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Review Article

A REVIEW ON SALMONELLA SPECIES AND INDICATOR ORGANISMS IN DRINKING WATER

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Abstract

Water is a chemical compound with the chemical formula H_20. A water molecule contains one oxygen and two hydrogen atoms connected by covalent bonds. The existence of liquid water and to a lesser extent its gaseous and solid forms, on earth are vital to the existence of life on earth as we know it. Safe drinking water is essential to humans and other life forms even though it provides no calories or organic nutrients. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion lack accesses to adequate sanitation. Due to growing populations and expanding land use, sources of pathogen contaminated wastes steadily increase hence also raising the potential pollution of groundwater reservoirs with infectious agents all around the world. The quality of many source waters will depend upon geology, soil type, natural vegetation, climate and run-off characteristics. Ideally, drinking water should be clear and acceptable to the palate. Water can dissolve many different substances, giving it varying tastes and odours. The use of indicator organisms, in particular the Coliform group, as a means of checking the potential presence of water-borne pathogens has been paramount to protecting public health. Frequent examinations of faecal indicator organisms remain the most sensitive way of assessing the hygienic conditions of water. Indicator organisms of faecal pollution include the Coliform group as a whole and particularly *Escherichia coli*, *Streptococcus faecalis* and some thermo tolerant organisms such as *Clostridium perfringes.*

Keywords: *Salmonella spec*ies, Drinking water, Indicator organisms.

Introduction

Water

Water is a chemical compound with the chemical formula H_20. A water molecule contains one oxygen and two hydrogen atoms connected by covalent bonds (Raven and Johnson, 2002). Water is a liquid at standard ambient temperature and pressure, but it often co-exist on earth with its solid state, ice, and gaseous state (water vapour or steam). Water also exists in a liquid crystal state near hydrophilic surfaces (Raven and Johnson, 2002). The existence of liquid water and to a lesser extent its gaseous and solid forms, on earth are vital to the existence of life on earth as we know it. The earth is located in the habitable zone of the solar system; if it were slightly closer to or farther from the sun (about 5% or about 8 million kilometers), the conditions which allow the three

forms to be present simultaneously would be far less likely to exist (Eja, 2002).

In the study by Shalom et al. (2011), the hydrosphere is estimated to contain about 1.36 billion Km^3, only about 0.3% of the water, existing as fresh water in rivers, streams springs and aquifers, are available for human use; the remaining 99.7% are locked up in seas and oceans. Water is one of the most abundant and essential resources of man, and occupies about 70% of earth's surface. About 97% of this volume of earth's surface water is contained in the oceans, 21% in polar ice and glaciers, 0.30.8% underground, 0.009% in inland freshwaters such as lakes, while 0.00009% is contained in rivers (Eja, 2002). Only 2.5% of the earth's water is freshwater, and 98.8% of that water is in ice and ground water. Less that 0.3% of all freshwater is in river, lakes and the atmosphere, and an even smaller amount of the earth's freshwater (0.003%) is contained within biological bodies and manufactured products (Shalom et al., 2011*)*.

Safe drinking water is essential to humans and other life forms even though it provides no calories or organic nutrients. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion lack accesses to adequate sanitation (Ukpong and Okon, 2013;Nwandikor and Obeagu,2015). However, some observers have estimated that by 2025, more than half of the world population will be facing water-based vulnerability *(*Nasser et al., 2002*)*. Water plays an important role in the world economy, since it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. Approximately 70% of the fresh water used by humans goes to agriculture *(*Baroni et al., 2007; Nwandikor et al.,2015*)*

Globally, ground water accounts for approximately one third of all freshwater resources and represents around 99% of available freshwater (Danielopol et al., 2003*)*. In most countries worldwide it is used as the main source of drinking water. Ground water usage in Europe ranges from only 13% in Norway up to 100% in Denmark, Lithuania and Austria (Ashbolt 2004, WHO 2006). Groundwater is already used extensively in Nigeria through wells and boreholes *(*Ukpong and Okon, 2013). The average proportion of groundwater as drinking water in US states is about 65% and hence quite similar to the European consumption. In Germany, about 70% of the drinking water derives are from aquifers. However, there is a huge variation between the federal states, with Bremen, Hamburg, Saarland and Schleswig groundwater in Berlin and Saxony is only about 25% and 33%, respectively (Steffen and Christian, 2011).

There is logic behind the utilization of groundwater as drinking water source. Naturally, groundwater ecosystems are well protected by overlaying soil and sediment layers. Water from precipitation recharging aquifers needs to pass these zones which act as effective mechanic and biological filters, hence providing a natural clean-up of newly generated groundwater. In aquifers, the biological components, mainly micro-organisms, provide the valuable ecosystem services of water purification and storage at high quality for decades and centuries (Herman et al., 2010). Nevertheless, today groundwater faces increasing threats from anthropogenic impacts including contamination with pathogenic microorganisms and viruses. Groundwater for a long time has been thought completely free of microbial contaminants and viruses, believing that vertical transport times are long enough and microbial survival too short to reach the aquifers. However, the risks of water contamination are obvious when having a look at the manifold small and huge endemic outbreaks from pathogenic microbes and viruses in the last two centuries which could be linked to contaminated groundwater and drinking consumption (OECD 2003, Craun et al., 2010). Today, there is no doubt that the pathogenic microorganisms and viruses can be found everywhere in the environment. Some relevant pathogenic microorganisms even became or always have

been members of natural microbial communities (e.g. species of the genus *legionella*) (Steinart et al., 2002). Others may survive for years and decades or even reproduce in the environment. There are multiple sources of contamination including wastewater entering different environmental compartments and manure applied to fields in agricultural areas. Pressures on terrestrial and aquatic environments may increase in the future due to global change. It may be suggested that pathogenic microorganisms and viruses will more often enter soil, river banks and aquifers along with irregular storms, flood and drought events (Foster and Chilton 2003, Schwarzenbach et al., 2010). The efficiency of natural attenuation of pathogenic agents in soils, zone sediments, fissured or karst aquifers are highly dependent on the matrix characteristics and environmental conditions. In regions with thin soil layers, fractured rocks or in karst regions, aquifers are especially vulnerable to contamination. In contrary, extended soil layers are a good protection shield against pathogens entry. More specific, biologically active environmental compartments, such as soils, often have been observed to support fast and efficient pathogen elimination (Nasser et al., 2002).

Sources and entry of contaminants into water body

Due to growing populations and expanding land use, sources of pathogen contaminated wastes steadily increase hence also raising the potential pollution of groundwater reservoirs with infectious agents all around the world. The quality of many source waters will depend upon geology, soil type, natural vegetation, climate and run-off characteristics. Disruption of natural geology and heavy rainfall can dramatically affect water quality. Wild animals and birds can also be natural sources of zoonotic pathogens (Environmental Agency, 2002). This is mainly true for pathogens originating from human and animal faeces. Sources of faecal contamination in groundwater potentially include: Leakage from on-site sanitation systems such as septic tanks or sewers, underground storage tanks, disposal systems, animal manure and compost, from (accidental and non-accidental) waste water discharge or sewage sludge applied to fields in agricultural areas (Reynolds and Barrett 2003, Gerba and Smith, 2005). Surface waters receiving treated or untreated sewage from human sources or livestock enterprises and discharge from non-point sources like urban and agricultural runoff are steady source and reservoir of pathogenic agents (Kirschner et al., 2009).

Chemical and physical properties of water

Water is transparent in the visible electromagnetic spectrum. Thus aquatic plants can live in water because sunlight can reach them. Infrared light is strongly absorbed by the hydrogen-oxygen or OH bonds (Raven and Johnson, 2002). Since the water molecule is not linear and the oxygen atom has higher electro negativity than hydrogen atoms, it carries a slight negative charge, whereas the hydrogen atoms are slightly positive. As a result, water is a polar molecule with an electrical dipole moment that can form intermolecular hydrogen bonds (four) for a molecule of water, giving rise to water's high surface tension (Campell et al., 2006) and capillary forces. The capillary action refers to the tendency of water to move up a narrow tube against the force of gravity. This property is relied upon by all vascular plants, such as trees. Water is a good polar solvent and is often referred to as the universal solvent. Substances that dissolve in water, e.g., salts, sugars, acids, alkalis, and some gases especially oxygen, carbon dioxide (carbonation) are known as hydrophilic (water loving) substances, while those that are immiscible with water (e.g. fat and oils, are known as hydrophobic (water fearing) substances. Most of the major components in cells (Protein, DNA and polysaccharides) are also dissolved in water. Pure water has a low electrical conductivity, but this increases with the dissolution of a small amount of ionic material such as sodium chloride. The boiling point of water (and all other liquids) is dependent on the barometric pressure. For example, on the top of Mt. Everest water boils at 68°C (154°F),

compared to 100°C (212°F) at sea level (Raven and Johnson 2002). At 4181,3J/(Kg.K), water has a high specific heat capacity, as well as high heat of vaporization (40.65 Kj.mol.$^{-}$1), both of which are as a result of the extensive hydrogen bonding between its molecules. These two unusual properties allow water to moderate Earth's climate by buffering large fluctuations in temperature (Raven and Johnson, 2002). The maximum density of water occurs at 3.98°C (39.16°F) (Kotz et al., 2005) it has the anomalous property of becoming less dense, not more, when it is cooled to its solid form, ice. During freezing, the open structure of ice is gradually broken and molecules enter cavities in ice-like structure of low temperature water. There are two competing effects: (1) Increasing volume of normal liquid and (2) Decrease overall volume of the liquid. Between 0 and 3.98°C, the second effect will cancel off the first effect so the net effect is shrinkage of volume with increasing temperature. The density of liquid water is 1,000 Kg/m^3 (62.43 Ib/cu ft) at 4°C. ice has a density of 917 Kg/m^3 (57.25 Ib/cu ft). Water is miscible with many liquids, such as ethanol, in all proportions, forming a single homogenous liquid. On the other hand, water and most oils are immiscible, usually forming layers according to increasing density from the top. As a gas, water vapour is completely miscible with air (Raven and Johnson ,2002). Water forms an azeotrope with many other solvents. Water can be split by electrolysis into hydrogen and oxygen. As an oxide of hydrogen, water is formed when hydrogen-containing compounds burn or react with oxygen containing compounds. Water is not a fuel; it is an end-product of the combustion of hydrogen. The energy required to split water into hydrogen and oxygen by electrolysis or any other means is greater than the energy that can be collected when the hydrogen and oxygen recombine.

Elements which are more electropositive than hydrogen such as Lithium, Sodium, Calcium, Potassium and Caesium displace hydrogen from water. Forming hydroxides, being a flammable gas, the hydrogen given off is dangerous and the reaction of water with more electropositive of these elements may be violently explosive (Raven and Johnson, 2002).

Qualities Of Portable Water

Taste and odour

Ideally, drinking water should be clear and acceptable to the palate. Water can dissolve many different substances, giving it varying tastes and odours (Environmental Agency, 2002). Humans and other animals have developed senses that enable them to evaluate the portability of water by avoiding water that is too salty or putrid. The taste of spring water and mineral water, often advertised in marketing of consumer products, derived from the minerals dissolved in it. However, portable water is tasteless and odourless. The advertised purity of spring and mineral water refers to absence of toxins, pollutants and microbes, not the absence of naturally occurring minerals.

Body requirement

Man requires a regular and accessible supply of water which forms a major component of the cell protoplasm and provides an essential requirement for vital physiological and biochemical processes. Man can go without food for twenty-eight days but only three days without water, and two third of a person's water consumption per day is through food while one third is obtained through drinking (Muyi, 2007).

The human body contains between 55% to 78% water, depending on body size. To function properly, the body requires between one and seven litres of water per day to avoid dehydration; the precise amount depends on the level of activity, temperature, humidity, and other factors. Most of this water is ingested through foods or beverages other than direct drinking. It is not clear how much water intake is needed by healthy people, though most advocates agree that approximately 2 litres (6 to 7 glasses) of water daily is the minimum to maintain proper hydration. Medical literature favours a lower

consumption, typically 1 litre of water for an average male, excluding extra requirements, due to fluid loss from exercise or warm weather (Rhoades, 2003). For those who have healthy kidney, it is rather difficult to drink too much water, but (especially in warm humid weather and while exercising) it is dangerous to drink too little. People can drink far more water than necessary while exercising, however, putting them at risk of water intoxication (Hyper hydration), which can be fatal (Noakes et al., 2005).

Parameters used for assessing potable water

pH

Although pH usually has no direct impact on water consumers, it is one of the most important operational water-quality parameters. Careful attention to pH control is necessary at all stages of water treatment to ensure satisfactory water clarification and disinfection (WHO, 2003). For effective disinfection with chlorine, the pH should preferably be less than 8. The pH of the water entering the distribution system must be controlled to minimize the corrosion of water mains and pipes in household water systems. Failure to do so can result in the contamination of drinking-water and in adverse effects on its taste, odour, and appearance. The optimum pH varies with different supplies according to the composition of the water and the nature of the construction materials used in the distribution system, but the acceptable range is between 6.5 and 3.5 (WHO, 2003).

Chlorine residual

The disinfection of drinking-water supplies constitutes an important barrier against waterborne diseases. Although various disinfectants may be used, chlorine in one form or another is the principal disinfecting agent employed in small communities in most countries. The absence of a chlorine residual in the distribution system may, in certain circumstances, indicate the possibility of post-treatment contamination (WHO, 2006). Typical level of free chlorine (or residual chorine) in drinking water is between 0.2 and 2.0 mg/dl (WHO, 2003)

Aesthetic parameters

Aesthetic parameters are those detectable by the senses, namely turbidity, colour, taste, and odour. They are important in monitoring community water supplies because they may cause the water supply to be rejected and alternative (possibly poorer-quality) sources to be adopted, and they are simple and inexpensive to monitor qualitatively in the field. Turbidity is important because it affects both the acceptability of waters to consumers, and the selection and efficiency of treatment processes, particularly the efficiency of disinfection with chlorine since it exerts a chlorine demand and protects microorganisms and may also stimulate the growth of bacteria (WHO, 2006). Potable water should be colourless, odourless, tasteless and not turbid.

Growth of microorganisms

In microbiology, growth refers to increase in numbers. Water drawn from groundwater already contains some bacteria. Their number multiplies during handling depending on the bacteria. Subsequently, their number may grow still further (either substantially or only slightly) depending on storage conditions. The changes which take place in the physio-chemical properties of water are the result of the activities of the individual microbial cells during their growth (development and reproduction) or of substances produced during such activity.

The growth of micro-organisms normally takes place in the following stages.
(i) Initial stationary phase (ii) lag phase (phase of adjustment and) (iii) accelerated growth phase (log phase) (iv) maximum stationary phase and (iv) phase of accelerated death.

The growth of micro organism is influenced by the following factors:

Temperature: An important means for controlling growth. Each species of microorganism has its optimum maximum and minimum temperatures of growth. According to their optimum growth temperature. Bacteria can be classified into: Psychpotropic can grow at refrigeration temperature (5-7^{0}C). Mesophytic can grow at temperatures ranging between 20^{0}C to 40^{0}C Thermophylic (heat loving) Can grow at temperatures above 50^{0}C. The optimum growth rate of the majority of microorganisms will be found at temperature between 15 to 38^{0}C or above.

Indicator organisms

The use of indicator organisms, in particular the Coliform group, as a means of checking the potential presence of water-borne pathogens has been paramount to protecting public health (Hijnen et al., 2000). These are based upon the principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests *(Environmental Agency, 2002)*. Many pathogens are present only under specific conditions and, when present in water, occur in low numbers compared with other micro-organisms. Whilst the presence of *Coliform* bacteria does not always indicate a public health threat, their detection is a useful indication that operations should be investigated (Edberg et al., 2000).

Coliform bacteria describe a group of enteric bacteria that includes *E. coli*, *Klebsiella species* and *Enterobacter species*. They are Gram negative, facultatively anaerobic, non-sporing rods that may be motile or not. They are able to ferment lactose to produce acid and gas within 48 h at 35^{0}C (Oyedeji et al., 2010). Although they are generally not harmful themselves, they indicate the possible presence of pathogenic bacteria, viruses and protozoans (Oyedeji et al., 2010). Members of the faecal Coliform group especially *E. coli* are used as indicators of possible recent sewage/faecal contamination because they are commonly found in human and animal faeces. Other microbial indicators of possible faecal contamination are faecal enterococci especially *E. faecalis* and *Clostridium perfringes* spores.

Frequent examinations of faecal indicator organisms remain the most sensitive way of assessing the hygienic conditions of water *(*Desalegn et al., 2012). Indicator organisms of faecal pollution include the Coliform group as a whole and particularly *Escherichia coli, Streptococcus faecalis* and some thermo tolerant organisms such as *Clostridium perfringes* (*WHO, 2004).* Commonly used faecal indicator bacteria (FIB), such as *Faecal Coliforms*, *Escherichia coli*, and *Enterococci*, are considered to be short-lived in the aquatic environment (Byamukama et al., 2005, Schultz-Fedemrecht et al., 2008) and this complicates the determination of the long-term fate of pollution in complex freshwater and marine systems. *Clostridium perfringes* has spore-forming capabilities, which allow for survival in harsh environments after deposition and facilitate prolonged detection in aquatic environments (Sabrina, 2010).

In Nigeria the rules and guidelines for drinking water is typically based on the UNICEF and WHO regulation and the Nigerian Industrial Standard (NIS) and Standards Organization of Nigeria (SON) in 2007 draw table out for Indicator Organisms Microbiological Limits in Drinking water in Nigeria.

Standard for drinking water quality

Parameter	Unit	Maximum Permitted Levels	Health Impact
Total Coliform Count	cfu/ml	10	Indication of faecal contamination
Thermo tolerant Coliform or *Escherichia coli*	cfu/100ml	0	Urinary tract infections, bacteraemia, meningitis, diarrhea, (one of the main cause of morbidity and mortality among children), acute renal failure and haemolytic anaemia.
Faecal Streptococcus	cfu/100ml	0	Indication of recent faecal contamination
Clostridium Perfringens spore	cfu/100ml	0	Index of intermittent faecal contamination.

Source: Nigerian Industrial Standard for Drinking Water Quality 2007.

Indicator organisms associated with portable water quality.

Streptococcus faecalis (*Enterococcus faecalis*)

Streptococcus faecalis formerly classified as group D streptococci are now included in genus Enterococcus and known as *Enterococcus faecalis* (Nannin and Murray 2006, Cheesbrough, 2005). *Enterococcus spp*, are facultatively anaerobic, catalase-negative gram positive cocci, arranged individually, in pairs or short chains (Nannin et al., 2006 and Teixeira et al., 2007). Optimal temperature for growth of *E. faecalis* 35°C (Teixeira et al., 2007). *E. faecalis* are normal inhabitants of the intestinal tract, female genital tract, and (less commonly) oral cavity and also in faeces of other warm blooded animals (Nannin and Murray, 2006).

Significance in water as indicator

The main reason for their enumeration is to assess the significance of the presence of Coliform bacteria in the absence of *E. coli*, or to provide additional information when assessing the extent of possible faecal contamination (Environmental Agency, 2002). As such, they are regarded as secondary indicators of faecal pollution. In human faeces, numbers of Entercocci rarely exceed 106 per gram of faeces, while in animal faeces, they are often more numerous than *E. coli* (Environmental Agency, 2002). Enterococci of faecal origin rarely multiply in water and are more resistant to environmental stress and chlorination than *E. coli* and Coliform bacteria. They generally persist longer in the environment, with the exception of *Streptococcus bovis* and *Streptococcus equines*, which die off relatively rapidly once outside the intestinal tract (Environmental Agency, 2002).

The species of Enterococci that occur in faeces and, therefore, are more likely to be found in polluted waters can be divided into two main groups. The first includes *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans*. These organisms are normally present in the faeces of humans and various animals. The second group includes *Streptococcus bovis*, *Streptococcus equines* and *Enterococcus avium* are not normally present in human faeces. The identification of species may be due to the source of contaminations (Environmental Agency, 2002).

Escherichia coli

Escherichia coli are gram negative straight rods that possess the enzyme β-glucuronidase measuring about 1-3 x 0.4-0.7 µm and arranged singly or in pairs. They are motile by flagella though few strains are non-motile. They possess capsules and fimbriae but no spores. They are facultitavely anaerobic (Ochei and Kolhatkar, 2007).

E. coli is a coliform bacterium and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water. As a coliform bacterium, it is a member of the family Enterobacteriaceae (Environmental Agency, 2002). As a member of the Enterobacteriaceae family, *E. coli* is naturally found in the intestines of humans and warm blooded animals. Unlike other bacteria in the family, the *E. coli* does not usually occur naturally on plants or in soil and water, although there is evidence that some strains may be able to survive and grow in *soils* (Winfield and Groiman, 2003; Byappanahali et al., 2006; Ishii et al., 2006). Within human and animal faeces, *E. coli* is present at a concentration of approximately 10^9 cells per gram (Edberg et al., 2000) and comprises about 1% of the total biomass in the large intestine (Leclere et al., 2001). Although *E. coli* are part of the natural faecal flora, some strains of this bacterium can cause gastrointestinal illness along with other, more serious health problems. Faecal concentrations of the typical non-pathogenic *E. coli*, used to indicate recent faecal contamination, will always be greater than those of the pathogenic strains, even during outbreaks.

Like other Gram negative bacteria, *E. coli* produces endotoxin. In addition, some strains produce exotoxins, enteroxin and haemolysin. The diarrhoea causing strains of *E. coli* fall into four distinct groups; the enterotoxigenic *E. coli* (ETEC), the enteropathogenic *E. coli* (EPEC), the enteroinvasive *E. coli* (EIEC) and enterohaemorrhagic *E. coli* (EHEC), (Ochei and Kolhatkar, 2007).

The EPEC are associated with outbreaks of infantile diarrhea in bottle feeding babies. Serotypes 0111:B4 and 055:B5 were the first to be identified as causing diarrhoea. Today over 60 other serotypes have been recognized, most common serotypes are 026, 055, 0111, 086, 0119, 0128 and 0142 (Ochei and Kolhatkar, 2007).

The enterotoxin producing *E. coli* (ETEC) are the chief causes of acute watery diarrhoea in the tropics. They also cause traveler's diarrhoea. Two types of enterotoxins of *E. coli* are produced namely:

1. A heat stable toxin (ST) which is non-antigenic.
2. A heat labile toxin (LT) which is antigenic.

The toxins inhibit active intestinal absorption and activate the secretion of water and electrolytes into the lumen of the intestine that result in watery stool (Environmental Agency, 2002).

ETEC strains of *E. coli* cause dysentery like diarrhoea with blood and mucus in the stool like *Shigellae*. They penetrate and multiply in the cells of the intestinal epithelium, destroy them and cause ulceration. But do not form toxins (Cheesbrough, 2005).

EHEC strains of *E. coli* cause haemolytic ureamic syndrome (HUS) and haemorrhagic colitis (HC) associated with bloody diarrhoea. Most cases are

caused by serotype 0157:H7 (Environmental Agency, 2002).

Significance in water as indicator

The survival time of *E. coli* in the environment is dependent on many factors including temperature, exposure to sunlight, presence and types of other Microflora, and the type of water involved (e.g. groundwater, surface water, or treated distribution water) (Foppen and Schijven, 2006). In general terms, *E. coli* survives for about 4-12 weeks in water containing a moderate micro flora at a temperature of 15-18^{0}C (Edberg et al., 2000). However, bacterial re-growth in water is not considered a concern in temperature environments. Limited information has been published showing *E. coli* survival and growth in soils in temperature environment (Byappanahalli et al., 2006) *E. coli* is generally the most sensitive of the Coliform bacteria to environmental stressors and does not survive as long in the environment as do Protozoan and some *viruses* (Edberg et al., 2000). *E. coli* does have similar survival rates to many faecal bacterial pathogens (Artz and Killham, 2002; Karim et al., 2004; Cook and Bolster, 2007). This survival character makes *Escherichia coli* to be a good indicator in water.

Clostridium perfringes

Clostridium perfringes is a non-motile Gram positive thick brick-shape rod, anaerobic spore-forming and some strains are capsulate in tissue (Cheesbrough, 2005; Ryan and Ray (2004). *Clostridium perfringes* is a member of the Sulphite-reducing Clostridia which is non-motile and is capable of fermenting lactose, reducing nitrate and liquefying gelatin. Sulphite is usually reduced to sulphide. *Clostridium perfringes* bacteria can be found in many different habitats, such as the normal flora of human gastrointestinal (GI) tract, and environment, such as sewage and soil (Tohru et al., 2002). Most of the Sulphite-reducing Clostridia are commonly found in human and animal faeces. *Clostridium perfringes* produces environmentally resistant spores that survive in water and in the environment for much longer periods than the vegetative cells of *E. coli* and other faecal indicators. Clostridia are removed from water by coagulation and filtration, but the spores of these bacteria can be resistant to Chlorine at concentrations normally used in water treatment. As *Clostridium perfringes* is generally present in faeces in much lower numbers than *E. coli* and *Enterococci*, it is less sensitive as an indicator of faecal contamination. Low numbers may occasionally occur in water supplies, but they do not represent a risk to health. These bacteria will not grow to significant numbers, or produce toxins, in water supplies, as conditions are usually unsuitable.

Clostridium perfringes have also been associated with food poisoning, and some strains of *Clostridium perfringes* can produce severe but self-limiting diarrhoea in humans and animals if ingested in large number. *Clostridium perfringes* is associated with 14 toxins, four of these genes Cpa, Cpb, Cpe, Cpi which code for the alpha, beta, episilon, and iota toxins, are used to characterized *C. perfringes* into five different toxin types (A through E) Sabrina et al., (2010). The presence of alpha toxin (phospholipase C) defines *Clostridium perfringes* and corresponds type A. other toxin genes of interest, such as enterotoxin, beta2, and netB, are not associated with a specific toxin type, but they can relate to overall virulence and differences among *Clostridium perfringes* strains (Jost et al., 2005). In humans, type A isolates that produce Enterotoxin encoded by the cpe gene cause food-borne illnesses, sporadic diarrhoea, and antimicrobial drug –associated diarrhoea (Harrison, 2005).

Significance in water as indicator

Although there is considerable controversy surrounding the use of *C. perfringes* as a water quality indicator because of its persistence in the environment, a number of scientists continue to recommend its use, particularly in situations where the prediction of the presence of viruses or remote fecal pollution is desirable (Fujioka and

Shizumura, 1985. Payment and Franco,1993). Consequently, the role of Enterotoxin in human disease has led to comprehensive analysis of *Clostridium perfringes* from multiple hosts and potential environmental reservoirs to characterize the source of this toxin. Overall, it is estimated that only 2 to 5% of *Clostridium perfringes* isolates worldwide carry the cpe gene. Cpe is the main virulent factor that binds to the target receptor on plasma membrane protein which result in the disruption of the membrane permeability (Bueschel et al., 2003, Miki et al., 2008). Many *C. perfringes* are saprophytic, normally inhabiting soil, water and decomposing plant and animal material. These bacteria will, therefore, be present in surface derived source waters. There has been some indication that humans are primary source of enterotoxin producing *Clostridium perfringes,* based upon the prevalence of individuals in which the cpe gene is detected in faecal samples (18%).

Salmonella

Classification

The Classification of *Salmonella* is complex because the organism is a continuum rather than a defined species. The members of the genus *Salmonella* were originally classified on the basis of epidemiology, host range, biochemical reaction and structure of the O, H and VI antigens (when present).

The names (e.g *Salmonella typhi, Salmonella typhimurium*) were written as if they were genus and species; this form of the nomenclature remains in widespread but incorrect use.

DNA-DNA hybridization studies have demonstrated that there are seven evolutionary groups- Groups: I, II, IIIa, IIIb, IV, V,VI. Nearly all of the *Salmonella* serotypes that infect humans are in DNA hybridization group 1; The species name *Salmonella enteric* has been widely accepted and the organisms in DNA hybridization group I are *S. enteric* sub species enteric. The organisms in the other groups have other sub-species enteric serotype typhimurium, which can be shortened to *Salmonella typhimurium* with the genus name in italics and the serotype name in Roman type. National and International reference laboratories may use the antigenic formulas following the sub species name.

There are more than 2500 serotype of *Salmonella*, including more than 1400 DNA hybridization group I that can infect humans.

Four serotype of *Salmonella* that cause enteric fever can be identified in the clinical laboratory using biochemical and serologic tests. These serotypes which should be routinely identified because of their clinical significance. as follows:

Salmonella paratyphi A (Serotype A), *Salmonella paratyphi* B(serotype B), *Salmonella choleraesius* (sero group C1) and *Salmonella typhi* (serotype D). The more than 1400 Salmonellae isolated in clinical laboratories are sero grouped by their O antisera. The isolates are then sent to reference laboratories for definitive serologist identification. This allows public officials to monitor and assess the epidemiology of *Salmonella* infections on a state wide and nation.

Salmonella is a genus of rod-shape, gram negative, non spore-forming, predominantly motile Enterobacteria with diameters around 0.7 to 1.5µm, lengths from 2 to 5 µm and flagella that move in all directions (i.e petrichous). They are chemorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources and are facultative anaerobes. Most species produce hydrogen sulfide (Clark et al., 2010) which can readily be detected by growing them on media containing ferrous sulfate, most isolates exist in two phases: a motile phase and a non motile phase. Cultures that are non-motile upon primary culture may be switched to the motile phase. *Salmonella* is closely related to the *Escherichia* genus and are found worldwide in cold and warm blooded animals (including humans), and in typhoid fever, paratyphoid fever and food borne illness (Ryan and Ray, 2004).

Initially, each *Salmonella* species was named according to clinical consideration (Kauffman, 1952) e.g *Salmonella typhimurium* (mouse typhoid fever), *S. cholerae-suit* (dog cholera). After it was recognized that host specifically did not exist for many species, new strains (or serovar, short for serological variants) received species names according to the location at which the new strain was isolated. Later, molecular findings led to the hypothesis that *Salmonella* consisted of only one species *S. enteric*, (Minor, 1987) and the serovar were classified into six groups, enteric (i), salamae (ii), arozonae (iiia), diarizonae (iiib), houtenae (iv) and indica (vi) (Janda, 2006) (Reeves et al., 1980) two of which are medically relevant. But as this now formalized nomenclature is not in harmony with the traditional usage familiar to specialist in microbiology and the traditional nomenclature is commonly used.

Currently, there are three recognized species: *S. enteric*, *S. bongori* and *S. subterranean*, with six main sub species: enteric (i), salamae (ii), arozonae (iiia), diarizonae (iiib), houtenae (iv) and indica (vi) (Janda, 2006). The serovar (serotype) is classification of Salmonella into sub species based on antigens that the organism presents.

TABLE: Representative antigenic formulas of Salmonellae.

O group	Serotype	Antigen formula
D	*S. typhi*	9 – 12
A	*S. paratyphi*	1, 2, 12
C	*S. choleraesuis*	6,7
	S. typhimurium	1, 4, 5, 12
	S. enteritidis	1, 9, 12

(Janda, 2006)

Pathogenesis and pathology

Salmonella typhi, *Salmonella choleraesius* and perhaps *Salmonella paratyphi A* and *Salmonella paratyphi B* are primarily infective for humans and infection with these organisms implies acquisition from a human source. The vast majority of *Salmonella*, however are chiefly pathogenic in animals that constitutes the reservoir for human infections, poultry, pigs, rodents, cattle, pets (from turtles to parrots) and many others. The organisms almost always enter into human system via the oral route, usually with contaminated food or drink. The mean infective dose to produce clinical or subclinical infection in humans is 10^5-10^8 *Salmonella* (but perhaps as few as 10^3 *Salmonella typhi* organisms) (Janda, 2006). Among the host factors that contribute to resistance to *Salmonella* infection are gastric acidity, normal intestinal microbial flora and local intestinal immunity.

Pathogenesis of *salmonella* involves invasion of the mucosal epithelial cells (e.g M. cells) by induced phagocytosis, escape from the phagocytic vacuole, multiplication and spread within epithelial cell cytoplasm and passage to adjacent cells. Micro abscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding and formation of a “Pseudo membrane” on the ulcerated area. This consists of fibrin, leukocytes, cell debris, a necrotic mucous membrane and bacteria. As the process subsides, granulation tissue fills the ulcers and scar tissue forms (Janda, 2006).

Salmonella infections are zoonotic and can be transferred between human and animals. Many infections are due to ingestion of contaminated food. For example, FDA studies linked Guatemalan Cantaloupes with fruits *Salmonella panama* (Rothschild, 2012). In speaking of other *Salmonella* serotypes, *Salmonella enteritis* and *Salmonella typhi/ Paratyphoid Salmonella*, (the latter-because of a special virulence factor on a capsule protein Virulence antigen) can cause serious illness such as *Salmonella enteritis*. *Salmonella typhi* is adapted to humans and does not occur in other animals.

Salmonella species are facultative intra cellular pathogen that enter the cells via Micro pinosomes (Kerr, 2010).

Epidemiology of salmonella

Salmonella is transmitted to human via the feco-oral route. An infected individual sheds the bacteria in his faeces, and the bacteria are viable for months in the environment in water, soil, and manure.

Estimates for the year 2000 suggest that there are approximately 21.5million infections and 200,000 deaths from typhoid fever globally each year (Crump et al., 2004), attributable deaths annually is predominantly among children under the age of five years (Clark et al., 2010). In Africa, about 4.36 million cases occur out of an estimated population of 427 million and it is often encountered in tropical countries including Nigeria where they constitute serious source of morbidities and mortalities (Ibekwe et al., 2008). *Salmonella* are widely distributed in the environment, but some species or serovars show host specificity. The pathogens typically gain entry into water systems through faecal contamination. Water-borne *Salmonella* out breaks have devastating public health implications. The World Health Organization (WHO) estimated that about 80% of ill-health especially in developing countries are water related (Cheesbrough, 2005).

Inadequate potable water supplies are a serious socio-economic problem in several communities in Nigeria and this has caused the inhabitants to resort to bore-holes, wells and polluted surface water for domestic water supplies. Polluted and untreated water supplies are responsible for water-borne infections such as enteric fevers. Morbidity associated with illness due to *salmonella* continues to be on the increase and, in some cases, resulting in mortality (Kabir et al., 2007). To date, it has been attributed to consumption of contaminated food products and water (Oluwayemisi et al., 2012). The issue of contamination of fresh water sources available is on the increase especially in rural communities (Kabir et al., 2007).

In the developing world, an estimated 10 million children under five die of diarrhea related disease and of these deaths, WHO estimates that 16.5 percent, or at least 1.65million, were caused by contaminated water (WHO 2008).

According to Nester et al. (2004), approximately 40,000 cases of *Salmonellosis* are reported in the United States every year. Typhoid and paratyphoid fever are communicable diseases and also constitute grave public health problem throughout the world, especially in developing nations like Nigeria. According to Obiajuru et al. (2000), many children especially in rural communities in Nigeria have missed examinations and some dropped out of school due to frequent infection of typhoid and paratyphoid fever and their associated problems. About 142,000 (Mermin et al., 1977) Americans are infected each year with *Salmonella enteritis* from chicken eggs and about 30 die, the shell of the egg may be contaminated with *Salmonella* by faeces from the environment, or its interior (yolk) may be contaminated by penetration of the bacteria through the porous shell or from a hen whose infected ovaries contaminate the egg during egg formation (Gantose et al., 2009).

Nevertheless, such interior egg yolk contamination is theoretically unlikely (Strokes et al., 2000) even under natural conditions, the rate of infection was very small (0.6%). In a study of naturally contaminated eggs (Humphrey et al., 2010*)* and 30% among artificially and heavily infected hens (Gast et al., 2007) in 2010, an analysis of deaths from older adults and those who were immune compromised (Cummings et al., 2010).

Sources of infection

The sources of infection are food and water that has been contaminated with Salmonella. The following sources are important;

Water Contamination with faeces often results in explosive epidemics. Polluted surface water and standing water (such as in shower houses and unused water dispenser).

Infected often gaining an unusual look or smell, and then is introduced into the stream of commerce. Animal dyes e.g carmine used in drugs, foods and cosmetics.

Shell fish from contaminated water. Unhygenically thawed fowl (the melt water contains many bacteria). Excretion from either skin or infected but apparently clinically healthy people and animals (especially endangered are care givers and animals). Poor kitchen hygiene, especially in institutional kitchens and restaurants because this can lead to a significant outbreak. Milk and other dairy products (ice cream, cheese, custard): inadequate pasteurization or improper handling. Meat and meat products: from infected animals or contaminated with faeces by rodents or humans.

Salmonella organisms can be transmitted from dairy cattle to human through several routes including the consumption of contaminated milk and group beef and direct contact with infected animals (Holmberg et al., 1987).

Incubation period

The incubation time is between 6 and 48 (usually 12-36) hours. The infective dose is thought to vary widely and can depend on the individual consuming the infected food, the type of food involved and possibly the serotype involved. Small numbers (between 10-100) of cells can cause illness if consumed by the young or the elderly, or if the food consumed has a high fat content (e.g. chocolate, cheese or peanut butter) because the fat is thought to protect the cells from the gastric acids(Jay et al., 2005).

In general however, it is thought that high numbers (between 10^5 – 10^6 cells) of Salmonella need to be consumed to cause illness (Jay et al., 2005).

Incidence of water samonellosis

In a study carried out in Owerri metropolis, Imo State Nigeria to determine the Ecology of *Salmonella bacili* in Owerri Metropolis, overall *Salmonella species* was most prevalent in surface water samples (20%) than other samples investigated (Ohalete et al., 2011). Water samples collected by stratified random sampling and processed according to standard Bacteriological techniques out of 20 water samples collected, *Salmonella* species was most prevalent in surface water 4(80%) followed by tap water 1(5%). There was no case of *Salmonella* in the borehole water sample collected (Ohalete et al., 2011).

Also in a cross-sectional study on comparison of DNA Extraction methods to detect Salmonella specie in tap water, both methods for direct DNA extraction generated positive results at 36 cfu/L and at higher bacterial loads. However, all parallels and replicates tested positive only with SH-d for water samples spiked with 144 cfu/L. in comparison to Aw-d, SH-d supported a markedly higher number positive results in all spike samples (Matjaz et al., 2011).

Studies carried out on Bacteriological quality of municipal borehole waters in Imo state, Nigeria revealed the presence of *Salmonella* (10%), *Bacillus* (70%) *Pseudomonas* (80%), *Micrococcus* (50%), *E. coli* (40%), and *Proteus* (20%) in 320 water samples analyzed (Duru et al., 2012).

Ohimain et al. (2013) carried out a study on the occurrence of *Salmonella* in surface and Borehole waters from four coastal communities in Bayelsa state using conventional method. All surface river water samples analyzed were positive for *Salmonella*, of which 75% was *S. typhi* and 33% positive for non-Typhi *Salmonellae*.

Also there was presence of *Escherichia coli* 0157:H7 and *Salmonella spp*. in surface waters of Southern Alberta, USA. Of the 1429 water sample analyzed, 0.9% *Escherichia coli* and 6.2% *Salmonella* were identified (Johnson et al., 2003).

Clinical symptoms

Infants and young children are more susceptible to infection, easily achieved by ingesting a small number of bacteria. In infants, contamination through inhalation of bacteria laden dust is possible (Cumming et al., 2010). After a short incubation period of a few hours to one day, the germs multiply in the intestinal lumen, causing an intestinal inflammation with diarrhoea that is often mucopurulent and bloody. In infants, dehydration can cause a state of severe toxicosis. The symptoms are usually mild. Usually, no sepsis occurs but it can occur exceptionally as a complication in weakened or elderly patients eg. Hodgkin's disease (Cumming et al., 2010). Extra intestinal localization are possible, especially *Salmonella meningitis* in children, Osteitis etc.

Enteritis *Salmonella* e.g (*Salmonella Enterica* Subspecie Enterica Serovar enteritidis) can cause diarrhoea, which usually does not require antibiotic treatment. However, in people at risk such as infants, small children, the elderly, Salmonella infections can become very serious, leading to complications. If these are not treated, HIV patients and those with suppressed immunity can become seriously ill. Children with sickle cell anaemia who are infected with *Salmonella* may develop Osteomyelitis (Cumming et al., 2010).

After a short incubation period (1-2days) there is a sudden onset of abdominal pain, fever and watery diarrhoea. The diarrhea has been attributed to an exotoxin acting in the small intestine. A day or so later, as the infection involves the ileum and colon, a number of stools contain mucus and blood. Each bowl movement is accompanied by straining and tenesmus (rectal spasm) with resulting lower abdominal pain. In more than half of adult cases, fever and diarrhoea subside spontaneously in 2—5 days. However, in children and the elderly, loss of water and electrolytes may lead to dehydration, acidosis and even death.

Conclusion

Water is a chemical compound with the chemical formula H_20. A water molecule contains one oxygen and two hydrogen atoms connected by covalent bonds. The existence of liquid water and to a lesser extent its gaseous and solid forms, on earth are vital to the existence of life on earth as we know it. Safe drinking water is essential to humans and other life forms even though it provides no calories or organic nutrients. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion lack accesses to adequate sanitation. The quality of many source waters will depend upon geology, soil type, natural vegetation, climate and run-off characteristics. Ideally, drinking water should be clear and acceptable to the palate. Water can dissolve many different substances, giving it varying tastes and odours. The use of indicator organisms, in particular the Coliform group, as a means of checking the potential presence of water-borne pathogens has been paramount to protecting public health. Frequent examinations of faecal indicator organisms remain the most sensitive way of assessing the hygienic conditions of water

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