



Microbial Analysis of Greywater from Local Bathrooms and Its Health Implications in Bali Local Government Area Taraba State Nigeria

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ABSTRACT

Introduction: Due to reckless ways of disposing water from the baths in Nigerian's Northern towns in Bali LGA Taraba State, which was caused by poor bathrooms occasioned by poverty, many lives had been lost from deaths caused by pathogenic microbes in these recklessly exposed used waters (greywater).

Objective: This research was conducted in order to determine microbial contaminants of greywaters from local bathrooms in Bali LGA of Taraba State, Nigeria, and their potential threat to the lives of people in this LGA.

Methods: Greywaters from bathrooms in ten (10) communities in Bali LGA were collected and analyzed for microbial contaminants. The communities are Bali, Garba-Chede, Pamgri, Garbabi, Maihula, Suntai, Jamtari, Mayokam, Daka, and Kungana, twenty areas were sampled in each of these communities. Simple Stain was done using 5 ml methylene blue solution by adding into the specimen bottles to know if there bacteria in the samples. Acid-fast Stain was done using 5 ml Ziehl Nielsen stain each added to the labeled bottles to identify *Mycobacterium* sp. in the greywater samples.

Stains for Cellular Features cellular features such as presence of capsule, spores and flagella were screened using India ink stain which creates a clear zone around the bacteria cell in gram negative strains, and Malachite stain which stains spores green and bacteria cell red. Differential Staining (Gram Staining Procedures) was done in which the bacteria were first stained with crystal violet and then treated with mordant in order to fix the stain inside the cell. Identification of microbial organisms down to species level was done using molecular biology technique by cleavage of microbial DNA Sequence using restriction enzymes (RE). **Results:** Results showed that most of the greywaters from these local bathrooms were mainly gram negative bacteria. The most disseminated species are *Salmonella typhi*, *Vibrio cholera*, *Escherichia coli*, *Campylobacter jejuni*, *Staphylococcus aureus* and *Shigella dysenteriae*, according to various restriction enzymes specific to each bacterium. The RE obtained at Collaborative Research Inc. 1365, Main Street, Massachusetts, USA; do show any abnormal cleavage pattern of the DNA sequence during electrophoresis and RE DNA digestion. Most species of the microbes were highly present in greywater from local bathrooms in Maihula, Mayokam and Garbabi. It was however not high in Bali and Suntai. Highest percentage of contamination was seen in Garbabi, Garba-Chede, Jamtari and Daka; with *Campylobacter jejuni* the dominant bacterium species present. **Conclusion:** The results showed that in areas sampled, the greywaters from local bathrooms contained mainly gram negative pathogenic bacteria with potential threat to lives, and *Campylobacter jejuni*, *Vibrio cholera*, *Salmonella typhi*, and *E. coli* were the dominant organisms.

Keywords: Bali LGA; bacteria; DNA sequence; *Campylobacter jejuni*; greywaters.

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Introduction

Bali Local Government Area (LGA) is one of the local governments in Taraba State. Taraba State was carved out of the former Gongola State on 27th August 1991 by the then regime of the then military head of state in Nigeria, General Ibrahim Babangida^[1]. Taraba State is named after the Taraba River which traverses the Southern part of the state. Taraba's capital is Jalingo; Situated in the North Eastern part of Nigeria. It occupies 54,473 km². The state is bounded in the West by Plateau, Nasarawa and Benue states, on the Eastern border by Adamawa State and the Republic of Cameroon, and on the Northern border by Gombe State. Taraba State lies largely within the middle of Nigeria and consists of undulating landscape dotted with a few mountainous features. This includes the scenic and prominent Mambilla Plateau. Across this plateau is found Bali LGA with a population of 208, 935 according to National Population Census report 2006.^[2]

The major occupation of the people of Bali LGA is agriculture. Cash crops produced in the state include coffee, tea, groundnuts and cotton. Crops such as maize, rice, sorghum, millet, cassava, and yam are also produced in commercial quantity. In addition, cattle, sheep and goats are reared in large numbers, especially on the Mambilla Plateau, and along the Benue and Taraba valleys. Similarly, the people undertake other livestock production activities like poultry production, rabbit breeding and pig farming in fairly large scale. Bali LGA is among the leading LGA in the production of livestock with its dairy farms. Communities living on the banks of River Benue, River Taraba, River Donga and Ibi engage in fishing all year round. Other occupational activities such as pottery, cloth-weaving, dyeing, mat-making, carving, embroidery and blacksmithing are also carried out in various parts of the LGA especially Bali, Garba-Chede and Suntai.

Bali LGA is richly endowed with potentials for the development of tourism, and mineral resources and that include the recently discovering of uranium in huge quantities in the state. In recognition of this, the government has made concerted efforts to improve areas of tourist attractions like Mambilla Tourist Center, Gumpti Park and game reserve in Gashaka, the Nwunyu Fishing festival in Ibi which usually holds in April of each year where activities such as canoe racing, swimming competition and cultural dances were held; villages which are enroute via Bali LGA^[3].

Also effort is made to attract foreign investors to go and invest in the natural resources that abound in the state. The main festival in the LGA is Purma celebrated annually. Despite these accolades, the challenges posed by dirty environment are enormous, and needed argent attention.



Figure 1: Map of Nigeria showing Taraba State in red colour

Greywater gets its name from its cloudy appearance and from its status as being between fresh, potable water (known as "white water") and sewage water ("black water"). In a household context, greywater is the leftover water from baths, showers, hand basins and washing machines only, although it is now becoming more common that washing machine effluent is being excluded from this category - this is due, in part to the rise in greywater recycling, where washing machine effluent is being excluded due to the filtration limitations. Some definitions of greywater include water from the kitchen sink. Any water containing human fecal waste is considered black water.^[4]

Domestic wastewater is usually combined at the sewer, so that grey- and blackwater are removed together using a shared sewerage system in a process called elimination. Sewage water can then be treated to limit pollution and health risks, before being returned to the environment at large. Most greywater ends up as effluent in rivers and oceans in this way. There are other alternatives to eliminating greywater that allow for efficient use; using it to irrigate plants is a common practice.^[5] The plants use contaminants of greywater, such as food particles, as nutrients in their growth. However, salt and soap residues can be toxic to microbial and plant life alike, but can be absorbed and degraded through constructed wetlands and aquatic plants such as sedges, rushes, and grasses.

Most greywater is easier to treat and recycle than blackwater, because of lower levels of contaminants. If collected using a separate plumbing system from blackwater, domestic greywater can be recycled directly within the home, garden or company and used either immediately or processed and stored. If stored, it must be used within a very short time or it will begin to putrefy due to the organic solids in the water. Recycled greywater of this kind is never safe to drink, but a number of stages of filtration and microbial digestion can be used to provide water for washing or flushing toilets. Some



greywater may be applied directly from the sink to the garden or container field, receiving further treatment from soil life and plant roots. Given that greywater may contain nutrients, pathogens such as bacteria, protozoan, fungi, algae, and viruses, and is often discharged warm, it is very important to store it before use for irrigation purposes, unless it is properly treated first^[6].

In Bali LGA, water from bathrooms are neither channeled underground nor discarded properly. These waters are exposed recklessly on the surface of the soil behind the local bathrooms; usually made from trees, bamboos, worn-out clothes with insides covered with or without soil. During rainfalls, the greywater from these baths flows into rivers and stream as run-offs, and they are in-turn used as drinking water since there is very little portable water to cater for these timing populations in the LGA. Many bacteria survived well in such dirty waters whether aerobic or anaerobic in nature^[6]. There are no doubts however, these that the presence of these pathogenic microbes in these environments posed great danger, and only will tell when it will explode. A lot of deaths had been reported to be caused by bacteria related epidemic in some parts of the LGA in 2008, yet the necessity for proper dispose of greywater from bathrooms, and the need for proper health education in the LGA is still far reached.^[7]

Microbial contaminations of greywater had been the cause of typhoid fever, food poisoning, cholera, viral hepatitis B, Staphylococcus infections, diarrhea, gastroenteritis, Guillain Barre Syndrome, and amoebic dysentery.^[8] The water from these bathrooms is often drunk by livestock, and food chain enters human physiology resulting in heavy casualties if not checked on time. The aim of this research is to determine the microbial species present in greywaters from local bathrooms and their health implications on people and lives in Bali LGA, Taraba State Nigeria, using molecular biological techniques and other analysis.

Materials and Methods

Sample Collection

Local bathrooms in villages as well as towns in Bali Local Government Area (LGA) were visited. The local bathrooms here imply those bathrooms whose bathwater are not channeled underground but on the soil surfaces. Areas where gray water was collected from the surface of the soil are: Bali, Suntai, Garba-Chede, Pamgri, Garbabi, Maihula, Jamtari, Mayokam, Daka, and Kungana. In each of the sampled areas, the sample size was twenty (20). Collection of gray water from the soil surface behind each local bathroom through the local outlets, using a moderately large spoon about 10 cm³ in volume. The samples collected were stored in 20 ml specimen bottles and labeled accordingly, and taken to the biology laboratory, Federal Polytechnic, Bali, Taraba State, for preliminary screening.

Microbial Analysis of Greywater Samples

The following analyses were carried out in samples in order to identify the microbes present in the collected greywaters.

Simple Stains: methylene blue solution 5 ml was added into the specimen bottles to know if there bacteria in the samples^[9].

Acid-fast Stains: 5 ml Ziehl Nielsen stain was each added to the labeled bottles to identify *Mycobacterium* sp. in the greywater samples. A dark-red colour confirmed the presence of *Mycobacterium* sp. while other bacteria are stained blue^[9].

Stains for Cellular Features: cellular features such as presence of capsule, spores and flagella were screened using India ink stain which creates a clear zone around the bacteria cell in gram negative strains, and Malachite stain which stains spores green and bacteria cell red^[9].

Differential Staining (Gram Staining Procedures): Bacteria were first stained with crystal violet and then treated with mordant in order to fix the stain inside the cell. The bacteria were then washed with a decolourizing agent alcohol and counter stained with safranin solution; a light dye. The walls of gram positive bacteria have peptidoglycans than that of gram negative strains. Gram positive bacteria retained the original violet dye, and cannot be counter stained. Gram negative bacteria have thinner walls containing an outer layer of lipopolysaccharides, which was disrupted by alcohol wash. This allows the original dye to escape so that the cell take up the second dye or counter stain. Thus, gram positive strains stain purple while gram negative strains stain pink. The sample bottles were then separated according to the strains, and kept for further analysis^{[9],[10]}.

Identification of Microbial Organisms Using Molecular Biology Technique:

Cleavage of Microbial DNA Sequence Using Restriction Enzymes

Since restriction endonucleases (enzymes) are specific in their mode of action, for each can only cleave a particular DNA sequence belonging to certain bacterial or microbial species. Restriction enzymes were purchased from the suppliers, and were used to cleave the DNA sequence in the microbes from each specimen collected and their band patterns were compared with that of the standard organisms. Briefly, restriction enzymes (RE) and substrates as well as assay buffer were kept in an ice bucket. 5 mg of DNA were each put in an appendorf tube and dissolved in 20 ml of water, and 2 ml of 10X assay buffer containing restriction enzymes were added. Sterilized water was added to make the final volume of reaction mixture to 30 ml. The mixture was then centrifuge gently at 120 rpm (revolution per minute). Mixture was then incubated for 1 hour at 37°C in an incubator. 1% agarose gel was prepared for loading and electrophoresis. After 1 hour, the reaction was stopped by adding 3.33 ml of 6X gel loading buffer to the appendorf tube. The vials are labeled and put

on ice. Electrophoresis was started until the bromophenol dye solution had reached three-fourth (3/4) of the gel (about an hour), and observed under UV transilluminator. The band appearances revealed the cleavage of DNA sequence by respective restriction enzymes. Results were compared with that of standard microorganisms ^[11].

RESULTS

The diagrams below ^[Figure 2] showed how bathwaters are discharged from these local bathrooms in Bali LGA.



A local water outlet from the baths

a.



b.

Figure 2: Water collection area behind the bathrooms (a) Bali (b) Maihula

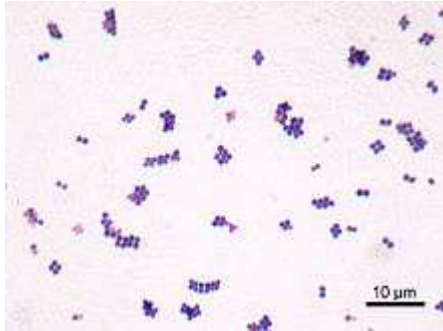
Bacteria were the dominant microorganisms present in all the sampled communities, and most of the bacteria are pathogenic with one form of structural adaptations in order to thrive well in the greywater from the baths, since some of the waters contained detergents ^[Figure 3]. Some algae were also noticed as indicator of polluted environment ^[Figure 3].



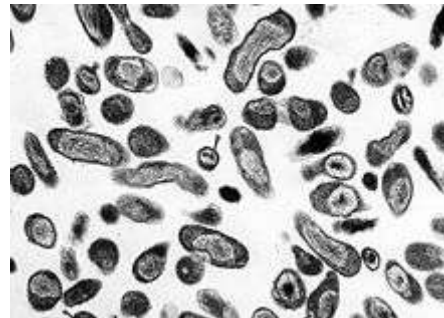
a. Bacilli with spores



b. Cubes of Cocci



c. *Staphylococcus aureus*.



d. *Campylobacter jejuni*



e. *Spirulina platensis* (an alga)



f. Cyanobacteria with heterocyst (an alga)



g. *Chlorella sorokiriana* (an alga)



h. *Vibrio cholerae*

Figure 3: Morphological screening of microbes using microscope \leq x100

_____ These microbes were viewed using compound microscope of double eye piece.

Restriction enzymes cleavage pattern did not differ greatly from the normal pattern, except for *Campylobacter jejuni* and *Shigella dysenteriae* which showed unique cleavage pattern of having affinity for cytosine base of the DNA sequence ^[Table 11].



Table 1: Microbial Identification Using Type I and II Restriction Enzymes

Microbes	RE	CS	CP	
<i>Bacillus amyloliquefaciens</i>	BamHI	5-G [^] GATCC-3 3-CCTAG [^] G-5	5-G 3-CCTAG G-5	GATCC-3 G-5
<i>A. Globigii</i>	BglII	5-A [^] GATCT-3 3-TCTAG [^] A-5	5-A 3-TCTAG	GATCT-3 A-5
<i>E. coli RY13</i>	EcoRI	5-G [^] AATTC-3 3-CTTAA [^] G-3	5-G 3-CTTAA	AATTC-3 G-5
<i>E. coli R₂45</i>	EcoRII	5'-G [^] GATCC-3' 3'-CCTAG [^] G-5'	5'-G-3' 3'-CCTAG-5'	5'-GATCC-3' 3'-G-5'
<i>Haemophilus influenzae Rd</i>	HindII	5-A [^] AGCTT-3 3-TTCGA [^] A-5	5-A 3-TTCGA	AGCTT-3 A-5
<i>H. parainfluenzae</i>	HpaI	5-GTT [^] AAC-3 3-CAA [^] TTG-5	5-GTT 3-CAA	AAC-3 TTG-5
<i>Klebsiella pneumoniae OK8</i>	KpnI	5-GGTAC [^] C-5 3-C [^] CATGG-3	5-GGTAC 3-C	C-3 CATGG-5
<i>Staphylococcus aureus</i>	Sau3AI	5- [^] GATC-3 3-CTAG [^] -5	5- 3-CTAG	GATC-3 5
<i>Salmonella typhi 57H</i>	Sty57HI	5'-GC [^] GCGC-3' 3'-CGCCGG [^] CG-5'	5'-GC-3' 3'-CGCCGG-5'	5'-GGCCGC-3' 3'-CG-5'
<i>Vibrio cholera Fd</i>	VcfdI	5'-C [^] CGG-3' 3'-GGC [^] C-5'	5'-C-3' 3'-GGC-5'	5'-CGG-3' 3'-C-5'
<i>Campylobacter jejuni</i>	CjeI	5'-C [^] CGG-3' 3'-GGC [^] C-5'	5'-C-3' 3'-GGC-5'	5'-CGG-3' 3'-C-5'
<i>Shigella dysenteriea 9710S</i>	Sdyl	5'-GG [^] CC-3' 3'-CC [^] GG-5'	5'-GG-3' 3'-CC-5'	5'-CC-3' 3'-GG-5'
<i>Streptomyces abus G</i>	SaLI	5-G [^] TCTGAC-3 3-CAGCT [^] G-5	5-G 3-CAGCT	TCGAC-3 G-5-----

RE (restriction enzymes), [^] **CS** (cleavage sites), **CP** (cleavage products), ***Source of RE** (Collaborative Research Inc. 1365, main street Waltham, Massachusetts, 02154, USA).



Figure 5: Electrophoresis of bacterial DNA using agarose gel

DNA separation was indicated bands on agarose gel for each bacterium (n=5)

Table 2: Distribution of bacteria in greywater from local bathrooms in Bali LGA

Bacteria type	Capsule	Spore	Flagella	Area found (n=20)
<i>E. coli</i>	+	+	+	**
<i>H. influenzae</i>	+	-	+	a, c, e, f
<i>K. pneumoniae</i>	+	+	+	**
<i>S. aureus</i>	-	+	+	**
<i>S. typhi</i>	+	+	+	**
<i>V. cholerae</i>	-	+	+	**
<i>B. globigii</i>	+	+	-	a, b, d, g, j
<i>C. jejuni</i>	+	+	+	**
<i>S. dysenteriae</i>	-	-	-	a, e, f, h, i
<i>P. aeruginosa</i>	+	+	+	c, g, i, j
<i>S. pyogenes</i>	+	-	+	a, d, e, f, h

+(present), -(absent), * (highly present), ** (occurs in all areas), a (Bali), b (Garba-Chede), c (Pamgri), d (Garbabi), e (Maihula), f (Suntai), g (Jamtari), h (Mayokam), i (Daka), j (Kungana). Only microbes with high incidence rate were represented.

Discussion

Restriction endonucleases are a class of enzyme that cut DNA molecules ^[12]. Each enzyme recognizes a unique sequence of nucleotides in the DNA strand, usually about 4-6 base-pairs long in various organisms ^[12]. The sequences are palindromic in that the complimentary DNA strand has the same sequence only in the reverse direction, so both strands of DNA are cut at the same location.

Restriction enzymes are found in many different strains of bacteria where their biological role is to participate in cell defense, and for this reason they can be used to identify bacteria species at DNA level ^[13]. These enzymes “restrict” foreign (e.g. viral) DNA that enters the cell, by destroying it. The host cell has a restriction-modification system that methylates its own DNA at sites specific for its respective restriction enzymes, thereby protecting it from cleavage. Over 800 known enzymes have been discovered that recognize over 100 different nucleotide sequences.

Restriction enzymes are used in biotechnology to cut DNA into smaller strands in order to study fragment length differences among individuals (Restriction Fragment Length Polymorphism – RFLP) or for gene cloning. RFLP techniques have been used to determine that individuals or groups of individuals have distinctive differences in gene sequences and restriction cleavage patterns in certain areas of the genome. Knowledge of these unique areas is the basis for DNA fingerprinting. Each of these methods depends on the use of agarose gel electrophoresis for separation of the DNA fragments ^[13].

There are three different types of restriction enzymes. Type I cuts DNA at random locations as far as 1000 or more base-pairs from the recognition site. Type III cuts at approximately 25 base-pairs from the site. Types I and III require ATP and



may be large enzymes with multiple subunits. Type II enzymes, which are predominantly used in biotechnology, cut DNA within the recognized sequence without the need for ATP, and are smaller and simpler. Type II restriction enzymes are named according to the bacterial species from which they are isolated. Hence, the use of type I and II RE had helped to identify these microorganisms to the species level ^[14]. The cleavage pattern of these enzymes on DNA molecules in the organisms were not uniquely different from that obtained by other researchers.

However, a variation was noticed in the cleavage pattern of *Campylobacter jejuni* and *Shigella dysenteriae* in which the ---CC--- (Cytosine) linkages were always affected as opposed to the usual guanine linkages. The reason for this abnormality was because the outer coatings of these bacteria were only susceptible to cleavage at this site by restriction enzymes, so cleavage is only possible at cytosine-cytosine linkages ^[15]. A bacterium uses a restriction enzyme to defend against bacterial viruses called bacteriophage, or phages. When a phage infects a bacterium, it inserts its DNA into the bacterial cell so that it might be replicated. The restriction enzyme prevents replication of the phage DNA by cutting it into many pieces.

Restriction enzymes were named for their ability to restrict, or limit, the number of strains of bacteriophage that can infect a bacterium. Each restriction enzyme recognizes a short, specific sequence of nucleotide bases (the four basic chemical subunits of the linear double-stranded DNA molecule—adenine, cytosine, thymine, and guanine). These regions are called recognition sequences and are randomly distributed throughout the DNA. Different bacterial species make restriction enzymes that recognize different nucleotide sequences. ^{[14], [15], [16]} Their functions in these microbes from greywaters from local bathrooms in Bali LGA were not different.

When a restriction endonuclease recognizes a sequence, it snips through the DNA molecule by catalyzing the hydrolysis (splitting of a chemical bond by addition of a water molecule) of the bond between adjacent nucleotides. Bacteria prevent their own DNA from being degraded in this manner by disguising their recognition sequences. Enzymes called methylases add methyl groups ($-\text{CH}_3$) to adenine or cytosine bases within the recognition sequence, which is thus modified and protected from the endonucleases. The restriction enzyme and its corresponding methylases constitute the restriction-modification system of a bacterial species ^[17].

When food becomes contaminated, it has the potential to make you sick. Depending on the source and level of contamination, the effects of contaminated food by greywaters from these local bathrooms in Bali LGA caused symptoms such as cramps, nausea, diarrhea, vomiting, nerve damage, allergies and paralysis. While eating foods contaminated with pathogenic bacteria, allergens or bacterial toxins has a relatively quick effect, food. Food-borne infection is a type of food poisoning that can result when you eat food that is contaminated with pathogenic bacteria, such as *E. coli* or *Salmonella*. When enough bacteria are present as was seen in Suntain, Daka, Maihula, and Bali, they can multiply in your digestive tract and make people sick ^{[Table 2], [Figure 2]}. Although many foods contain some bacteria, certain species are particularly hazardous and cause symptoms such as stomach cramps, nausea, fever, diarrhea and vomiting, such *Vibrio cholerae*, *Campylobacter jejuni*, and *Haemophilus* ^[18]. Results can be serious and life threatening for babies, elderly people, pregnant women and people with compromised immune systems.

Depending on the species of bacteria, symptoms of food-borne infection can take as long as a month to develop. Food intoxication is a type of food poisoning caused by bacterial toxins. Although the bacteria *Staphylococcus aureus* and *Clostridium botulinum* will not make people sick, the toxins that they release into food can ^[18]. If you eat food that is contaminated with *C. botulinum*, the person will experience symptoms such as weakness, vertigo and vomiting within 36 hours. Without medical treatments, food intoxication from botulism can lead to paralysis and possibly death ^[19]. The bacteria seen in these greywaters especially in Garba-Chede, Maihula, Bali, and Kungana, possess various types of pathogenic life cycles in the environment. ^[Figure 1-2]

Klebsiella species are non-motile, rod-shaped, gram-negative, catalyase-positive, oxidase-negative, lactose fermenting, facultative anaerobic bacteria with a prominent polysaccharide capsule. The genus *Klebsiella* is named after Theodor Albrecht Edwin Krebs, a German-Swiss pathologist and microbiologist who identified the bacterium causing diphtheria. When *K. pneumoniae* colonizes the respiratory system it can cause bronchopneumonia or bronchial pneumonia - as distinct from lobar pneumonia ^[20]. This results in the acute inflammation of the walls of the bronchioles (small air passages leading from the windpipe and bronchi towards the alveoli: the terminal air sacs) and consequent congestion with pus. Usually there are several sites of infection, on both lungs. Endotracheal intubation (insertion of a tube into the windpipe to assist breathing) sometimes results in this infection. Bronchopneumonia may also be caused by *Staphylococcus aureus*, *E. coli*, and *Pseudomonas*. *Klebsiella pneumoniae* can also cause urinary tract infections often associated with catheters (UTIs) as well as infecting surgical wound sites. The presence of this bacterium in Bali greywater from baths can cause these pathologies in the lives of people, since the bacterium are spread via water, and animal agents ^[21].

Escherichia coli is widely known as *E. coli*, although scientific names should not be abbreviated until they have been stated once in full. In the past it has been called *Bacterium coli* and *Bacillus coli*, but it was (re-) named after Theodor Escherich, a leading German bacteriologist in the field of pediatrics, who first described it in 1886 ^[22]. The specific part of the binomial name "coli" means "of the colon" - the large intestine (also known as the bowel). It has been widely used in laboratories for over 60 years because it is easy to grow and offers little danger of infection, and it has been extensively used in virology and recombinant DNA work.

As it leaves the body in faeces and can survive for some time afterwards, it serves as an indicator of faecal contamination of the environment and foods. A number of microbiological test procedures have been developed for this purpose. It lends itself to tests involving selective media based on recreating conditions within the colon. It is a Gram-negative, facultative anaerobic, rod-shaped bacterium. It is non-sporulating i.e. it does not produce spores ^[23].



Some strains are motile i.e. they possess flagella, which are described as peritrichous i.e. projecting outwards all round the surface of the cell wall. Enteropathogenic forms of *E. coli* have recently become better known than the "ordinary" forms. These cause severe stomach cramps, diarrhea (often bloody), and vomiting and perhaps slight fever. They produce a toxin that can attack the body in several areas: the gut (causing bloody diarrhea), the kidneys (causing kidney failure), and sometimes the nervous system. One strain in particular (known as O157, O157 H7, STEC, VTEC or EHEC) has been responsible for most problems i.e. outbreaks of "*E. coli*" infections around the world, first noted in 1982. In addition, other kinds of *E. coli* (called sero groups) can cause disease of varying seriousness. These serotypes are based on antigens associated with various components: (O: outer cell wall layer, H: flagellin, K: capsule). A recent outbreak in Germany was caused by a strain now known as O104:H4 from contaminated seed sprouts. The faecal-oral transmission route is the main way in which pathogenic strains of the bacterium cause disease ^[23]. It is possible that random distribution *E. coli* in these greywaters in Bali LGA could cause one these epidemics if not checked on time ^[Table 2].

Because most of the microbes seen all the areas are mostly gram negative bacteria; gram-negative bacteria can cause many types of infections and are spread to humans in a variety of ways. Several species, including *Escherichia coli*, are common causes of food-borne disease. *Vibrio cholerae*—the bacteria responsible for cholera—is a waterborne pathogen. *Staphylococcus aureus* may cause minor skin infections such as pimples and boils, but these may become deep-seated, causing abscesses etc. If it enters the blood it can cause a number of problems in the body: bacteremia and sepsis, toxic shock syndrome (TSS), pneumonia, meningitis, osteomyelitis, endocarditis. Gram-negative bacteria can also cause respiratory infections, such as certain types of pneumonia, and sexually transmitted diseases, including gonorrhea. *Shigella dysenteriae*, the Gram-negative bacterium responsible for plague, is transmitted to people through the bite of an infected insect or contaminated water. Generally, the cell wall of Gram-negative organisms is much more complex than the cell wall of Gram-positive bacteria. From the plasma membrane outwards, it consists of the following: a periplasmic space containing enzymes and other components, a peptidoglycan layer 2 nm in thickness, forming 5% of the cell wall mass, that is often linked to outwardly projecting lipoprotein molecules, an outer membrane consisting of a lipid bilayer, similar in some respects to the plasma membrane, that contains protein molecules and (on its inner aspect) lipoproteins linked to the peptidoglycans ^[Figure 2 a and b].

Other proteins form transmembrane water-filled channels, termed porins, through which hydrophilic antibiotics can move freely. Complex polysaccharides forming which are important components of the outer surface differ between strains of bacteria and are the main determinants of the antigenicity. The complex polysaccharides constitute the source of endotoxin, which, *in vivo*, trigger various aspects of the inflammatory reaction by activating complement, causing fever ^[23]. These were the main cause of deaths in these areas sampled; with average life span less than 60 years.

CONCLUSION

The study showed that greywaters from local bathrooms in Bali LGA contained massively gram negative bacteria and other pathogenic microbes, and their presence in these water possessed great threat to lives of people; since these microbes are circulated by various agents to humans causing various types of diseases through their contaminations.

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