio vulnificus*

i. Leads to sepsis, serious wound infections, and death.

ii. Raw oyster consumption or wound exposure to seawater frequently results

infection.

4. *Vibrio alginolyticus*

i. Usually results in infections of the ear, eyes, and wounds, particularly in people with weakened immune systems.

Humans can contract infections from a number of *Vibrio species*. Among these species, *V. cholerae* is by far the most significant. Numerous forms of soft tissue infections have been linked to *V. alginolyticus.* Gastrointestinal tract infections or diarrhea can be brought on by *V. fluvialis, Grimontia hollisae* (*V. hollisae*), and *V. mimicus.* There is no proof that *V. furnissii* can genuinely cause diarrhea, despite the fact that it has been isolated from a small number of sufferers. Especially in Japan and Southeast Asia, *V. parahaemolyticus* is a well-established cause of acute food-borne gastroenteritis. Consumption of raw or undercooked shellfish, including lobster, crabs, shrimp, and oysters, is linked to cases. *V. vulnificus* is a significant contributor to wound infections and septicemia, which are frequently fatal. *V. vulnificus* plays a significant role in wound infections and septicemia, which are frequently fatal. Humans are unrelated to other vibrios, such as *V. natriegens* and *Allivibrio fischeri* (*Vibrio fischeri*).

The majority of *Vibrio* bacteria are found in water. The distribution of species is influenced by water temperature and sodium content. In marine and estuarine environments, vibrios are extremely prevalent. They can live freely on surfaces or in the digestive tracts of marine animals. Freshwater environments are also home to species with low sodium requirements.

2.6.3.3 The Species Vibrio Cholerae

Cells of *Vibrio cholerae* can grow at 40°C and pH 9–10. When sodium chloride is present, the growth is stimulated. The bacterial species *Vibrio cholerae* is extremely varied. The structure of the lipopolysaccharide (LPS) (O antigens) distinguishes it into about 200 serovarieties. The only serovarieties implicated in true cholera are O1 and O139. Cholera cannot be caused by certain serovarieties, but they can cause gastroenteritis. El Tor and Classical biotypes are distinguished by virological and biochemical traits (Todar *et al.,* 2009).

2.6.3.4 Etiological Agent

i. Species: *Vibrio cholerae*

ii. Pathogenic strains: O139 and O1 (El Tor and Classical biotypes) serogroups

iii. Virulence factor: Cholera toxin (CT), which is encoded by the ctxAB genes that the CTXϕ bacteriophage carries.

2.6.3.5 Cholera

2.6.3.5.1 Characterization of the disease

The acute, secretory diarrheal illness known as cholera is brought on by drinking water or eating food tainted with toxic strains of *Vibrio cholerae*, mainly serogroups O1 and O139. It can be fatal if left untreated. It is characterized by the abrupt onset of watery, profuse diarrhea that causes electrolyte imbalance and rapid dehydration.

Table 2.3: Clinical Features of Cholera

Features	Description
Incubation period	**2 hours to 5 days (average 1–3 days)**
Onset	**Sudden with painless, profuse watery diarrhea**
Stool appearance	**"Rice-water" stool (grayish-white, flecked with mucus, odorless)**
Dehydration signs	**Sunken eyes, dry mouth, low blood pressure, rapid heartbeat**
Other symptoms	**Vomiting, leg cramps, weakness, no fever**

	(usually)
Complications	**Hypovolemic shock, acidosis, renal failure, death if untreated**

2.6.3.5.2 Severity

i. Cholera that is mild to moderate: self-limiting diarrhea

ii. Severe cholera (about 10% of cases): need for immediate rehydration due to rapid dehydration

Numerous factors determine how severe the disease is;

i. Personal immunity: this can be given by vaccinations or past infections.

ii. Inoculum: the illness doesn't manifest until a certain quantity of cells, about 108, are consumed.

iii. The gastric barrier: Because *V. cholerae* cells prefer basic media, the stomach, which is typically highly acidic, is a hostile environment for bacterial survival. Antacid medication users are more prone to infection than healthy individuals.

iv. Blood group: O-group individuals are more vulnerable than others for as-yet-unidentified reasons.

Without treatment, cholera patients have a mortality rate of up to 50%; with treatment, it is less than 1%. It is necessary to replenish lost salts, particularly potassium, in addition to lost water. Water and salts can be taken orally for mild cases of dehydration, but rapid intravenous administration is required for severe cases. At the moment, doxycycline is the most effective antibiotic. In many situations, administering water containing salts and sugar can save the patient and aid in their recovery if antibiotics are not available for treatment (WHO, 2017).

Infection is primarily determined by two factors;

i. The presence of adhesins and pili on the surface of the bacterial cells is necessary for their adherence to the intestinal mucous membrane.

ii. The cholera toxin's production

2.6.3.6 Cholera toxin

Cholera toxin is an exotoxin that targets cells with extreme precision. The toxin activates the enzyme adenylate cyclase by binding to a particular receptor (ganglioside Gl) on intestinal cell membranes. As a result, internal ATP continuously degrades, releasing inorganic phosphate and cAMP. The primary cause of diarrhea is an efflux of water, sodium, potassium, chloride, and carbonate ions from the mucous membrane cells brought on by an increase in the internal concentration of cAMP.

2.6.3.7 Cholera pandemics and the new facts about cholera epidemiology

A well-known illness since the 19th century is cholera. There have been seven significant pandemics in the 19th and 20th centuries. These are the times when the first six pandemics happened: First: 1816-1826; Second: 1829-1851; Third: 1852-1875; Fourth: 1863-1875; Fifth: 1881-1896; Sixth: 1899-1923. All of these pandemics began in Asia and traveled to South America via Europe. There was involvement of the classical biotype. The seventh pandemic began in 1961 in Asia's Celebes Islands and is still going strong. The disease began to spread throughout Asia in the 1960s, then to the Middle East and Africa in the 1970s, and finally to South America in 1991, where it rapidly spread. El Tor has now taken the place of the *classical biotype.* Although the *El Tor biotype* had been identified in 1905, it wasn't until the seventh pandemic that it overtook the Classical biotype and took over as the dominant one.

Bangladesh was the first place where a new serovariety (O139), known as the Bengal serovariety, was discovered in 1992. This novel serovar swiftly replaced O1 in Southeast Asia and India. Despite the resurgence of serovariety O1 El Tor in 1994 and 1995, the Bengal serovariety continues to hold its dominant position. Serovarieties O139 and O1 cause illnesses that are identical (Farmer *et al.,* 2005).

In 1991, the seventh pandemic made its way into South America via Peru's coastal region. The disease was confirmed when *Vibrio cholerae O1 El Tor* was isolated from cholera-symptomatic patients in Chancay, northern Peru, on January 23. 66 people died and 1,859 were hospitalized in this area between January 24 and February 9. The disease quickly spread from Peru to other South American nations. There are two possible ways that the bacteria could enter Peru;

i. A boat's ballast water arriving from Asia

ii. Zooplankton carrying V. cholerae cells might have been carried by the El Niño current. Fish and shellfish that consumed this zooplankton became contaminated, and people who consumed these marine foods contracted the bacteria.

Important epidemiological studies were conducted during this outbreak, and the tragedy of those who perished in the initial months of this devastating cholera epidemic in South America seemed to have spurred scientists to work harder on the disease's research. These studies demonstrated that cholera can also spread through contaminated uncooked food and drink.

2.6.3.8 Ecology of the bacterium and the cycle of the disease

Particularly in estuaries, *V. cholerae* strains that are not O1 or *O139* are prevalent in the environment. They live and proliferate on the surface of phytoplankton and zooplankton cells and have been isolated from a variety of estuarine animals, including fish, birds, frogs, and shellfish.

Only in areas where there is an epidemic can *V. cholerae O1* and O139 strains be isolated from the environment. They endure for a long time in water and among aquatic and marine organisms in the cultivable state. Unfavorable environmental conditions cause *V. cholerae* cells to shrink, turn coccoid, and go into a dormant state within exopolysaccharide biofilms. On the surface of agarized media, cells exhibit a particular metabolism but are unable to proliferate and form colonies. For extended periods of time, cells in this viable but non-culturable state maintain both their viability and their capacity for pathogenicity (Alam *et al.,* 2006).

Viable but non-culturable cells have the ability to remultiply after going dormant, which causes their concentration in the environment to skyrocket. A horizontal transfer of cholera exotoxin-producing genes between toxigenic and non-toxigenic strains can result in a sharp and quick

increase in the number of toxigenic cells in the environment because non-toxigenic strains are frequently found in aquatic environments, particularly estuaries. These events are most likely connected to the episodic character and abrupt onset of violent cholera outbreaks, which are then rapidly slowed down.

2.6.4 Pathogenic Escherichia coli Strains

Escherichia coli is a facultative anaerobe that is Gram-negative and usually a harmless gut commensal. However, certain virulence determinants that are acquired by pathogenic *E. coli*, usually on plasmids or bacteriophages, allow for intestinal and extraintestinal disease (Alhadlaq *et al.,* 2024; Anueyiagu *et al.*, 2024).

Based on phenotypic characteristics, clinical manifestations of the illness, epidemiological evidence, and particular virulence factors, *E. coli* strains isolated from intestinal diseases have been divided into at least six major groups. These include the highly significant enterotoxigenic (ETEC, specifically O148), enterohemorrhagic (EHEC, specifically O157), and enteroinvasive serotypes (EIEC, specifically O124) that can spread through tainted water (Bettelheim *et al.,* 2003).

Table 2.4: Major Pathogenic E. coli Strains (Pathotypes)

Pathotype	Disease Type	Key Virulence Factors	Main Populations Affected
ETEC (Enterotoxigenic)	**Traveler's diarrhea, infant diarrhea**	**Heat-labile (LT) and heat-stable (ST) enterotoxins**	**Travelers, children in developing countries**
EPEC (Enteropathogenic)	**Infantile diarrhea**	**Bundle-forming pili (BFP), type III secretion system**	**Infants, especially in low-income regions**
EHEC/STEC	**Bloody diarrhea,**	**Shiga toxin (Stx1,**	**All ages, often**

(Enterohemorrhagic/Shiga toxin-producing)	**hemolytic uremic syndrome (HUS)**	**Stx2), intimin**	**foodborne**
EIEC (Enteroinvasive)	**Dysentery-like illness**	**Invasion plasmid antigens (ipa genes)**	**Children and adults**
EAEC (Enteroaggregative)	**Persistent diarrhea**	**Aggregative adherence fimbriae (AAF), EAST1 toxin**	**Children, immunocompromised**
DAEC (Diffusely Adherent)	**Mild diarrhea**	**Diffuse adherence pattern**	**Mainly children**

2.6.4.1 Enterotoxigenic E. coli (ETEC) Strains

Gastroenteritis in infants can be caused by *enterotoxigenic E. coli* (ETEC) serotypes. It is a major cause of diarrhea in the developing world, where there is inadequate clean water and poor sanitation, but there are relatively few reports of its occurrence in developed countries. These strains cause 300,000–400,000 deaths annually and 200 million cases of diarrhea in developing nations, where they are the most frequently isolated bacterial enteropathogenic in children under the age of five (WHO, 2020).

Ingestion of contaminated food or water can spread the ETEC disease through the fecal-oral route. The symptoms include nausea, cramping in the abdomen, and a lot of watery diarrhea. The illness typically lasts for three to five days and causes dehydration and malnutrition in young children. According to Scheutz *et al.* (2005), ETEC is also the most frequent cause of travelers' diarrhea (30–60%) among those who are visiting developing nations. ETEC's involvement in pediatric diarrheal illness is highlighted by recent African genomic studies that confirm the

presence of eltA/B and cfa adhesion factor genes in the majority of isolates (Chekole *et al.,* 2025).

2.6.4.1.1 Laboratory Diagnosis

ETEC cannot be detected by a typical stool culture. PCR assays for toxin genes (elt for LT, est for ST), ELISA or bioassays for toxin activity, and frequently a referral to specialized labs are necessary for detection.

2.6.4.1.2 Prevention and Control

Although there isn't a vaccine that is currently widely accessible, oral vaccines (like ETVAX) are being developed or are undergoing clinical trials. Clean water and food hygiene are still crucial. Rehydration therapy (ORS) is the cornerstone of care, and while antibiotics can shorten the duration of illness, they are not usually advised.

2.6.4.2 Enterohemorrhagic E. coli (EHEC) Strains

Enterohemorrhagic E. coli (EHEC), also called Shiga toxin-producing E. coli (STEC), is a highly pathogenic strain of *E. coli* that can cause hemolytic uremic syndrome (HUS) and hemorrhagic colitis, as well as bloody diarrhea. Although *E. coli O157:H7* is the most well-known EHEC strain, other serotypes such as O26, O45, O103, O111, O121, and O145 can also cause illness (Gyles, 2007).

The consumption of contaminated foods, such as raw or undercooked ground beef or meat products, raw milk, and apple cider, has been primarily linked to reported outbreaks. Healthy cattle have been identified as this bacterium's main reservoir. WHO (2010). Although outbreaks (like the 2011 O104:H4 in Germany) show possible severity, the prevalence is low (0.5–1.2% in some African/Asian studies) (Iranian meta-review; *Alhadlaq et al.,* 2024).

Severe cramping in the abdomen, bloody diarrhea, hemolytic uremic syndrome, and no or low-grade fever are all symptoms of *E. coli* serotype O157:H7. This bacterium makes toxins that resemble Shiga. Three to four days after ingestion is the incubation period, and symptoms appear seven to ten days later. Between 2 and 7% of *E. coli* O157:H7 infections are thought to cause thrombocytopenia, hemolytic anemia, and acute renal failure.

Despite the fact that *E. coli* O157:H7 is usually not a problem in treated drinking water, outbreaks have been documented where drinking water tainted with human sewage or cattle feces was consumed. The consumption of fruits and vegetables (sprouts, lettuce, coleslaw, and salad) tainted with domestic or wild animal excrement at some point during production or handling is linked to an increasing number of outbreaks. In sediments found in water troughs and manure, EHEC can survive for several months. Additionally, it has been isolated from water bodies (ponds, streams), water troughs, and wells.

One significant method of transmission via the oral-fecal route is person-to-person contact. There have been reports of an asymptomatic carrier state, in which people are able to infect others despite not exhibiting any clinical symptoms of the illness (WHO, 2010).

2.6.4.2.1 Diagnosis

PCR to identify the stx1, stx2, and eae genes; stool culture on Sorbitol-MacConkey (SMAC) agar (O157:H7 does not ferment sorbitol); and enzyme immunoassays (EIA) to identify toxins.

2.6.4.2.2 Treatment and Prevention

Antibiotics should not be used because they may increase the release of Shiga toxin and the risk of HUS (Tarr *et al.,* 2005). Instead, oral or IV rehydration and monitoring for HUS symptoms are recommended. Hand hygiene and food safety, avoiding unpasteurized dairy and juice, and thoroughly cooking meat.

2.6.4.3 Enteroinvasive E. coli (EIEC) Strains

A pathogenic strain of *E. coli* known as *enteroinvasive E. coli* (EIEC) produces an illness that is comparable to bacillary dysentery and closely resembles infections brought on by *Shigella spp.* In humans, they can invade and proliferate in the intestinal epithelial cells of the distal large bowel. According to Taneja *et al.* (2016), the illness has a one- to three-day incubation period and is characterized by abdominal cramps, watery or bloody diarrhea, vomiting, fever, chills, and a generalized malaise.

For example, during a period of high dysentery in the Jesreel district of Israel, EIEC strains were isolated from 28 subjects. *E. coli* O124 was often isolated from gastroenteritis, enterocolitis, and

dysentery cases, according to a Croatian investigation. While the two other disease types were equally prevalent in all age groups, dysentery was more prevalent in the older age groups. In Bangkok, Thailand, 410 children with diarrhea and an equal number of control children without diarrhea participated in a 1985 survey to check for the presence of *Shigella, EIEC,* and other pathogen strains. Six children without diarrhea and seventeen with diarrhea were found to have produced EIEC.

Any food that has come into contact with human waste from a sick person, either directly or through tainted water, has the potential to infect others. Unpasteurized milk, food, water, and hamburger meat have all been linked to outbreaks, particularly in South America, Southeast Asia, and Africa.

2.6.4.3.1 Diagnosis

Serotyping and cell invasion assays, as well as PCR detection of invasion genes (ipaH, ial), are employed in research settings because culture and biochemical tests might not be able to differentiate EIEC from commensal *E. coli.*

2.6.4.3.2 Prevention & Treatment

Improved sanitation, safe food handling, clean water access, and the use of antibiotics (such as ciprofloxacin or azithromycin) in severe cases, particularly in travelers, are the mainstays of supportive care with oral rehydration therapy (ORT).

2.6.5 Campylobacter

Gram-negative rods with a curved or spiral shape that are motile through polar flagella are found in the genus *Campylobacter.* They thrive in conditions with lower oxygen levels (5%) and higher carbon dioxide levels (10%) because they are microaerophilic (Kaakoush *et al.*, 2015). These bacteria, which are catalase and oxidase-positive, metabolize amino acids rather than fermenting carbohydrates . The genera *Arcobacter* and *Campylobacter* belong to the family *Campylobacteraceae.* Bacterial gastroenteritis is known to be caused by members of both genera that can spread from person to person, through contaminated food and water, or through contact with animal reservoirs (Igwaran *et al.,* 2019). The typical symptoms of campylobacteriosis include fever, cramping in the abdomen, nausea, malaise, and bloody diarrhea. These symptoms

usually manifest 2 to 5 days after exposure. The illness usually goes away on its own after a week or so.

Rarely, though, campylobacteriosis can cause inflammatory bowel disease (IBD), esophageal disorders, periodontal disorders, celiac disease, colorectal cancer, Miller-Fisher syndrome, Guillain-Barré syndrome (GBS), an autoimmune neuropathy, bacteremia and septicemia in immunocompromised individuals, hemorrhage, meningitis, and reactive arthritis (Kaakoush *et al.*, 2015). Studies on human volunteers have shown that as few as 500 *C. jejun*i can cause illness, indicating that the infectious dose of *Campylobacter* is believed to be low.

Unpasteurized milk, untreated drinking water, and undercooked poultry are the main causes of campylobacteriosis. Furthermore, water may serve as a common source of infection for domestic animals, poultry, wild birds, and humans. Defects in water treatment processes, fecal contamination, wastewater discharge into the water source, broken water pipes, or malfunctioning disinfection equipment have all been linked to waterborne outbreaks that have been documented globally. Notably, *Campylobacter* has been identified as the most common bacteria linked to waterborne outbreaks, with the exception of parasites (Moreira *et al.,* 2017).

Overall, 78% of all acute gastrointestinal illnesses and 12% of waterborne disease outbreaks in the United States have been linked to *Campylobacter. Campylobacter* has been identified as one of the main causative agents in Canada's waterborne outbreak investigations. Over the past ten years, outbreaks of waterborne campylobacteriosis have been documented in numerous developed nations, according to a review by Kaakoush *et al.* (2015). Due to either water distribution issues or fecal contamination from wild birds, Nordic countries and Australia/New Zealand have experienced the highest number of waterborne *Campylobacter* outbreaks and cases among developed nations (Jalava *et al.,* 2014).

In Japan, foodborne campylobacteriosis has been reported, but only one case of the same strain of *C. jejuni* was reported in patients who had stomach pain and water that had been tainted due to chlorination failure (Abe *et al.,* 2008). It is challenging to diagnose campylobacteriosis in the developing world due to the pathogen's widespread presence in food and water sources, as well as confounding risk factors like malnutrition, undernutrition, and poor sanitation, which make it hard to draw clear conclusions. However, research conducted in rural coastal areas of Kenya, the

Northwest Province of South Africa, and Northwest Ethiopia has linked drinking water to campylobacteriosis in children under the age of five (Chukwu *et al.,* 2019).

2.6.5.1 Pathogenic Mechanisms and Virulence Factors

Campylobacter expresses adhesins, invasins, and cytolethal distending toxin (CDT), which damages host DNA and induces cell cycle arrest. It also uses flagellar motility to break through intestinal mucus (Kaakoush *et al.,* 2015). Bacterial lipopolysaccharide (LOS) mimics host gangliosides and can lead to autoimmune complications, such as Guillain-Barré syndrome (GBS).

2.6.5.2 Diagnosis

The basis for the diagnosis is stool culture in microaerophilic conditions with selective media at 42°C and PCR or ELISA assays for quicker detection (WHO, 2020).

2.6.5.3 Treatment

In most cases, supportive care is all that is needed. Nonetheless, azithromycin is the recommended antibiotic for infections that are severe or persistent. Although they are substitutes, fluoroquinolones such as ciprofloxacin are becoming more and more resistant worldwide (CDC, 2022).

2.6.5.4 Prevention

Poultry prepared properly, Stay away from untreated water and raw milk. Maintain clean hands after handling raw food or animals, and keep raw meats apart to avoid cross-contamination.

2.6.6 Helicobacter Pylori

*Helicobacter pylor*i has been cited as a major etiologic agent for gastritis and has been implicated in the pathogenesis of peptic and duodenal ulcer disease and gastric carcinoma. However, most individuals that are infected by this pathogen remain asymptomatic.

Water is one of the environmental sources from which *H. pylor*i has not been isolated using culture-based techniques. However, this pathogen has been successfully detected using molecular methods. In drinking water distribution systems and other water bodies, this pathogen has been successfully identified using fluorescence in situ hybridization. The presence of *H.*

pylori DNA in drinking water, particularly that linked to biofilms, has also been found using the polymerase chain reaction method. Viable but non-culturable *H. pylori c*ells quickly lose their ability to be cultured in drinking-water biofilms. With densities exceeding 106 cells per cm^2, cells can survive in these biofilms for over a month (Gião *et al.,* 2008).

The transmission mechanism of the organism remains incompletely understood. Nonetheless, it is highly suggestive of oral-oral or fecal-oral transmission because it has been found in saliva, dental plaques, the stomach, and fecal samples. Despite their apparent lack of direct significance, food and water can nevertheless have a big impact when there is poor sanitation and hygiene.

2.7 Microbiological Water Analysis

The three most common bacterial gastrointestinal illnesses spread by water are shigellosis, salmonellosis, and cholera. The primary means of spreading these illnesses is through food and water tainted with patient excrement. It is extremely concerning that these harmful bacteria can contaminate drinking water. However, the levels of pathogenic bacteria in water are low, their presence is irregular and sporadic, and it is difficult to isolate and cultivate them. For these reasons, the identification of harmful bacteria is not a part of routine water microbiological analysis. However, water must be free of harmful bacteria in order to be considered safe.

Finding and testing indicator bacteria allowed for the conciliation of the two needs. Additionally, the normal inhabitants of the human intestine are present in water contaminated with pathogenic species. A reliable bacterial indicator of faecal contamination should meet the following requirements;

i. Are abundant in human intestines and feces.

ii. Be non-toxic to people.

iii. Doesn't grow outside of the intestinal environment

iv. The indicator should be more prevalent than potentially harmful bacteria in environmental waters.

v. The die-off behavior of the indicators and the pathogens should be comparable.

vi. Farm and domestic animals' intestines should not contain a lot of the indicator if human fecal pollution is to be distinguished from animal pollution (WHO, 2008).

vii. In environmental waters, it is inexpensive, simple, and reliable to detect. The following requirements should also, if at all possible be fulfilled.

Numerous studies, including one by Wilkes *et al.* (2009), have demonstrated the value of indicator bacteria in anticipating the presence of pathogens.

2.8. Culture-Based Microbial Source Tracking (MST)

Microbial source tracking (MST) refers to methods for identifying the source of faecal contamination in environmental waters. The growth and identification of bacteria from fecal sources on selective media are the main goals of culture-based MST. Because of its affordability, ease of use, and low technical requirements, this approach continues to be the mainstay of microbial analysis in developing nations (Rompré *et al.,* 2002; APHA, 2017).

Selective media like MacConkey agar for coliforms, Eosin Methylene Blue (EMB) agar for *E. coli,* Nutrient agar for general bacterial growth, and Xylose Lysine Deoxycholate (XLD) agar for isolating gram-negative enteric bacteria are commonly used in culture-based procedures. These techniques enable the isolation of pathogens for additional biochemical or antibiotic susceptibility testing, as well as the quantification of faecal indicators (Adewoyin and Ogedengbe, 2020).

Culture-based approaches have drawbacks despite their benefits. Because some organisms cannot grow on synthetic media, they are labor-intensive, time-consuming, and may understate microbial diversity (Field and Samadpour, 2007). Furthermore, their inability to distinguish between animal and human sources of faecal contamination restricts their use in focused mitigation techniques.

2.9 Microbial Source Tracking (MST) Methods

Human waste is frequently connected to the prevalence o*f Enterococcus faecalis* and *E. coli* serotype O157:H7 (Scott *et al.*, 2002). Molecular methods like DNA fingerprinting, quantitative

PCR (qPCR), and polymerase chain reaction (PCR) are now considered advanced MST tools. But these techniques are frequently costly and technically complex.

Despite being less precise, culture-based MST is still useful in many developing nations with inadequate laboratory infrastructure (Harwood *et al.,* 2014).

2.10 Advantages and Limitations of Culture-Based Detection

Culture-based detection techniques are preferred because they are easy to use, reasonably priced, and can recover viable organisms that can be used for additional analysis, including genotyping, serotyping, and antimicrobial resistance profiling (APHA, 2017). For routine water quality monitoring, these techniques are also beneficial, especially in environments with limited resources.

However, the limitations include the inability to track specific sources of contamination and decreased sensitivity, particularly in detecting viable but non-culturable (VBNC) bacteria (Noble *et al.,* 2003). Furthermore, in outbreak situations, the necessary incubation period (typically 24 to 48 hours) may cause a delay in prompt public health interventions.

2.11 Public Health Implications of Fecal Contamination

Numerous infectious diseases, such as cholera, dysentery, typhoid fever, and hepatitis A, are linked to drinking or coming into contact with tainted water. Immunocompromised people, the elderly, and children are disproportionately affected by these illnesses. According to WHO (2017), over 2 billion people worldwide drink water tainted with feces, which causes about 485,000 cases of diarrheal illness each year.

In Nigeria, diarrheal illness continues to be a major cause of death for children under five, primarily as a result of inadequate water and sanitation facilities. According to Okeke *et al.* (2007), the health burden is further increased by the growth of antibiotic-resistant pathogens in surface waters.

2.12 Regional and Global Case Studies

Studies conducted in Europe, North America, and Asia have shown that a range of human activities can contaminate rivers and lakes with feces. Field and Samadpour (2007), for instance,

employed MST techniques to link sewage treatment facilities and livestock to fecal contamination in the Ohio River in the United States.

Olalemi *et al.* (2020) discovered *Shigella, E. coli,* and *Vibrio species* in the Awba River in Ekiti State, Nigeria. The contamination was linked to open defecation and refuse dumps, which are closely associated with market activity and human settlements.

In a different study, Igbinosa and Okoh (2009) discovered that the levels of pathogens in the rivers in the South African province of the Eastern Cape varied seasonally, with the rainy season showing the highest contamination.

E. coli and *Salmonella* were found in the Tyume River by Igbinosa *et al.* (2012), highlighting the dangers to downstream users, and Adewumi *et al.* (2014) reported elevated coliform levels in the Odo Ona River, Ibadan, connected to abattoir waste.

2.13 Relevance of Culture-Based MST in Nigeria

Culture-based MST is still a useful and efficient technique for detecting microbial contamination in rivers like the Ogba, despite the financial and technological limitations in the majority of Nigeria. Culture-based approaches can offer adequate baseline data for decision-making and public health interventions, despite not being as discriminatory as molecular methods (Adewoyin and Ogedengbe, 2020).

Antimicrobial resistance testing can also be performed on isolated strains from culture-based techniques, providing more information about potential threats to public health (Okeke *et al.,* 2007). Despite the fact that river microbial pollution in Nigeria is well-established, little research has been done on the Ogba River specifically using culture-based MST techniques. The majority of studies use molecular techniques or concentrate on physico-chemical parameters, which are frequently impractical in local settings. This study fills this knowledge gap by identifying faecal bacteria using easily accessible culture-based methods, which makes it pertinent for immediate public health use.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

In order to analyze water samples from the Ogba River microbiologically using culture-based detection methods, this study used an experimental research design. The design made it possible to isolate, identify, and count bacterial pathogens in feces in order to assess the river water's microbiological quality. For evaluating environmental contamination, the method was suitable, especially for pathogenic bacteria and fecal indicator organisms (Bartram and Rees, 2000).

3.2 Study Area

Benin City, the capital of Edo State in southern Nigeria, is where the Ogba River is situated. The river passes through a number of commercial, industrial, and residential areas before joining other water systems. Along its banks, notable human activities include bathing, washing, open defecation, and the disposal of waste from homes and slaughterhouses. Fecal contamination is more likely as a result of these human activities. The study was conducted at the Central Research Laboratory, University of Benin, Benin City, Edo State. The sampling sites' geographic coordinates were 6°20'N to 6°25'N and 5°35'E to 5°40'E.

3.3 Sample collection

A water sample was taken from the OGBA River in Benin City, Edo State, using a sterile container. The sample was sent right away to the laboratory for microbiological analysis within six hours (APHA, 2017).

It was crucial to transport the samples quickly in order to maintain their integrity, guarantee accurate test results, and reduce the possibility of microbial content changes brought on by exposure to the environment.

3.4 Apparatus

Beakers, conical flasks, measurements cylinder, micropipette, sterilize petri dish, aluminum foil paper, analytical weighing balance, Bunsen burner, test tubes, volumetric flasks, incubator, thermometer, autoclave, colony counter, vortex mixer, microscope, glass slides, cotton wool and personal protective equipment such as laboratory coat, gloves, nose masks and shoes.

3.5 Sterilization of Work Bench and Materials

In accordance with laboratory rules and regulations, all laboratory work was completed in an aseptic environment. The work surface was cleaned with 70% ethanol, and glassware was thoroughly cleaned and autoclaved at 121°C and 15 mmHg for 15 minutes prior to use. During the practical procedures, a laboratory coat was worn, and proper hygiene was maintained.

3.6 Laboratory Analysis

3.6.1 Preparation of Culture Media

The used media were prepared in accordance with the manufacturer's instructions and autoclaved for 15 minutes at 121°C and 15 psi to sterilize them. The media utilized were Salmonella Shigella agar, Eosin Methylene Blue agar, Mac Conkey agar, Nutrient Agar, and Mannitol sat agar.

3.6.1.2 Preparation of Nutrient Agar

28 grams of nutrient agar (NA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.6.1.3 Preparation of MacConkey agar

MacConkey agar (MCA) was prepared by dissolving 51.55 grams of agar powder in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.6.1.4 Preparation of Eosin methylene blue agar

35.96 grams of Eosin methylene blue agar (EMBA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.6.1.5 Preparation of Mannitol salt agar

111 grams of Mannitol salt agar (MSA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.6.1.6 Preparation of Salmonella Shigella agar

28 grams of Salmonella Shigella agar (SSA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.7 Sample preparation

3.7.1 Serial dilution

In a test tube, 9 ml of sterile distilled water was mixed with 1 ml of OGBA water sample. In order to achieve a 10^3 dilution, a sterile pipette was used to transfer the contents of the first test tube to the second, which contained 9 ml of distilled water. To obtain 10^2, 1 ml was taken with a pipette from the test tube that contained the 10^1 dilution and moved to the third tube that contained 9 ml of sterile diluted water.

3.8 Inoculation and Incubation

A 1 ml aliquot of a 10^3 diluted sample was inoculated into 6 sterile petri dishes. Prepared and sterilized nutrient agar was poured into the inoculated petri dishes and mixed gently to evenly spread the organisms and then allowed to solidify. The petri dishes were incubated at 37 degrees Celsius for 24 hrs.

3.9 Subculturing

To obtain the pure culture, the bacterial colonies were streaked on a new nutrient agar medium. On the surface of a nutrient agar plate medium, a single colony was recognized and re-streaked as the primary inoculant. Nutrient agar plates were examined for pure cultures. The same colony was streaked onto a nutrient agar slant once a pure culture was achieved. For a full day, these cultures were incubated at 37°C.

3.10 Bacterial Identification

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar, MacConkey agar, Mannitol salt agar and Salmonella Shigella agar. Biochemical tests were also carried out to further identify the bacterial isolates.

3.11 Gram staining test for bacterial isolate

The smear of each of the isolates was prepared by picking a small portion of microbial growth from the plates, then the slides were heated and fixed carefully. The heat-fixed smears were stained with crystal violet for 60 seconds, washed off with water, and drained, then flooded with Lugol's iodine for about 60 seconds and washed off gently with water and drained. The slides were rinsed with 50-50 alcohol-acetone for 3 seconds and were rinsed with water and drained. The slides were then counterstained with safranin for 1 min; after that, the stains were washed off with water. The slides were air-dried; immersion oil was dropped on the smears, and the smears were examined for cell morphology and arrangement, presence of capsule, and staining reaction.

3.12 Biochemical test for identification of isolates

The selected bacterial isolates were subjected to biochemical and staining techniques as described and keyed in the Bergey’s Manual of Determinative Bacteriology. The following biochemical tests were carried out: catalase test, oxidase test, Urease test, coagulase test, methyl red test, indole test, and citrate test.

3.12.1 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubbles indicated a catalase-positive enzyme.
$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.12.2 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

3.12.3 Urease Test

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48 hours. Urease-positive cultures produced a red-pink color due to changes in the color of the indicator.

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

3.12.4 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hours. The development of deep blue color after incubation indicates a positive result.

3.12.5 Hydrogen Sulfide (H_2S)

Test Hydrogen sulfide production can be detected by incorporating a heavy metal salt containing iron (Fe^{2+}) or lead (Pb^{2+}) ions as an H_2S indicator into a nutrient culture medium containing cysteine and sodium thiosulfate as the sulfur substrates. Hydrogen sulfide, a colorless gas, when

produced, reacts with sulfur metal salt (ferrous sulfate), forming a visible insoluble black sulfide precipitate.

3.12.6 Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the family Enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethylaminobenzaldehyde and amyl alcohol is used. The broth was inoculated with the test organism and incubated for 18 hours at 37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red color at the interface of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

3.13 Quality Assurance and Control

Strict aseptic techniques were employed throughout the sampling and analysis processes. Media sterility was verified by incubating uninoculated plates. All glassware was sterilized before use. Replicates of each sample and control strain of known bacterial species were used to validate results. Standard operating procedures (SOPs) were adhered to as recommended by APHA (2017).

CHAPTER FOUR

RESULTS

Table 1 shows the cultural examination as carried out on the bacterial isolates. Cultural characteristics such as colony shape, elevation, optical activity margination and pigmentation were evaluated and recorded. Biochemical test were carried out to further identify the bacterial isolates on table 3 after Gram staining was done to observed the gram reaction of the bacterial cell as shown in table 2. The isolates identify are Escherichia coli, Salmonella, Shigella and Staphylococcus aureus.

TABLE 1

4.1 Cultural characteristics of the bacterial isolates

Bacterial	Agar	Colony morphology	Elevation	Margination	Pigmentation	Optical density
Staphylococcus aureus	NA	Round, smooth convex	Raised	Entire	Golden yellow	Opaque
	MSA	Round	Raised	Entire	Golden yellow	Opaque
Salmonella	NA	Smooth, colorless	Flat	Entire	None	Translucent
	MAC	Small, colorless	Slightly raised	Entire	None	Translucent
Shigella	NA	Small, smooth, translucent	Flat	Entire	None	Translucent
E.coli	NA	Smooth, greyish	Slightly raised	Entire	None	Translucent
	MAC	Pink, small	Slightly raised	Entire	Pink	Opaque
	EMB	Pink/ red with green metallic sheen	Slightly raised	Entire	Pink/ red	Opaque with sheen

TABLE 2

4.2 Gram stain reaction of all isolates

SHAPE OF THE ISOLATES	COLOR OBSERVED	SUSPECTED ORGANISM
Coccus/Round	Purple	Staphylococcus
Rod	Pink/ red	Escherichia coli

Rod	**Pink/ red**	**Salmonella**
Rod	**Pink/ red**	**Shigella**

TABLE 3

4.3 BIOCHEMICAL TEST

TEST	**E.coli**	**SALMONELLA**	**SHIGELLA**	**STAPHYLOCOCCUS aureus**
Catalase	+	+	+	+
Oxidase	–	–	–	–

Mannitol salt agar	_	_	_	+
Indole	+	_	_	_
Citrate	_	+	_	_
Urease	_	_	_	+
Methyl red	+	+	+	+
H_2S production	_	+	_	_
Vogues Proskauer [VP]	_	_	_	+
Lactose	+	_	_	_
Coagulase	_	_	_	+

Key:-

+

-

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The present study focused on the microbial source tracking of faecal bacterial pathogens in Ogba River, Benin City, Nigeria. The investigation involved the isolation and identification of bacteria from water samples collected from different points along the river, using cultural, morphological,

and biochemical methods. The isolates obtained were *Staphylococcus aureus, Escherichia coli, Salmonella spp.* and *Shigella spp.,* which are well-recognized indicators of faecal and anthropogenic contamination in surface water systems. The presence of these pathogens clearly demonstrates the deteriorating microbial quality of the river and suggests contamination from both human and animal sources. The cultural characteristics of the bacterial isolates revealed distinctive features that aided their identification. *Staphylococcus aureus* produced raised, smooth, and golden-yellow colonies on nutrient and mannitol salt agar, consistent with its ability to ferment mannitol and produce pigment. *Salmonella spp.* formed smooth, translucent, and non-lactose fermenting colonies on nutrient and MacConkey agar while *Shigella spp.* also produced small, translucent colonies, indicating their close morphological relationship as members of the Enterobacteriaceae family. *E. coli* showed pink to red colonies on MacConkey agar and a characteristic metallic sheen on eosin methylene blue (EMB) agar, confirming its lactose-fermenting property. These cultural observations correlate with the findings of Adenugba and Akinmoladun (2023), who reported similar characteristics in isolates obtained from rural streams in Ekiti State, Nigeria. The distinct colony appearances on selective and differential media validate the use of these methods for rapid identification of faecal indicator bacteria.

The Gram staining reaction further supported the identification of these isolates. *Staphylococcus aureus* appeared as Gram-positive cocci arranged in clusters while *E. coli, Salmonella spp.* and *Shigella spp*. were all Gram-negative rods, which is typical of enteric pathogens. The dominance of Gram-negative bacteria in the isolates is a strong indicator of fecal pollution, as members of this group, particularly the Enterobacteriaceae, are commonly associated with intestinal tracts of humans and warm-blooded animals. This pattern aligns with the observations of Abdulkareem *et al.* (2023), who reported a predominance of Gram-negative enteric bacteria in surface water samples influenced by agricultural runoff and domestic discharges.

The biochemical characterization provided confirmatory evidence for the identity of the isolates. *E. coli* was catalase positive, oxidase negative, indole positive, methyl red positive, and capable of fermenting lactose, which are diagnostic features of this species. *Salmonella spp*. exhibited catalase positivity, indole negativity, H_2S production, and citrate utilization, traits that distinguish it from other enteric bacteria. *Shigella spp.* on the other hand did not produce H_2S, was indole positive and did not utilize citrate, while *Staphylococcus aureus* tested positive for catalase and

coagulase, confirming its identity as a pathogenic *staphylococcus*. These biochemical profiles agree with the descriptions in Bergey's Manual of Systematic Bacteriology and have been corroborated by Adeniyi and Ighalo (2021), who identified similar reaction patterns in bacterial isolates from contaminated surface waters in Nigeria. The combination of biochemical tests thus provides a reliable approach for accurate differentiation of these pathogenic bacteria.

The detection of *E. coli, Salmonella spp., Shigella spp.* and *Staphylococcus aureus* in Ogba River is of serious environmental and public health significance. The presence of *E. coli* is widely recognized as a key indicator of faecal contamination, implying that the river is exposed to waste from human or animal sources. *Shigella spp.* is almost exclusively human-associated, and its occurrence in the samples points strongly toward contamination from human faecal matter, possibly due to open defecation and direct washing or bathing activities along the riverbanks. *Salmonella spp.* which can originate from both humans and animals, suggests mixed contamination sources, likely from domestic sewage, livestock waste, and runoff from nearby settlements. The isolation of *Staphylococcus aureus* further indicates anthropogenic impact, possibly from skin flora shed during bathing, laundry and other human contact activities within the river. These interpretations are consistent with microbial source tracking principles described by Adeniyi and Ighalo (2021), which link specific microbial signatures to defined pollution sources in aquatic environments.

The presence of these pathogens underscores the river's vulnerability to pollution from surrounding human settlements and agricultural activities. The results suggest that the Ogba River serves as a reservoir for potentially pathogenic microorganisms, which could pose health risks to communities relying on it for domestic, recreational, or agricultural purposes. Diseases such as typhoid fever, bacillary dysentery, gastroenteritis, and skin infections are possible outcomes of exposure to or consumption of untreated water from the river. Similar findings were reported by Oliver *et al.* (2021) in their assessment of water quality in Adamawa State, where *E. coli, Salmonella,* and *Shigella* were identified as major contaminants in untreated surface waters. These pathogens are known to cause outbreaks in areas lacking adequate water treatment and sanitation facilities.

From an environmental perspective, the continual discharge of untreated effluents and domestic waste into the river alters the microbial ecology and can result in oxygen depletion, increased

organic load, and loss of aquatic biodiversity. Additionally, rivers contaminated with fecal bacteria are potential hotspots for antimicrobial resistance development, as antibiotic-resistant genes can be exchanged among microbial populations in such environments. This concern is supported by recent studies (Abdulkareem *et al.,* 2023) that emphasize the emergence of multidrug-resistant bacteria in Nigerian rivers exposed to human and animal waste.

The findings from this research therefore reveal that Ogba River is microbiologically unsafe for human use. Its contamination reflects the direct influence of poor sanitation, indiscriminate waste disposal, and human activities along the riverbanks. The study further demonstrates the usefulness of microbial source tracking as a valuable approach for identifying the origins and types of fecal contamination in aquatic ecosystems. The results highlight the need for urgent remediation measures, including the enforcement of sanitation laws, construction of proper sewage systems, regular monitoring of water quality, and sensitization of local communities on the health risks associated with using contaminated water.

5.2 CONCLUSION

In conclusion, this study has shown that Ogba River is heavily contaminated with faecal bacterial pathogens, notably *E. coli, Salmonella spp., Shigella spp.* and *Staphylococcus aureus.* These findings confirm that the river is under severe microbial stress and is not suitable for domestic, recreational, or agricultural purposes without adequate treatment. The observed microbial load and diversity indicate both human and animal sources of contamination, with direct anthropogenic activities playing a significant role.

5.3 RECOMMENDATION

I recommend for continuous microbial surveillance, effective waste management, treating the water by boiling, filtration or chlorination before use for domestic or agricultural purposes and community-based interventions are recommended to mitigate pollution and safeguard public health. The study thus provides vital baseline information for water quality management and underscores the urgent need for sustainable environmental policies to restore and protect the Ogba River ecosystem.

REFERENCES

Abe, T., Haga, S., Yokoyama, K. and Watanabe, N. (2008). An outbreak of Campylobacter jejuni subsp. jejuni infection via tap water. *Japanese Journal of Infectious Diseases* **61:** 327.

Abdulkareem, M. A., Omotunde, O. T., Momoh-Salami, T. M., Bamikole, O. J. and Olajide, T. H. (2023).Bacteriological and physicochemical analysis of surface water; implications on public health. *Biomedical Research* **34**(1):65–75.

Adefisoye, M. A. and Okoh, A. I. (2016). Identification and antimicrobial susceptibility of Salmonella spp. from environmental water sources in South Africa. *Scientific Reports* **6**, 30212.

Adenugba, A. A. and Akinmoladun, O. O. (2023). Stream and well water samples from two rural communities in Ekiti State, Nigeria: Physico-chemical parameters and bacteriological quality. Acta Scientiarum. Biological Sciences **45**(1):e64296.

Adewoyin, O. B. and Ogedengbe, K. (2020). Evaluation of low-cost methods for microbial water quality monitoring in Nigeria. *Water Practice and Technology* **15**(4):1010–1019.

Adewumi, J. R., Adeogun, A. O. and Akande, S. O. (2014). Impact of abattoir effluent on water quality of Odo Ona River, Ibadan, Nigeria. *African Journal of Environmental Science and Technology* **8**(7):451–457.

Adeniyi, A. G. and Ighalo, J. O. (2021). A comprehensive review of water quality monitoring and assessment in Nigeria. *Environmental Monitoring and Assessment* **193**(8):1–15.

Alam, M., Sultana, M., Nair, G. B., Sack, R. B., Sack, D. A., Siddique, A. K., Ali, A., Huq, A. and Colwell, R. R. (2006). Toxigenic Vibrio cholerae in the aquatic environment of Mathbaria, Bangladesh. *Applied and Environmental Microbiology* **72**(4):2849–2855.

Alhadlaq, M. A., Aljurayyad, O. I. and Almansour, A. (2024). Overview of pathogenic Escherichia coli, with a focus on Shiga toxin- producing serotypes, global outbreaks (1982–2024) and food safety criteria. *Gut Pathogens* **16:** 57.

American Public Health Association (APHA). (2017). Standard methods for the examination of water and wastewater (23rd ed.). Washington, D.C.

Anueyiagu, K. N., Agu, C. G., Umar, U. and Lopes, B. S. (2024). Antimicrobial resistance in diverse Escherichia coli pathotypes from Nigeria. *Antibiotics* **13**(10):922.

Arvanitidou, M., Kanellou, K. and Vagiona, D. G. (2005). Diversity of Salmonella spp. and fungi in Northern Greek rivers and their correlation to faecal pollution indicators. *Environmental Research* **99**(2): 278–284.

Ashbolt, N. J. (2004). Microbial contamination of drinking water and disease outcomes in developing regions . *Toxicology* **198**(3):229–238.

Ashida, H., Ogawa, M., Kim, M., Mimuro, H. and Sasakawa, C. (2012). Bacteria and host interactions in the gut epithelial barrier. *Nature Chemical Biology* **8**(1):36–45.

Bartram, J. and Rees, G. (2000). Monitoring bathing waters: A practical guide to the design and implementation of assessments and monitoring programmes. World Health Organization.

Barry, E. M., Pasetti, M. F., Sztein, M. B., Fasano, A., Kotloff, K. L. and Levine, M. M. (2013). Progress and pitfalls in Shigella vaccine research. *Nature Reviews Gastroenterology & Hepatology* **10**(4):245–255.

Ben Aissa, R., Al-Gallas, N., Troudi, H., Belhadj, N. and Belhadj, A. (2007). Trends in Salmonella enterica serotypes isolated from human, food, animal, and environment in Tunisia, 1994–2004. *Journal of Infection* **55**(4):324–339.

Bettelheim, K. A. (2003). The genus Escherichia. In M. Dworkin, S. Falkow, & E. Rosenberg (Eds.), The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community (3rd ed., Release 3.14).

Brandl, M. T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology* **44:**367–392.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R. and Harwood, V. J. (2012). Enterococci in the environment. *Microbiology and Molecular Biology Reviews* **76**(4):685–706.

Cabral, J. P. S. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health* **7**(10):3657–3703.

Cabral, J. P. and Marques, C. (2006). Faecal coliform bacteria in Febros River (Northwest Portugal): Temporal variation, correlation with water parameters, and species identification. *Environmental Monitoring and Assessment* **118**(1):21–36.

Centers for Disease Control and Prevention. (2022). Healthy swimming: Microbes in pool water.

Centers for Disease Control and Prevention (CDC). (2023). Shigella – Antibiotic-resistant infections.

Chekole, W. S., Potgieter, L., Adamu, H., Sternberg-Lewerin, S., Tessema, T. S., & Magnusson, U. (2025). Genomic insights into antimicrobial resistance and virulence of Escherichia coli in central Ethiopia: A One Health approach. *Frontiers in Microbiology* 16, Article 1597580.

Chompook, P., Todd, J., Wheeler, J. G., von Seidlein, L., Clemens, J. and Chaicumpa, W. (2006). Risk factors for shigellosis in Thailand. *International Journal of Infectious Diseases* **10**(5):425–433.

Chukwu, M. O., Abia, A. L. K., Ubomba-Jaswa, E., Obi, L. and Dewar, J. B. (2019). Characterization and phylogenetic analysis of Campylobacter species isolated from paediatric stool and water samples in the Northwest Province, South Africa. International *Journal of Environmental Research and Public Health* **16**(12):2205.

Crump, J. A., Luby, S. P. and Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization* **82**(5):346–353.

Edokpayi, J. N., Odiyo, J. O. and Durowoju, O. S. (2015). Impact of wastewater on surface water quality in developing countries: *A case study of South Africa. Water Quality* **15**(2):1–13.

Emch, M., Ali, M. and Yunus, M. (2008). Risk areas and neighborhood-level risk factors for Shigella dysenteriae 1 and Shigella flexneri. *A Journal of Health & Place* **14**(1):96–105.

Faruque, S. M., Khan, R., Kamruzzman, M., Yamasaki, S., Ahmad, Q. S., Azim, T., Nair, G. B., Takeda, Y. and Sack, D. A. (2002). Isolation of Shigella dysenteriae type 1 and S. flexneri strains from surface waters in Bangladesh: Comparative molecular analysis of environmental Shigella isolates versus clinical strains. *Applied and Environmental Microbiology* **68**(8):3908–3913.

Faruque, S. M., Albert, M. J. and Mekalanos, J. J. (2003). Epidemiology, genetics, and ecology of toxigenic Vibrio cholerae. *A Journal of Microbiology and Molecular Biology Reviews* **67**(4):703–731.

Farmer, J. J. and Hickam-Brenner, F. W. (2003). The genus Vibrio and Photobacterium. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), The Prokaryotes: An evolving electronic resource for the microbiological community (electronic release 3.14, 3rd ed.).

Farmer, J. J., Janda, J. M., Brenner, F. W., Cameron, D. N. and Birkhead, K. M. (2005). Genus Vibrio. In D. J. Brenner, N. R. Krieg and J. T. Staley (Eds.), Bergey's manual of systematic bacteriology (2nd ed., Vol. 2, Part B, pp. 494–546).

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S. and Gordon, M. A. (2012). Invasive non-typhoidal salmonella disease: An emerging and neglected tropical disease in Africa. *The Lancet* **379**(9835): 2489–2499.

Field, K. G. and Samadpour, M. (2007). Fecal source tracking, the indicator paradigm, and managing water quality. Water Research **41**(16):3517–3538.

Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J. and Ricke, S. C. (2011). Population dynamics of Salmonella enterica serotypes in commercial egg and poultry production. *Applied and Environmental Microbiology* **77**(13): 4273–4279

Fricker, C. R. (2003). Detection of Escherichia coli in water using chromogenic and fluorogenic substrates. *Journal of Applied Microbiology* **85**(s1): 147S–157S.

Gião, M. S., Azevedo, N. F., Wilks, S. A., Vieira, M. J. and Keevil, C. W. (2008). Persistence of Helicobacter pylori in heterotrophic drinking water biofilms. *Applied and Environmental Microbiology* **74**(19):5898–5904.

Germani, Y. and Sansonetti, P. J. (2003). The genus Shigella. In M. Dworkin, S. Falkow, & E. Rosenberg (Eds.), The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community (electronic release 3.14, 3rd ed.).

Griffith, D. E., Aksamit, T., Brown-Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F. and Winthrop, K. L. (2007). An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American Journal of Respiratory and Critical Care Medicine* **175**(4):367–416.

Grimont, P. A. D. and Weill, F. X. (2007). Antigenic formulae of the Salmonella serovars (9th ed.). WHO Collaborating Centre for Reference and Research on Salmonella, Institute Pasteur.

Gu, B., Cao, Y., Pan, S., Zhuang, L., Yu, R., Peng, Z. and Wang, X. (2012). Comparison of the prevalence and antimicrobial resistance of Shigella from outpatient and inpatient children under 5 years of age in eastern China. *BMC Pediatrics* **12**:110.

Gyles, C. L. (2007). Shiga toxin-producing Escherichia coli: An overview. *Journal of Animal Science* **85**(13): E45–E62.

Harwood, V. J., Staley, C., Badgley, B. D., Borges, K. and Korajkic, A. (2014). Microbial source tracking markers for detection of fecal contamination in environmental waters. *FEMS Microbiology Reviews* **38**(1):1–40.

Health Canada. (2006). Guidelines for Canadian drinking water quality: Guideline technical document. Bacterial waterborne pathogens. Current and emerging organisms of concern. Ottawa, ON, Canada: Health Canada.

Igbinosa, E. O. and Okoh, A. I. (2009). Impact of discharge wastewater effluents on the physicochemical qualities of a receiving watershed in a typical rural community. *International Journal of Environmental Science and Technology* **6**(2):175–182.

Igbinosa, I. H., Igumbor, E. U., Aghdasi, F., Tom, M. and Okoh, A. I. (2012). Emerging Aeromonas species infections and their significance in public health. *The Scientific World Journal,* 2012, Article ID 625023.

Igwaran, A. and Okoh, A. I. (2019). Human campylobacteriosis: A public health concern of global importance. *A Journal of Heliyon* **5**(7):e02814.

Jalava, K., Rintala, H., Ollgren, J., Maunula, L., Gomez-Alvarez, V., Revez, J., Palander, M., Antikainen, J., Kauppinen, A. and Rasanen, P. (2014). Novel microbiological and spatial statistical methods to improve strength of epidemiological evidence in a community-wide waterborne outbreak. *PLoS ONE* **9**(8): e104713.

Kaakoush, N. O., Castano-Rodriguez, N., Mitchell, H. M. and Man, S. M. (2015). Global epidemiology of Campylobacter infection. *Clinical Microbiology Reviews* **28**(3):687–720.

Kariuki, S. and Gordon, M. A. (2015). Invasive non-typhoidal Salmonella infections in Africa: Epidemiology, pathogenesis and control options. *Clinical Infectious Diseases* **61**(4): S235–S240.

Kim, M., Ogawa, M., Fujita, Y., Yoshikawa, Y., Nagai, T. and Sasakawa, C. (2009). Bacteria hijack integrin-linked kinase to stabilize focal adhesions and block cell detachment. *A Journal of Nature* **459**(7246):578–582.

Kotloff, K. L., Nataro, J. P. and Blackwelder, W. C. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the GEMS study). *A Journal of the Lancet* **382**(9888): 209–222.

Livio, S., Strockbine, N. A. and Panchalingam, S. (2014). Shigella isolates from the Global Enteric Multicenter Study inform vaccine development. *Clinical Infectious Diseases* **59**(7):933–941.

Majowicz, S. E., Musto, J. and Scallan, E. (2010). The global burden of non-typhoidal Salmonella gastroenteritis. *Clinical Infectious Diseases* **50**(6):882–889.

Melton-Celsa, A. R. (2014). Shiga toxin (Stx) classification, structure, and function. *Microbiology Spectrum* **2**(3).

Moreira, N. A. and Bondelind, M. (2017). Safe drinking water and waterborne outbreaks. *Journal of Water and Health* **15**(1):83–96.

Nwachukwu, E. and Ume, C. (2018). Isolation of enteric bacteria from polluted rivers and their public health significance. *African Journal of Microbiology Research* **12**(15):370–378.

Noble, R. T., Moore, D. F., Leecaster, M. K., McGee, C. D. and Weisberg, S. B. (2003). Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research* **37**(7):1637–1643.

Obi, C. L., Potgieter, N., Bessong, P. O. and Matsaung, G. (2002). Gene encoding virulence markers among Escherichia coli isolates from diarrhoeic stools and river sources in rural Venda communities of South Africa. *Water SA* **28**(3):287–292.

Okeke, I. N., Aboderin, O. A., Byarugaba, D. K., Ojo, K. K. and Opintan, J. A. (2007). Growing problem of multidrug-resistant enteric pathogens in Africa. *Emerging Infectious Diseases* **13**(11):1640–1646.

Olalemi, A. O., Olorunfemi, D. I. and Ojo, D. A. (2020). Assessment of microbial pollution and public health risks of water sources in southwest Nigeria. *African Journal of Environmental Science and Technology* **14**(3):75–85.

Oliver, K., Malawi, M. W. and Tammi, H. (2021). Assessment of the physical, chemical, and biological properties of sachet water in Mubi Metropolis, Adamawa State, North East, Nigeria. *International Journal of Innovative Research & Development* **10**(6):1–10.

Opara, A. A., Eze, V. C. and Nwuba, R. I. (2011). Occurrence of enteric bacterial pathogens in the Otamiri River and the public health implications. *Nigerian Journal of Microbiology* **25**(1):2201–2210.

Parry, C. M., Hien, T. T., Dougan, G., White, N. J. and Farrar, J. J. (2002). Typhoid fever. *New England Journal of Medicine* **347**(22):1770–1782.

Phalipon, A. and Sansonetti, P. J. (2007). Shigella's ways of manipulating the host intestinal innate and adaptive immune system: A toolbox for survival? *Immunological Reviews* **225:**132–147.

Pinto, B., Pierotti, R., Canale, G. and Reali, D. (1999). Characterization of faecal streptococci as indicators of faecal pollution and distribution in the environment. *Letters in Applied Microbiology* **29**(4):258–263.

Podolak, R., Enache, E., Stone, W., Black, D. G. and Elliott, P. H. (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods. *Journal of Food Protection* **73**(10):1919–1936.

Popoff, M. Y. and Le Minor, L. E. (2005). Genus Salmonella. In D. J. Brenner, N. R. Krieg, & J. T. Staley (Eds.), Bergey's manual of systematic bacteriology (2nd ed., Vol. 2, Part B, pp. 764–799).

Riddle, M. S., Kaminski, R. W. and Di Paolo, C. (2016). Recurrent Shigella infections in travelers: The role of antimicrobial resistance. *Clinical Microbiology Reviews* **29**(3):403–418.

Rompré, A., Servais, P., Baudart, J., de-Roubin, M. R. and Laurent, P. (2002). Detection and enumeration of coliforms in drinking water: Current methods and emerging approaches. *Journal of Microbiological Methods* **49**(1):31–54.

Sack, D. A., Rahman, M., Yunus, M. and Khan, E. H. (2004). Antimicrobial resistance in Shigellosis, cholera and Campylobacter infection. *Bulletin of the World Health Organization* **82**(9):738–746.

Sansonetti, P. J. (2001). Microbes and microbial toxins: Paradigms for microbial–mucosal interactions. III. Shigellosis: From symptoms to molecular pathogenesis. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **280**(3):G319–G323.

Scheutz, F. and Strockbine, N. A. (2005). Genus Escherichia. In D. J. Brenner, N. R. Krieg, & J. T. Staley (Eds.), Bergey's manual of systematic bacteriology (2nd ed., Vol. 2, Part B, pp. 607–623).

Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R. and Lukasik, J. (2002). Microbial source tracking: Current methodology and future directions. *Applied and Environmental Microbiology* **68**(12):5796–5803.

Svec, P. and Devriese, L. A. (2009). Genus Enterococcus. In P. DE Vos, G. M. Garrity, D. Jones, N. R.Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer and W. B. Whitman (Eds.), Bergey's manual of systematic bacteriology (2nd edition) **3**: 594–607).

Taneja, N. and Mewara, A. (2016). Enteroinvasive Escherichia coli: An uncommon cause of dysentery. *Indian Journal of Medical Research,* **144**(1):1–2. https://doi.org/10.4103/0971-5916.193279 Tarr, P. I., Gordon, C. A., & Chandler, W. L. (2005). Shiga‐toxin‐producing Escherichia coli and haemolytic uraemic syndrome. *A Journal of the Lancet* **365**(9464):1073–1086.

Tindall, B. J., Grimont, P. A. D., Garrity, G. M. and Euzéby, J.-P. (2005). Nomenclature and taxonomy of the genus Salmonella. *International Journal of Systematic and Evolutionary Microbiology* **55**(2):521–524.

Todar, K. (2009). Shigella and shigellosis. *In Todar's Online Textbook of Bacteriology*. Retrieved September 1, 2010.

Troeger, C., Khalil, I. A. and Rao, P. C. (2018). Global burden of diarrhoeal diseases due to Shigella and enterotoxigenic Escherichia coli in children under 5 years: A modelling study. *The Lancet Global Health* **6**(3): e255–e265.

United States Environmental Protection Agency. (2006). National primary drinking water regulations: Total coliforms (including fecal coliforms and E. coli) (EPA 816‐F‐06‐029). United States Environmental Protection Agency. (USEPA). (2012). Recreational water quality criteria (EPA 820‐F‐12‐058).

Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E. and Lapena, D. R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research* **43**(9):2209–2223.

Wilson, M. (2005). Microbial inhabitants of humans: Their ecology and role in health and disease. C*ambridge University Press* 1-30

World Health Organization. (2005). The treatment of diarrhoea: A manual for physicians and other senior health workers (4th rev.). World Health Organization.

World Health Organization. (2008). Guidelines for drinking-water quality: Incorporating 1st and 2nd addenda. Volume 1: Recommendations (3rd ed.). World Health Organization.

World Health Organization. (2010a). Enterotoxigenic Escherichia coli (ETEC). In Diarrhoeal diseases (Fact sheet). World Health Organization. Retrieved September 4, 2010.

World Health Organization. (2010b). Enterohaemorrhagic Escherichia coli (EHEC) (Fact sheet No. 125). World Health Organization. Retrieved September 4, 2010.

World Health Organization. (2017a). Guidelines for drinking-water quality (4th ed., incorporating 1st addendum). World Health Organization.

World Health Organization. (2020). Diarrhoeal disease (updated August 20, 2020). World Health Organization. Retrieved December 5, 2024.

World Health Organization. (2020). Diarrhoeal disease. World Health Organization. Retrieved August 20, 2020.