Presence of Pathogenic Bacteria in Drinking Waters of Selected Public Elementary Schools of Iligan City, Philippines

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Abstract

This study reports the presence of pathogenic bacteria in the drinking water of Iligan City Central School and Iligan City North 1 Central School. After the inoculation and identification, the bacteria found in drinking water sample from Iligan City Central School were identified as Klebsiella pneumonia, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca and Salmonella sp. While in Iligan City North 1 Central School sample, E. coli, Salmonella sp. and Shigella sp. were found. In the body, these microorganisms can be pathogenic and has the ability to cause diseases if the immune system is low. A regular sanitation team from the local water district, non-government organizations and other private sectors must conduct evaluation in the public drinking water system of the said locality.

Keywords: drinking water, public schools, pathogenic bacteria

1. Introduction

Contamination by human or animal feces is the most regular and pervasive health risk associated with drinking water (Lee *et. al*., 2005). Recognition that water was a source of pathogenic microorganisms was made in the late 1800's. The pathogenic agents implicated consist of bacteria, viruses and protozoa. The most common microorganisms are total coliform bacteria, fecal coliforms and *Escherichia coli*. As early as 5000 years ago, centralized systems supplied drinking water to communities in parts of the Middle East. Twenty-five hundred years ago, Athens, Greece rebuilt its city with sewers

that transported sanitary waste to rural areas for disposal onto orchards and agricultural fields. In the centuries since, these two services— supply of drinking water and disposal of wastewater—have become intrinsic responsibilities of communities worldwide. As recently as the mid-nineteenth century, however, drinking water supply and wastewater disposal were largely matters of transportation—of bringing drinking water to citizens and removing wastewater.

In the United States, health concerns and technological advances brought changes to drinking water infrastructure around the turn of the twentieth century. In 1872, Poughkeepsie, New York introduced slow sand filtration to reduce turbidity in drinking water. This treatment via filtration removed microbial contaminants that had caused typhoid, dysentery, and cholera epidemics. In 1908, Jersey City, New Jersey introduced drinking water disinfection treatment, and chlorination further reduced drinking water disease outbreaks. If a community's wastewater received any treatment prior to 1900, this treatment consisted of physically separating solids and floating debris from wastewater before discharge into a nearby water body.

Microbiological contamination of water has long been a concern to the public. From the 1920's-1960's, the bacillus which causes typhoid fever was considered a major problem in the water supply. Once it was eradicated, new microbes were present to take its place. In parts of the United States, concern is increasing due to outbreaks of coliform bacteria, giardiasis, cryptosporidiosis, and hepatitis A (1, 2, 3). Some of these are bacteria, while others are viruses or protozoa (Keyser, 1997).

In an analysis of 90 soda and water samples taken from fountains in 30 different fast food restaurants in the Roanoke Valley region of Virginia, researchers from Hollins University found that 48% tested positive for coliform bacteria, or bacteria found in human and animal feces, 11% tested positive for *Escherichia coli*, and more than 17% tested positive for *Chryseobacterium meningosepticum*, which has been shown to cause pneumonia and even meningitis in people with compromised immune systems. They believed that the presence of *E. coli* in more than 11% of samples and in soda fountains at 13% of restaurants included in the study is a cause for concern, as is the presence of *Chryseobacterium meningosepticum*, which could prove dangerous for individuals with compromised immune systems (O'Callaghan, 2010).

Due to the poorly studied water system of Iligan City, this paper evaluated the status of the drinking water of the said locale and assessed its potential risk to pupils of the selected public elementary schools.

2. Methodology

2.1 Research Locale

The laboratory-based experimental activity was conducted at the laboratory of Mindanao Sanitarium and Hospital College located at Tibanga, Iligan City. The water samples were collected from Iligan City North 1 Central School, Brgy. Saray, Tibanga, Iligan City and Iligan City Central School, Roxas Avenue, Mahayahay, Iligan City.

2.2 Specimen Collection

As a means to initiate the experimental process, water samples were collected from selected schools in Iligan City. The specimens were collected in a 250 milliliters sterile bottle. Two water samples were obtained, one from the Iligan City North 1 Central School and the other from Iligan City Central School. Specimens were brought at the Medical Technology laboratory of Mindanao Sanitarium and Hospital College.

2.3 Specimen Improvised Filtration

Specimen preparation was done through filtration of water samples using sterile filter paper. Each water sample of 250 milliliters was slowly poured into their corresponding Erlenmeyer flask with the sterile filter paper. Swabbing that filter paper was done for testing.

2.4 Bacterial Inoculation, Culture and Isolation Method

After specimen preparation, Blood agar (BA), Eosin Methylene Blue agar (EMB), Chocolate agar (CA), Salmonella Shigella agar (SSA) and Thiosulfate Citrate Bile Sucrose agar were used in this study for letting the bacteria grow. The sterile cotton swab was used as the instrument for the inoculation of the bacteria. After inoculation of bacteria, the culture media were incubated at 37 degree Celsius for 24 hours to enhance bacterial

growth. Bacteria grew on the culture medium. For pure culture, nutrient broth was used for BA, EMB and CA and Selenite broth for SSA. Selenite broth is for enrichment of isolation of Salmonella species (Forbes, Sahm, Weissfeld, 2007). Using a sterile loop, small amount of sample from the agar plate was placed on the nutrient broth by streaking it. After the procedure, broths were incubated at 37 degree Celsius for 24 hours to enhance bacterial growth. Bacterial growth was observed in the broths.

2.5 Isolate Identification Tests

To identify them, gram staining and microscopic evaluation of cultured bacteria was performed. First, a smear preparation was done; a sterile needle was used to transfer a small amount of growth from the culture media to the surface of the slide. Gram staining is used for the differentiation of the composition of two major groups of bacteria, Gram- positive cell walls and Gram- negative cell walls (Forbes *et. al*, 2007). If the gram staining result is Gram- positive, catalase test is done. Catalase test is used to differentiate Staphylococcus (catalase positive) from Streptococcus (catalase negative). If it is catalase positive, coagulase test is done. Coagulase test is used to differentiate *Staphylococcus aureus* (coagulase positive) from Staphylococcus species (coagulase negative). On the other hand, if the gram staining result is gram- negative, cytochrome oxidase test is done. Cytochrome Oxidase test is initially used for differentiating between groups of Gram- negative bacteria. And biochemical tests were done after Cytochrome Oxidase test for identifying the bacteria.

Biochemical methods for identification of organism includes Triple Sugar Iron (TSI), Lysin Iron Agar (LIA), Methyl Red (MR), Motility Testing, Indole Production, Citrate Utilization and Urea Hydrolysis. TSI used to detect fermentation of glucose, lactose and sucrose, production of gas from the fermentation of sugars and production of hydrogen sulfide. LIA used to determine whether a Gram- negative rod decarboxylates or deaminates lysine and form hydrogen sulfide. MR used to determine the ability of an organism to produce and maintain stable end products from glucose fermentation. Motility testing used to determine if an organism is motile. Indole production used to determine the ability of an organism to split tryptophan to form the compound indole. Citrate utilization used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Urea hydrolysis used to

determine the ability of organism to produce the enzyme urease, which hydrolyzes urea. Hydrolysis of urea produces ammonia and carbon dioxide.

3. Results and Discussion

Table 1 shows the growth of bacteria in each culture media from the two samples collected by the researchers. This table shows that all agars used except the TCBS has growth. TCBS Agar was used for the selective isolation, differentiation, and cultivation of pathogenic Vibrios. This result gave the researchers an idea that there is no Vibrio spp. present in the samples. The samples were both inoculated in all five culture media namely, Eosin Methylene Blue (EMB), Blood Agar (BA), Chocolate Agar (CA), Salmonella- Shigella Agar, and Thiosulfate-Citrate-Bile salts-Sucrose agar (TCBS).

Table 1. Growths (types of colonies) in each agar from the samples

Agars	North 1	City Central
1. Eosin Methylene Blue Agar (EMB)	2 growths	2 growths
2. Blood Agar (BA)	1 growth	1 growth
3. Chocolate Agar (CA)	1 growth	1 growth
4. Salmonella- Shigella Agar (SS)	1growth	2 growths
5. Thiosulfate-Citrate-Bile salts-Sucrose agar (TCBS)	No growth	No growth

Of the two samples collected, 11 colonies were isolated. Five colonies were isolated from Iligan City North 1 Central School and six colonies were isolated from Iligan City Central School. Isolation of colonies in a culture media means that bacteria are present in the sample. The isolated colonies should be subjected in to pure culture. Obtaining a pure culture is mandatory to establish the identity and susceptibility. If you attempt to identify a culture that is not pure, in other words, containing two or more different species of bacteria, then you may incorrectly identify the bacteria you intend to find. A pure culture ensures that only one type of bacteria is present and ensures one answer regardless of how many times you test it (Antranik, 2012).

After obtaining a pure culture, the colonies should be subjected to Gram stain and examine microscopically to identify whether it is a Gram- positive or Gram- negative bacteria.

Gram- positive bacteria should be subjected to catalase and coagulase tests. Gram- negative bacteria should be subjected to oxidase and biochemical tests. The specific type of bacteria present is shown in Table 2.1 and Table 2.2.

Table 2.1 Result of bacterial identification tests in the sample from North 1

Bacterial Identification Tests	Bacteria 1	Bacteria 2	Bacteria 3
1. Agar	EMB, BA	EMB, SSA	CA
2.Gram Staining	Gram (-) bacilli	Gram (-) bacilli	Gram (-) bacilli
3.Catalase Test			
4.Coagulase test			
5.Oxidase Test	-	-	-
6.Biochemical Tests			
Indole	+	-	-
Motility	+	+	-
MR	+	+	+
TSI	A/A*, lactose fermenter	K/A*	K/A
LIA	-/+	-/+	-/+
Citrate	-	+	-
Urease	-	-	-
Result	*Escherichia coli*	*Salmonella*	*Shigella*

*A/A – acid slant/acid butt; K/A – alkaline slant/acid butt

Table 2.2 Result of bacterial identification tests in the sample from City Central

Bacterial Identification Tests	Bacteria 1	Bacteria 2	Bacteria 3	Bacteria 4	Bacteria 5
1. Agar	EMB	EMB	BA, SSA	CA	SSA
2.Gram Staining	Gram (-) Bacilli	Gram (-) bacilli	Gram (-) bacilli	Gram (-) bacilli	Gram (-) bacilli
3.Catalase Test					
4.Coagulase Test					
5.Oxidase Test	-	-	-	-	-
6.Biochemical Tests					
Indole	-	-	+	+	-
Motility	-	+		-	+
MR	-	-	+	-	+
TSI	A/A*, lactose fermenter	A/A, lactose fermenter	A/A,lactose fermenter	A/A,lactose fermenter	K/A*
LIA	-/+	-/+	-/+	-/+	-/+
Citrate	+	+	-	+	+
Urease	+	+	-	-	-
Result	*Klebsiella Pneumonia*	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella oxytoca*	*Salmonella*

*A/A – acid slant/acid butt; K/A – alkaline slant/acid butt

Tables 2.1 and 2.2 show the overall results of the bacterial identification tests from the two sampling sites. The inoculated water from North 1 isolated 3 kinds of bacteria. These are *Escherichia coli* which was isolated in EMB and BA, *Salmonella* which was isolated in both BA and SSA, and *Shigella* sp. which was isolated in CA.

The inoculated water from the City Central isolated five kinds of bacteria. These bacteria were: 1) *Klebsiella pneumoniae*, which was isolated in EMB; 2) *Enterobacter aerogenes*, which was also isolated in EMB; 3) *Escherichia coli*, which was isolated in both BA and SSA; 4) *Klebsiella oxytoca*, which was isolated in CA and 5) *Salmonella*, which was isolated in SSA.

Difference of location and orientation of the sample sites could possibly contribute to the slight difference of bacterial species isolated.

3.1 Possible Health Effects of the Isolated Bacteria

3.1.1 Escherichia coli

Eschirichia coli is an enteric bacteria which makes up some of the normal flora in the human intestines, but can cause infection if it enters other parts of the body. There are 5 strains or categories of *E. coli* that cause diarrheal illnesses or disease.

First, enterotoxigenic *E. coli* is an important cause of diarrhea in infants and travelers in underdeveloped countries or regions with poor sanitation. It is acquired by ingestion of contaminated food and water. The disease requires colonization and elaboration of one or more enterotoxins. This illness is characterized by severe diarrhea without fever, which may lead to dehydration (Todar, 2008).

Second, enteroinvasive *E. coli* is similar to shigellosis and is caused by bacterial penetration and destruction of intestinal mucosa. Chills, fever, headache, muscle pain, abdominal cramps, and profuse diarrhea are the symptoms of this disease (Todar, 2008).

Third, enterohemorrhagic *E. coli* is the primary cause of hemorrhagic colitis or bloody diarrhea, which can progress to the potentially fatal hemolytic uremic syndrome and characterized by the production of verotoxin or Shiga toxins (Todar, 2008).

Fourth, enteropathogenic *E. coli* causes severe diarrhea in infants that can last for over 2 weeks and results to death if dehydration is severe. In adults, the illness is characterized by severe diarrhea, nausea, vomiting, abdominal cramps, headache, fever, and chills. This is caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins (Todar, 2008).

Fifth, enteroaggregative *E. coli*are associated with persistent diarrhea in young children. The bacteria adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation. *E. coli* usually remains harmlessly confined to the intestinal lumen; however, in the

debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even normal "nonpathogenic" strains of *E. coli* can cause infection (Kaper, *et.al,* 1998).

3.1.2 Salmonella

In humans, *Salmonella* are the cause of two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a food- borne infection/intoxication. Gastroenteritis, more commonly known as food poisoning, its symptoms include diarrhea, fever and abdominal cramps which usually subside on their own without medical treatment. The bacteria can cross from the intestines into the bloodstream causing serious illness or even death if not treated with antibiotics. Typhoid fever is also transmitted through contaminated food and water but causes a more serious systemic throughout the body infection. Symptoms include fever, sweating, gastroenteritis, diarrhea and rash. If left untreated, the fever can last for weeks or even month causing complications that can result in death (Chandler, 2011).

3.1.3 Shigella

Shigella causes bacillary dysentery, the symptoms of which include abdominal pain, diarrhea, fever, vomiting and blood or mucus in the stool. The bacteria are transmitted by the fecal-oral route, and through contaminated food and water. Once ingested, the bacteria survive the gastric environment of the stomach and move on to the large intestine. There, they attach to and penetrate the epithelial cells of the intestinal mucosa. After invasion, they multiply intracellularly and spread to neighboring epithelial cells, resulting in tissue destruction and characteristic pathology of shigellosis (Todar, 2008).

Following host epithelial cell invasion and penetration of the colonic mucosa, *Shigella* infection is characterized by degeneration of the epithelium and inflammation of the lamina propria. This results in desquamation and ulceration of the mucosa, and subsequent leakage of blood, inflammatory elements and mucus into the intestinal lumen. Patients suffering from *Shigella* infection will therefore pass frequent, scanty, dysenteric stool mixed with blood and mucus, since, under these conditions, the absorption of water by the colon is inhibited (Todar, 2008).

3.1.4 Klebsiella pneumoniae

Klebsiella pneumoniae is a ubiquitous bacterium normally found in the mouth, throat, skin and especially the intestines of most healthy people. *Klebsiella* can become a problem and cause serious infections in those with compromised or weakened immune systems. *K. pneumoniae* can infect the lungs and cause pneumonia. There can also be the formation of lung abscesses and emphysemas, a collection of pus between the lung and chest wall. Other symptoms include chest pain, shortness of breath, difficulty breathing, fever and chills. *K. pneumonia* can also be the cause of some urinary tract infections that can affect the bladder, urethra, ureters or kidneys. A urinary tract infection has sudden onset of symptoms, to include an urgency to urinate, abdominal discomfort, pain or burning on urination, back pain and possibly fever. The urine may appear to be cloudy or bloody. *K. pneumoniae* bacteria can enter the bloodstream through any opening in the skin, such as a wound or a surgical site. Sepsis is a bacterial infection carried by the blood throughout the body, causing widespread inflammation. The symptoms of sepsis are shaking chills, fever, low blood pressure, confusion and extreme weakness. (Meininger, 2011).*K. pneumoniae* and *K. oxytoca*, both commensals of the human gastrointestinal tract, occasionally cause diarrhea in humans (Goldstein, 1998)

3.1.5 Klebsiella oxytoca

Klebsiella oxytoca is typically found in the intestinal tract, where they are part of a healthy colon's ecosystem. The bacteria can spread to other parts of the body, however, and cause life-threatening diseases. These infections may be community-acquired or sometimes a person may develop the infection while he is a patient in a hospital. *K. oxytoca* may cause infections of the urinary tract, the respiratory tract, the gastrointestinal tract or, in serious cases, the blood (Marie, 2010)

3.1.6 Enterobacter aerogenes

Enterobacter aerogenes forms part of the endogenous human gastrointestinal microflora. It also resides in soil, water and in dairy products. Generally infections arise from the patients' own flora; however cross-infection can occur via the hands of healthcare workers, during insertion of medical devices and in surgical procedures (Sanders, *et.al,* 1997). *Enterobacter* is a

frequent cause of infection in immunocompromised individuals, low birth weight and premature babies, and those with serious underlying health conditions. It has frequently been implicated in urinary tract infections, skin and soft tissue infections, respiratory infections, gastrointestinal infections, adult meningitis, wound infections and bacteremia (Sanders *et.al*, 1997). Particular risk factors for *E. aerogenes* infection include prolonged hospital stay, intravenous catheter use, invasive surgical procedures and previous antibiotic usage.

4. Conclusion and Recommendation

This study attempted to determine the possible bacteria present in drinking fountains and faucet water from Iligan City Central School, Roxas Avenue, Mahayahay, Iligan City and Iligan City North 1 Central School, Brgy. Saray, Iligan, City.

The specific bacteria found in Iligan City Central School were *Klebsiella pneumonia, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca* and *Salmonella*. On the other hand, in Iligan City North 1 Central School*; Escherichia coli, Salmonella* and *Shigella* were present.

These bacteria except *Salmonella* and *Shigella* are normal flora in the specific areas of the body and it will be pathological if there is increase in numbers in their population and if they will be moved to another sterile parts of the body. Moreover, the body of an individual will be endangered through these bacteria if the immune system of the person is very weak. *Salmonella* and *Shigella* are found in humans at times of infection and not part of the normal bowel flora.

Considering the bacteriological findings and conclusion, this study firmly suggests that the presence of bacteria in the drinking water of the schools at the Iligan City Central School and Iligan City North 1 Central School may indicate that it is not totally safe for drinking because of contamination which is probably due to leakage of the pipes, contamination in the nozzle of the drinking water fountains, and biofilm formation in faucets and pipes which cause the bacteria to live. It is then recommended that the local water district of Iligan City should conduct deeper survey on the safety level on the drinking faucets. Thereby, drinking water of the research locale of this study may result to possible health risk.

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