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ISOLATION AND IDENTIFICATION OF BACTERIA FROM UDUTH HOSTEL WATER SOURCES (TAPS AND RESERVOIRS) WITH POSSIBLE TREATMENT USING ANTIBIOTICS AND PLANT EXTRACTS (*Vernoniaamygdalina& Allium sativum extracts*)

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ABSTRACT

Water is essential to life and health; however, more than one billion people worldwide do not have access to safe drinking water (WHO, 2000). Waterborne diseases have been estimated to cause more than two million deaths and four billion cases of diarrhea annually (WHO, 2000). This worked aimed at isolating and identifying bacteria from the tap and reservoir water in UDUTH hostel with possible way of treatment using both conventional antibiotics and plants extracts. Sample were collected from UDUTH hostel, five serial dilutions were done and cultured, the visible colonies were counted and subcultured. Slant bottles were made, the isolates were identified by Gram staining and biochemical tests. After identification the isolates were tested for antibacterial susceptibility test using both conventional antibiotics and plants extracts (*Vernoniaamygdalina& Allium sativum* extracts). Following Gram staining and biochemical tests, 50% of the isolates were accounted for E.coli, 33% were Staph.aureus, and only 17% of the isolates were Klebsiella spp. The isolates were found to be susceptible to four of the antibiotics used (Ofloxacin, Ciprofloxacin,Gentamacin and Streptomacin) and resistant to two of the antibiotics (Nalixidic acid and Ampicillin). The isolates were also sensitive to two plant extracts used at variable strength.

Keywords: Waterborne diseases, Gram staining, antibiotics, UDUTH

INTRODUCTION

Water is one of the most principal natural amenities available on earth. It covers approximately 70% of surface of earth and remaining amount is found in environment. Out of this only 2% of world's water is drinkable. Water is required for all living organisms and thus needed for economic development. Traditionally, chlorination was the preferred method for decontaminate potable water supplies and coliform population estimates. However, observed that coliform free potable water supplies may not definitely be free from human pathogens and sometimes become the main source of water borne diseases in humans. Water supports growth of many microorganisms (Chapelle, 2000).

Water sources may become contaminated by enteric pathogens i.e. coliform group due to natural activities or human activities. About 75% of worldwide communicable diseases are waterborne (Shenjgi*et al*., 2004).

World Health Organization (WHO) reported that 80% of all human diseases in developing countries are caused due to infected water consumption. While assessing microbial quality of drinking water, there are often present very low numbers of microorganisms. Water may also contain chemical substances and pathogenic microorganisms (Karnwal, 2017).

It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe to use. The interpretation and the action trigger levels for different waters vary depending on the use made of the water. Whilst very stringent levels apply to drinking water, more relaxed levels apply to marine bathing waters, where much lower volumes of water are expected to be ingested by users. The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern. Indicator organisms are bacteria such as non-specific coliforms, *Escherichia coli* and *Pseudomonas aeruginosa* that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage. Indicator organisms are used because even when a person is infected with a more pathogenic bacterium, they will still be excreting many millions times more indicator organisms than pathogens. It is therefore reasonable to surmise that if indicator organism levels are low, then pathogen levels will be very much lower or absent. Judgments as to suitability of water for use are based on very extensive precedents and relate to the probability of any sample population of bacteria being able to be infective at a reasonable statistical level of confidence.

Water is essential to life, an adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place. In

general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Waste water discharges in fresh waters and costal sea waters are the major source of fecal microorganisms, including pathogens. Acute microbial diarrhea diseases are a major public health problem in developing countries (Cabral, 2010).

Water is considered a vehicle for the propagation and dissemination of human associated bacteria. Safe drinking water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumers. Human health should therefore be protected by preventing microbial contamination of water that is intended for consumption. In rural communities, untreated surface water from rivers, dams, and streams is directly used for drinking and other domestic purposes. These unprotected water sources can be contaminated with microbes through rainfall run- off and agricultural inputs, mixing with sewage effluents and faeces from wild life, which render them unacceptable for human consumption. Faecal coliforms, *Aeromonas* and *Pseudomonas*, are used as indicators of faecal contamination in water and the presence of these pathogens may have severe health implications on consumers especially those that are immunocompromised(Mulamattathil *et al*., 2014).

Major factors affecting microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements. Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution (Antony & Renuga, 2012).

Additionally, water quality is known to be affected by increased microbial pollution under extreme weather conditions (climate change) and requires more systematic studies. Monitoring objectives consist in directly targeting the sources of contamination, by using simple and rapid indicators but are mainly focused on parameters such as faecal bacteria (*E. coli* or Enterococci). Enteric viruses, that play a major role in waterborne diseases, are rarely investigated due to the detection limits of commonly applied methods (Cann *et al*., 2014).

Surface water bodies are presumed to be more vulnerable to fecal contamination than groundwater reservoirs due to the absence of natural soil protection and filtration and the possibly short distances between the occurrence of contamination and water extraction. For this reason monitoring microbiological raw water quality is an essential component of the protection strategy in catchment areas of surface drinking water reservoirs (Kistemann *et al*., 2002).

Water is susceptible to contamination with microorganisms and organic matter among other pollutants regardless of the source. Significantly, microbial contaminants such as coliforms, *E.coli*, *Cryptosporidium parvum*, and *Giardia lamblia* compromise the safety of the water. Presence of *Escherichia coli*, *Klebsiella*, and Enterobacter species in water is a likely indicator of the presence of pathogenic organisms such as *Clostridium pafringens*, *Salmonella*, and Protozoa (Onyango *et al*., 2018).

Most of diseases in human beings are caused due to unhygienic water supplies used for drink purpose that cause infection like dysentery, diarrhea, cholera, typhoid etc. Currently about 20% world population scarcity of safe drinking water and >5 million people die every year from illness associated with drinking water due to inadequate sanitation (Karnwal, 2017).

It is argued that especially in the case of heavy rainfall the microbial loads of running waters may suddenly increase substantially and reach reservoir bodies very quickly (Kistemann*et al*., 2002).

Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrhea diseases. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year (Cabral, 2010).

As far as possible, water source must be protected from contamination by human and animal waste, which may contain bacterial, protozoan pathogens and helminthes parasites (Ahmed, 2005). The success of the water and sanitation decadence is largely depending on people etttbecominga ware of the relationship that exists between health, water, hygiene and sanitation. Bacterial contamination of water can be a serious problem .Water testing is the only way to evaluate whether bacteria is present in a water supply. This work emphasized the urgent need in the continuous monitoring of the water purification performance, including the evaluation of the effectual treatment of both Gram-Positive and Gram-Negative bacteria using both antibiotic discs and plant extracts.

The aim of this study is to isolate and identify bacteria from tap and reservoir water in UDUTH hodtelSokoto and the possible way of treatment using both conventional antibiotics and plants extracts (*Vernoniaamygdalina& Allium sativum*extracts).

Water

Water is an indispensable natural resource essential for the existence of all living creatures(raji, *et al,*. 2017).

Water is one of the most important elements for all forms of life and is indispensable in the maintenance of life on Earth and essential for the composition and renewal of cells. Water represents 70% of our body, participates in the composition of our tissues, and transports the most diverse substances throughout our organism. Human beings increasingly continue to pollute the reserves which still remain, provoking illnesses that can jeopardize the population. Bioaccumulation, sewerage, agricultural, industrial, radio-active, and thermal residues are the principal polluters of water on our planet (Thereza *et al*., 2002).

Nowadays, public health protection demands that drinking water must be free of all pathogenic bacteria. Polluted water bodies, unprofessional water disposal, and defective management impart major public health problems. Pollution of water supplies also imparting an important role on the natural environment and increasing risks for life (Karnwal, 2017).

Importance of water

Water is one of the most important elements for all forms of life. It is indispensable in the maintenance of life on earth(Abera *et al*., 2011).

Water is essential to life. An adequate, safe and accessible supply must be available to all.

Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible(Cabral, 2014). Safe drinking water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumer(Mulamattathil *et al*., 2014).

Bacteriological water analysis

Is a method of analyzing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe to use.

The interpretation and the action trigger levels for different waters vary depending on the use made of the water. Whilst very stringent levels apply to drinking water, more relaxed levels apply to marine bathing waters, where much lower volumes of water are expected to be ingested by users(Okene, n.d.).

Water Quality

Can be defined as the chemical, physical and biological characteristics of water, usually in respect to its suitability for a designated use. Water can be used for recreation, drinking, fisheries, agriculture or industry. Each of these designated uses has different defined chemical, physical and biological standards necessary to support that use. For example, there are stringent standards for water to be used for drinking or swimming compared to that used in agriculture or industry (Roy, 2018).

Purified Water

Water is essential for industrial, pharmaceutical and hospital purposes, in the preparation and processing of medicines and other health products. In the majority of cases, water is an input, which should be incorporated into the product during processing. At other times, even if it is not present in the preparation, it is especially used for cleaning and hygiene purposes. It is recognized that the greatest demand on water is destined for human consumption, its quality being relatively guarantied up to the point where the pipe transportation network terminates(Thereza *et al*., 2002).

Sources, Fate and Behavior of Microorganisms in Water

The microbial contamination of water is often of faecal nature related to humans (water sewage

treatment plants, combined sewage overflow (CSO), non-collective sewage systems), domesticated animals (manure spreading, pit stock overflow), or wildlife. The main origins of microbial contamination of natural aquatic resources are discharges of water treatment plants, decontamination stations, hospitals, industries considered as point sources(Cann *et al*., 2014).

Drinking Water as a Vehicle of Diseases

Water is essential to life. An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal diseases. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place (Cabral, 2010).

Water Borne Disease

Water borne diseases remain a major cause of death and illness in developing countries. The global spatial distribution show that Africa and Asia account for a large percentage of these diseases, which include cholera, typhoid fever, paratyphoid, bacillary dysentery, amoebic dysentery, gastro-enteritis, and infective hepatitis(O.oguntoke, 2009).

Microbial waterborne diseases also affect developed countries. In the USA, it has been estimated that each year 560,000 people suffer from severe waterborne diseases, and 7.1 million suffer from a mild to moderate infections, resulting in estimated 12,000 deaths a year (Cabral, 2014). However, poor water quality continues to be a leading cause of health problems especially in developing countries where it is estimated that 80% of all illnesses are linked to water and sanitation and 15% of all child deaths under the age of 5 years result from diarrheal diseases(Raji,*et al*., 2017).

According to UNICEF report, about 800 million people in Asia and Africa are living without access to safe drinking water. Consequently, this has caused many people to suffer from various diseases. However, inadequate quantity, poor quality of drinking water, and poor sanitation are the main reasons in incidence and prevalence of diseases in the world(Al-bayatti *et al*., 2012).

In Africa, it has been estimated that every child has five episodes of diarrhoea per year and that 800,000 children die each year from diarrhea and dehydration(Iyanda & Raji, 2015).

Infection Control Mechanisms

Bacteriological Assessment

Bacteriological Assessment is an important part of infection control program, providing data regarding types, and counts of microbial flora present in a particular environment (Najotra*et al*, 2017).Sampling procedures which includes swabbing, open plate, and both microbiological (culturing, gram staining) and biochemical (Coagulase, Catalase, etc.) investigations are used to assess microbial load of an environment (Baban*et al*, 2019).Microbiologists should be aware oforganisms, sites and populations found in OT as surveillance culturesshould be chosen carefully to allow meaningfulinterpretation of results. Environmental monitoringmeans the microbiological testing of air, surfaces andequipment to detect changing trends of microbial

countsand micro-flora (Sandle T, 2006).It is usually necessary to study the epidemiology of infection as a multidisciplinary approach. Surveillance as an essential element of an infection control program, also provides data to identify infected patients and determine the site of infection and the factors thatcontributed to the infection. Surveillance data most useful for decision making, the hospitalshould focus on their most important and predominant problems and use surveillancemethods that adhere to sound epidemiology principles (Nithya SG, 2010).

USE OF DISINFECTANTS (IZAL)

Contamination of the OT and ICU with nosocomialmultidrug-resistant bacteria and fungi can be prevented throughadequate application of infection control standards. A studyreported that reduction of airborne bacteria in the OT wouldlead to prevent 50% of postoperative wound contamination bycompliance to proper cleaning and disinfection standards.Use of disinfectants (such as izal) is one of the important practices for infection control to minimize the risks for exogenous and nosocomial infections.

Water Treatment

Through the early to mid-1800s drinking water systems in developed nations consisted mainly of pipes for delivering water (e.g., New York and London). Modern technologies for contaminant removal and remediation include filtration, flocculation and sedimentation, and disinfection. Flocculation is utilized to coagulate particles that settle out of the water as sediment in the treatment process. Alum, metal salts, and synthetic organic polymers are often used as flocculants(Rajagopal, 2016).

Chlorination

can be achieved by using liquefied chlorine gas, sodium hypochlorite granules and onsite chlorine generators. The gas is supplied in pressurized containers. The gas is withdrawn from the cylinder and is dosed into water by chlorinator, which both controls and measures the gas flow rate(Elmahdi, 2007).

Filtration methods

Bacteria are generally recovered on 47 mm diameter membrane filters with porosities of 0.22 to 0.45 µm. Membrane filters may be incubated on solid media, pads soaked in liquid media or as a MPN system in enrichment broth. Cysts of protozoan parasites can be recovered on similar membranes but with larger surfaces (up to 293 mm diameter) and porosities as high as 2 µm(Ashbolt, n.d.).

Pathogens of Mineral Water

Escherichia coli

E. coli have been identified on the basis of different virulence factors. Enterotoxigenic E. coli (ETEC) produces heat-labile or heat-stable enterotoxin, or both toxins simultaneously, and is an important cause of diarrhea in developing countries, especially in young children. Infection with enteropathogenic E. coli (EPEC) has been associated with severe, chronic, non-bloody diarrhea, vomiting and fever in infants. Enteroinvasive E. coli (EIEC) causes watery and occasionally bloody diarrhea where strains invade colon cells by a pathogenic mechanism similar to that of Shigella(Aditi *et al*., 2017).

The Staphylococci species

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 µm and characterized by individual cocci, which divide in more than one plane to form grape-like clusters. To date, there are 32 species and eight sub-species in the genus Staphylococcus, many of which preferentially colonize the human body (Kloos and Bannerman, 1994), however *Staphylococcus aureus* and *Staphylococcus epidermidis*are the two most characterized and studied strains. The staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however, in general they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine, and B vitamins, including thiamine and nicotinamide (Kloos and Schleifer, 1986; Wilkinson, 1997). Members of this genus are catalase-positive and oxidase-negative, distinguishing them from the genus streptococci, which are catalase-negative, and have a different cell wall composition to staphylococci (Wilkinson, 1997). Staphylococci are tolerant to high concentrations of salt (Wilkinson, 1997) and show resistance to heat (Kloos and Lambe 1991). Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood (Kloos and Musselwhite, 1975). This distinguishes the coagulase positive strains, S. aureus (a human patho- gen), and *S. intermedius* and *S. hyicus*(two animal patho- gens), from the other *staphylococcal* species such as *S. epidermidis*, that are coagulase-negative (CoNS) (Cells *et al*., 2002).

Klebsiellapneumoniae

Klebsiella is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and showing characteristic radiographic abnormalities due to a severe pyogenic infection which has a high fatality rate if untreated.The vast majority of Klebsiella infections, however, are associated with hospitalization. As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiellapneumoniae*, the medically most important species of the genus.

Antibiotics/Antimicrobial Agents

Antimicrobial Prophhylaxis

Antimicrobial prophylaxis is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intraoperative contamination to a level that cannot overwhelm host defenses (Sanderson PJ *et al*, 1993).Prolonged use of antibiotics used in prophylaxis is not appropriate, may mask the diagnosis of postoperative infection, lead to emergence of multi-resistant bacteria and can be financially wasteful. Aseptic technique and prophylactic antibiotics provide the first line of defense, but it has been shown that removing bacteria and fungi from operation room air helps to minimize infection (Nithya SG, 2010).

Antibiotics Susceptibility Testing (AST)

Plays an important role to check the effectiveness of a drug against a bacterium and select the best drug that act against the bacterium. One of the significant roles of clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of various bacterial isolates. The main objectives of the testing are to find out possible drug resistance in common pathogenic microorganism and the susceptibility to drug of choice for a particular infectious microorganism can be assured.

Mechanism of antimicrobial resistance:

There are number of ways by which microorganisms are resistant to antimicrobial agents. These includes:

1. Bacteria produce enzymes which destroy the antimicrobial agents before it reaches its targets e.g. Beta lactamase enzyme hydrolyses beta lactam drugs which develop resistance.
2. Impermeable cell for antimicrobial drugs e.g. Gram negative bacteria may become resistant to Beta lactam antibiotics by developing permeability barrier.
3. Mutation e.g. Ribosome methylation of ribosomal RNA develop macrolide resistant.
4. Bacterial efflux pump that expels antimicrobial drugs from cell before it can reach its targets. 5. Specific Metabolic pathways in the bacteria are genetically altered so that antibacterial agents cannot exert an effect (Bagul *et al*., 2016).

Water are the major drugs of choice of the physician's desk to treat the pathogenic infection. It has been observed that some of the clinicians prescribe the medicine based on the symptoms instead of performing diagnostic tests. This prescribing pattern may be one of the reasons for the development of resistant for the antibiotics.(Bagul*et al*., 2016).

Ciprofloxacin

CIPRO is indicated in adult patients for treatment of skin and skin structure infections caused by Escherichia coli, Klebsiellapneumoniae, Enterobacter cloacae, Proteus mirabilis, Proteus vulgaris, Providenciastuartii, Morganellamorganii, Citrobacterfreundii, Pseudomonas aeruginosa, methicillin? susceptible Staphylococcus aureus, methicillin-susceptible Staphylococcus epidermidis, or Streptococcus pyogenes(*CIPRO (Ciprofloxacin Hydrochloride)*, 1987).

Plant Extracts

Antibacterial Activity of VernoniaAmygdalina

Africa has arguably one of the richest phyto diversities in the world. Africa's forests geographically span approximately 216,634,000 ha (Farombi, 2003). More than 50% of all modern clinical drugs are of plant origin (Suffness and Douros, 1982) Plant products therefore play an important role in drug development programs of the pharmaceutical industry (Baker *et al*., 1995; Cordell, 1995). Furthermore, the consumption of plant materials is believed to contribute immensely to the improvement of the health of man and his plants and animals. Yedjou*et al*. (2008) estimated that 80% of the population of Africa depends on medicinal plants to satisfy their health care requirements(Del *et al*., 2011).

Many experimental studies of V. amygdalina, have reported that this plant posseses antibacterial activity. Newbold*et al*. (1997) showed that this plant has mild antimicrobial effect on rumen bacteria and protozoa while Kambizi and Afolayan (2001) proved that acetone extract of V. amygdalina possesses antibacterial activity towards Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Micrococcus kristinae, Staphylococcus aureus, Enterobacter cloacae and Escherichia coli growth with minimum inhibition concentration (MIC) of 5 mg/ml. Although, Cos *et al*. (2002) concluded that V. amygdalina was more sensitive towards the gram positive bacteria than gram negative bacteria; some researchers found that the activity of V. amygdalina against gram-negative bacteria was comparable to that towards the gram- positive species(Yeap*et al*., 2010).

Antibacterial activity of Allium sativum extract

The usage of garlic for medicinal purposes dates back to antiquity, to the year 1550 BC, when it was already a valuable component of food. The Bible mention garlic with regard to the Jews' flight from Egypt. Drawings of garlic were found 3700 years BC in Egyptian tombs. In vitro studies show garlic activity against many types of Gram-negative, Gram-positive bacteria inclu- ding species such as: Escherichia, Salmonella, Staphy- lococcus, Streptococcus, Klebsiella, Proteus, Bacillus, Clostridium and Mycobacterium tuberculosis. The in vitro antibacterial activity of essential oils obtained from fresh bulbs of garlic show a good antimicrobial activity against Staphylococcus aureus, Pseudomo- nasaeruginosa and Escherichia coli (Majewski, 2015).

MATERIALS AND METHODS

Materials

The basic materials utilized for the study are discussed in this section. In addition, the methods employed in carrying out the study are elaborated.

Apparatus and Materials

The apparatus and Materials used in the research are listed below;

Nutrient agar
Triple sugar iron agar
Petri dishes
Syringe
Glass slide
Broth media
Plasma
Autoclave
Inoculating loop
Test tubes
Test tube rack
Microscope
oven
Oil-immersion
Incubator
Bunsen burner

Chemicals and Reagents

The chemicals and reagents used in the research are listed below;

Normal saline

McFarland solution
Crystal violet
Acetone
Hydrogen Peroxide

Methodology

Study Area

The study area is within the Sokoto metropolis Specialist Hospital Sokoto (SHS) and UsmanuDanfodiyo University Teaching Hospital Sokoto(UDUTHS)].

Sokoto State is located in the extreme North West of Nigeria, near the confluence of the Sokoto River and Rima River. The state is located between longitude11'30°,13'50° East and latitude 4'0° to 6'0° North, bordered to the North by Niger Republic, Zamfara state to the east while Kebbi State borders most of the South and Western parts (Mamman 2000).

The State falls within the Savannah zone. Rainfall starts late and ends early with mean annual rainfall ranging from 500 to 1,300mm and annual average temperature of 28.3°C(82.9°F). It has a land area of about 28,232.37to kilometers and stands at altitudes of 272m above the sea level. The state has three peculiar season; the cold-dry (December-February), hot- dry (October-April) and wet. The wet season begins in most part of the state in May and lasts up to September. The vast Fadama land of the Sokoto Rima River system dissects the plain and provide rich alluvial soil and extensive grassland fits for the variety of crop cultivation, hence farming and livestock rearing are the principal activities in the state (Sokoto state diary,2003).

Other commercial activities are cement and leather production. The major indigenous tribes in the state are Hausa and Fulani. Majority of the Hausas are farmers while Fulanis are nomadic and are engaged in animal rearing (Imam,2006). Based on 2006 population census Sokoto state had a population of 3,696,999, with an estimate of 4,584,278.8 in 2014 based on the population growth rate of 3%(NPC,2006). The last known population is approximately 552400(year 2015). This was 0.303% if total Nigeria population. If population growth rate would be same as in period 2006-2015(+1.1%/year), Sokoto population in 2021 would be 583445.

Culture Media Preparation

The following culture media were used; Nutrient agar, Nutrient broth, Triple Sugar Iron agar (TSI), Simmon Citrate agar. All the media were prepared as directed by the manufacturers.

Sample Collection

The sample was collected from different taps and reservoir at the students' hostel in UDUTH, Sokoto.The issue of ethical clearance was taken care of by our supervisor.Five bottle samples was used to collect the water sample after allowing the water to run for about five minutes.

Bacteriological Analysis of Samples

Samples were immediately taken to Pharmaceutical Microbiology Laboratory. Five serial dilutions was done for each sample. 1ml of water sample was transferred into the first test tube containing 9mls of normal saline, 1ml was then withdrawn from the first test tube and transferred into the next test tube, this was maintained for the whole test tubes aseptically. This procedure was repeated to all samples. 0.1ml was then withdrawn from first test tube and then inoculated in to the plate and labelled, glass spreader was then used to spread the sample into the plate, this maintained for all the serial dilutions and respected plates. The plates were then allowed for some minutes. The plates were incubated at 37^{O}C for 24 hours(Crown, 2017).

Total Bacterial Count

Total Bacterial Count was carried out to determine the total number of bacteria present in each dilutions of each five samples.

Gram Staining

Gram staining is a differential staining procedure that separates bacteria to either,Gram positive or Gram negative. It was done by using a sterile wire loop to drop aloop full of sterile distilled water on a clean, grease free glass slide. A colony of thebacteria was smeared on the slide and allowed to air dry. The smear was heat fixedby gently passing over flame thrice. The smear was flooded with crystal violet for60 seconds and washed off immediately with water, it was then flooded with Lugols iodine for 60seconds and washed off with water. It was decolourized with acetone for few seconds and washed off. The smear was counterstained with neutral red for 60 seconds, then washed off with water. The slide was allowed to air dry. It was

viewed under the X100 objectives with oil immersion. Gram positive cells picked upthe colour of the primary stain while Gram negative cells pick up the colour of thesecondary stain(Smith & Hussey, 2016).

Catalase Test

A drop of 3% hydrogen peroxide was made on a clean glass slide. With a woodenapplicator, a colony of the test bacteria was brought in contact with the hydrogenperoxide and observed for bubble. The presence of bubble was a positive catalasetest and no bubbles showed a negative catalase test(Reiner, 2016).

Coagulase Test

Two drops of distilled water were made at the ends of a clean grease-free glass slide. A colony of the test organism was emulsified on each of the drops. To one of the suspensions a loop-full of plasma was added and mixed gently. The other suspension was left as the control. Observation of agglutination within ten (10) seconds of addition of plasma indicated a positive coagulase test. Agglutination indicates a positive test(Unit *et al*., 2018).

Indole Test

The isolate was inoculated in 5ml peptone water (tryptophan broth) and incubated at37°C for 24 hours. After 24 hours, 3 drops of Kovac's reagent was added to the inoculum, shaken and observed for reaction. A positive reaction is indicated by the246development of a red colour in the reagent layer above the broth within one minute and a negative reaction is indicated by the reagent retaining its yellow colour(Macwilliams, 2016).

Citrate utilization

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2 ml in each vial was prepared by autoclaving at 15 psi 121°C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the vials by means of a streak inoculation method with an inoculating needle and the vials were incubated for 48 hours at 37°C. Following incubation, citrate positive culture was recognized by the presence of growth on the surface of the slant of Simmons citrate agar and deep Prussian blue coloration of the medium(Aditi *et al*., 2017).

Sugar fermentation

Triple sugar iron test was done to differentiate among the different groups or genera of the Enterobacteriaceae based on the ability to reduce sulfur and ferment carbohydrates. Slants were prepared in the test tubes by autoclaving. Using sterile technique; small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes by means of a stab and streak inoculation method with an inoculating needle. The screw caps were not fully tightened and the tubes were incubated for 24 hours at 37°C. Fermentation is indicated by yellowing of the butt and the slant of Triple Sugar Iron (TSI) agar media. If gas was formed during the fermentation, it is shown in the butt either by the formation of bubbles or cracking of the agar(Aditi *et al*., 2017)**.**

Antimicrobial Susceptibility Test

An antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method. The following antibiotic discs (Mast Diagnostics,UK)at the final concentrations that are indicated were used: ampicillin (PN) 10 ug, streptomycin (S) 10 ug, ciprofloxacin (CPX) 10 ug, ofloxacin (OFX) 10 ug, Gentamacin (CN) 10ug, Nalixidic acid (NA) 10 ug. These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

Three colonies were picked from each sample and each colony was transferred in to 3 mL of sterile normal saline to prepare bacterial suspension. Aliquots of 100 L from each suspension were spread-plated on Mueller-Hinton agar plates. Antibiotic discs were applied on to the plates using sterile needles and the plates were incubated at 37◦C for 24 hours. After incubation, the antibiotic inhibition zone diameters (IZD) were measured. Results obtained were used to classify isolates as being resistant, intermediate resistant, or susceptible to a particular antibiotic using standard reference values according to National Committee for Clinical Laboratory Standards, now Clinical and Laboratory Standards Institute (CLSI). Multiple antibiotic resistance (MAR) phenotypes were generated for isolates that showed resistance to 3 or more antibiotics.

Determination of Antimicrobial Activity of *Vernoniaamygdalina* Extract

The antimicrobial activity of the crude extracts was done using agar-well diffusion method described by (Gashe&Zeleke, 2017) with few modifications. Briefly, 0.2 mL of the adjusted suspensions were separately inoculated using a sterile wire loop on the solidified media plates (Nutrient Agar (NA) for bacteria and Potato Dextrose Agar plate (PDA) for fungi) and spread uniformly using a sterile glass rod. The agar medium was punched out using a sterile hole cork-borer of 8 mm diameter and cut agar discs were aseptically and carefully removed with sterile forceps. A sterile Pasteur pipette was used to introduce 0.5 mL of the crude plant extract samples into the 8 mm holes bored on the surface of the agar media containing the cultures. The plates were allowed to stand for one hour at room temperature to allow for diffusion of the substrates to proceed before the growth of the organisms commenced. The plates were finally incubated at 37°C for 24 h for bacteria and at room temperature for fungi. The presence of zone of inhibition around the holes containing the extracts indicates the antimicrobial activity against the test organisms. Antimicrobial activity was expressed in terms of diameter of zones of inhibition (mm)(Inusa *et al*., 2018).

RESULTS

Table 1 Total Bacterial Count

S/N	SAMPLE CODE	MORPHOLOGICAL CHARACTER	NUMBER OF COLONY	NUMBER OF DISTINCT COLONY
1	A1	Circular yellow	22	02
2	A2	Circular yellow	50	02
3	A3	Circular yellow	06	02
4	A4	Circular yellow	08	02
5	A5	Circular yellow	08	02
6	B1	Circular yellow	120	02
7	B2	Circular yellow	90	02
8	B3	Circular yellow	45	02
9	B4	Circular yellow	21	02
10	B5	Circular yellow	04	02
11	C1	Circular yellow	120	03
12	C2	Circular yellow	88	02
13	C3	Circular yellow	09	02
14	C4	Circular yellow	08	02
15	C5	Circular yellow	03	02

16	D1	Circular yellow	11	02
17	D2	Circular yellow	08	02
18	D3	Circular yellow	04	02
19	D4	Circular yellow	04	02
20	D5	Circular yellow	02	01
21	E1	Circular yellow	06	02
22	E2	Circular yellow	06	02
23	E3	Circular yellow	02	02
24	E4	Circular yellow	02	02
25	E5	Circular yellow	01	01

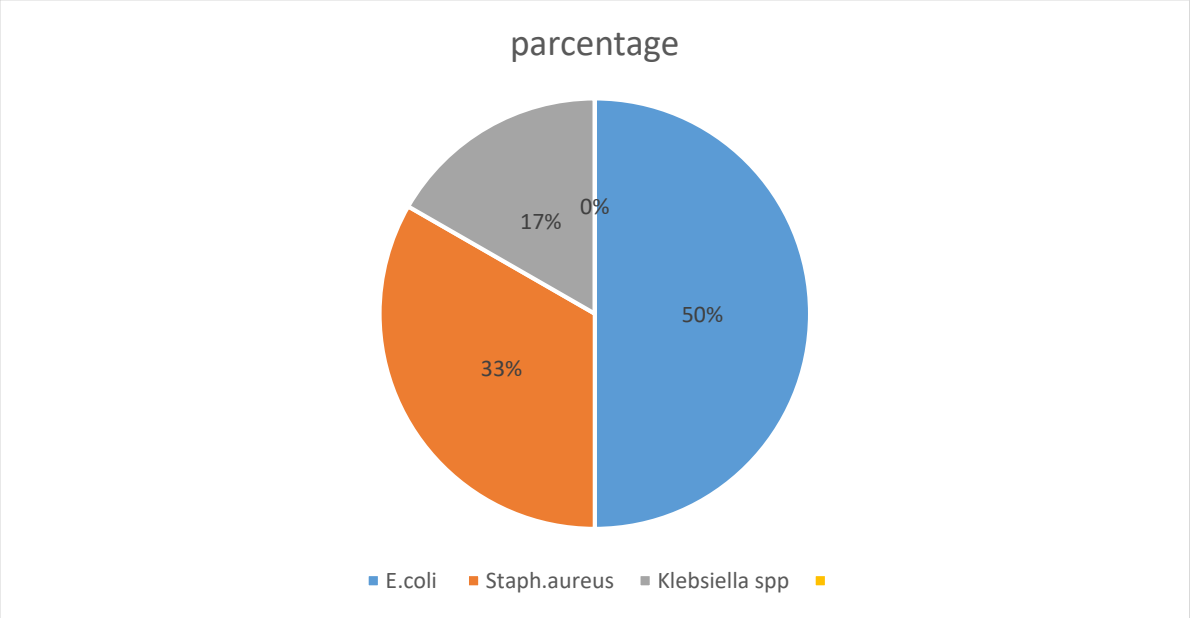

Figure 1: Distribution of Isolated Organisms

Table 2: Antimicrobial Susceptibility Test

S/N	CODE	ANTIBIOTIC AGENTS ZONE OF INHIBITION(mm)											
		OFX 10ug		CPX 10ug		CN10ug		S 10ug		NA 10ug		PN 10ug	
1.	**A1b**	28	S	31	S	22	S	26	S	-	R	-	R
2.	**A2a**	16	S	30	S	20	S	32	S	-	R	-	R
3.	**A2b**	31	S	60	S	25	S	31	S	-	R	16	I
4.	**A3a**	27	S	33	S	30	S	12	I	-	R	10	R
5.	**A3b**	28	S	31	S	30	S	26	S	-	R	-	R
6.	**A4b**	27	S	32	S	18	S	20	S	-	R	-	R
7.	**A5a**	31	S	53	S	25	S	33	S	-	R	-	R
8.	**B1a**	38	S	31	S	20	S	53	S	-	R	-	R
9.	**B3a**	32	S	38	S	20	S	28	S	-	R	-	R
10.	**B4b**	38	S	30	S	22	S	51	S	-	R	-	R
11.	**C1a**	29	S	31	S	19	S	20	S	-	R	-	R
12.	**C1c**	28	S	30	S	17	S	21	S	-	R	-	R
13.	**C2a**	29	S	31	S	21	S	42	S	-	R	-	R
14.	**C3a**	28	S	32	S	21	S	42	S	-	R	-	R
15.	**C4b**	28	S	32	S	21	S	42	S	-	R	-	R
16.	**D1a**	38	S	30	S	22	S	52	S	-	R	-	R
17.	**D1b**	37	S	31	S	23	S	49	S	-	R	-	R
18.	**D2a**	35	S	35	S	28	S	28	S	10	R	-	R
19.	**D3a**	38	S	30	S	24	S	37	S	32	S	-	R

20.	**D3b**	24	S	30	S	21	S	31	S	-	R	-	R
21.	**D4**	28	S	30	S	22	S	26	S	-	R	-	R
22.	**D5a**	37	S	31	S	23	S	40	S	-	R	-	R
23.	**E1**	38	S	31	S	24	S	42	S	-	R	-	R
24.	**E5a**	36	S	30	S	25	S	45	S	-	R	-	R

Table 3: Antimicrobial Activity *Aliumsativum* and *Vernoniaamygdalina*extracts

S/N	CODE& ORGANISMS		PLANT EXTRACTS *Aliumsativum (mg)*				*Vernoniaamygdalina (mg)*			
			20	**25**	**30**	**35**	**20**	**25**	**30**	**35**
1.	**A1b**	E.coli	4	6.5	8	10	2	3	3	6
2.	**A2a**	Klebsiel laspp	3.9	6	8	10	2	2.5	3	5
3.	**A2b**	E. coli	4	6.5	8	10	2	3	3	6
4.	**A3a**	*Staph. aureus*	4	4	5	6	3	3	4	7
5.	**A3b**	*Staph. aureus*	4	4	5	6	3	3	4	7
6.	**A4b**	Klebsiel laspp	3.9	6	8	10	2	2.5	3	5
7.	**A5a**	*Staph. spp*	4	4	5	6	3	3	4	7
8.	**B1a**	*E.coli*	4	6.5	8	10	2	3	3	6
9.	**B3a**	*E.coli*	4	6.5	8	10	2	3	3	6
10.	**B4b**	*E.coli*	4	6.5	8	10	2	3	3	6
11.	**C1a**	Klebsiel laspp	3.9	6	8	10	2	2.5	3	5
12.	**C1c**	Klebsiel laspp	3.9	6	8	10	2	2.5	3	5
13.	**C2a**	*Staph. spp*	4	4	5	6	3	3	4	7
14.	**C3a**	*Strep. spp*	4	4	5	6	3	3	4	7
15.	**C4b**	*Strep. spp*	4	4	5	6	3	3	4	7
16.	**D1a**	*E.coli*	4	6.5	8	10	2	3	3	6
17.	**D1b**	*E.coli*	4	6.5	8	10	2	3	3	6
18.	**D2a**	*E.coli*	4	6.5	8	10	2	3	3	6
19.	**D3a**	*Eoli.c*	4	6.5	8	10	2	3	3	6
20.	**D3b**	*Staph. spp*	4	4	5	6	3	3	4	7
21.	**D4**	*Staph. spp*	4	4	5	6	3	3	4	7
22.	**D5a**	*E.coli*	4	6.5	8	10	2	3	3	6
23.	**E1**	*E.coli*	4	6.5	8	10	2	3	3	6
24.	**E5a**	*E.coli*	4	6.5	8	10	2	3	3	6

DISCUSSION

Five water samples were collected from different sources of water at UDUTH hostel Sokoto, from the bacterial count result above shows that sample C has large number of colony count followed by sample B, sample A, sample D then sample E. This high bacterial count in sample C might be as a result of the distance away from the main source, poor hygiene and sanitation such as cloth and cooking puts washing, which are common practices around the study area. Sample B possess higher bacterial count than sample A (reservoir water) despite being it tap water this might be due to its close proximity to latrine and laundry which could all contaminate the water.In relation to distance of water source from latrine, found at a distance of less than 30m which is below WHO recommendation for minimum distance that should be exist between latrine and water source. Sample E has the lowest bacterial count this resulted due to short distance from the borehole. The finding of this study corroborate with that of study that was taken place in sokoto river(raji, m.i.o.et oyeniyi, 2017), where there was high bacterial count as a result of human acts such washing, bathing fishing and swimming around the river (Raji, 2017). From table 2; it is seen that samples A and C isolates were mostly Gram positive cocci and few Gram Negative Rod (*klebsiella*), only Gram negative rod (*E. coli*) was obtained from samples B&E. However, sample D isolates were mostly Gram negative rod (*E. coli*) and few Gram positive cocci. The presence of these 2 species indicate that the drinking

water are most probably contaminated with human and animal feces(Al-bayatti*et al*., 2012).

Table 4.3 indicates that all of the isolates were susceptible to four antibiotic agents used in the study and almost all of them were resistant to two of the antibiotic agents, the interpretation was carried out based on the clinical laboratory standard institutes (CLSI), this finding corresponds to study that carried out from south Africa by Mulamattathil *et al*.,(2014) which stated that 8.Theresults revealed that a large proportion of the environmental isolates were resistant to erythromycin, followed by trimethoprim and amoxicillin. None of the isolates were resistant to ciprofloxacin and only very few isolates from Modimola dam were resistant to streptomycin and neomycin.

Table 4 indicates the antibacterial activity of vernoniaamygdalina and allium sativunagaiant gram positive cocci and gram negative *E.coli* with the formation of 6-7mm zone of inhibition, this study was supported by,(Syahirah, 2019) who said V. amygdalina leaves are the most used part for extraction which has been shown to exhibit an inhibitory effect on both Gram-positive Staphylococcus aureus and Gram-negative *Escherichia coli* bacteria (Oboh and Enobhayisobo, 2009).

Also this study was corroborate with (Del *et al*., 2011) who said the sap of the leaves of VA was found to show inhibitory capacities against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, and *Pseudomonas aeroginosa*(Ijeh*et al*., 1996). A 60% methanolic extract of the leaves gave antimicrobial activity against *Bacillus subtilis, Klebsiella pneumonia, P. aeruginosa, Proteus vulgaris, Shigelladysenteriae and S. aureus* (Akinpelu, 1999).

RECOMMENDATION

The presence of large number of E.coli and staph.aureus makes the water to be unsafe for human consumption as such regular monitoring of the microbial quality of water, sanitation and water treatment should be conducted from time to time, to minimize health risk resulting from the consumption of such contaminated water, appropriate treatment processes should therefore be utilized for disinfection of ground water for quality and safe food processing and drinking water. The reservoir should be covered to reduce the risk of contamination of the water, the pipes should be checked periodically for leakage and corrosion that could also contribute in the contamination of the water.Unhygienic acts should be avoided around the reservoir such as washing to avoid diffusion of the waste water in to the reservoir.The taps should be at least 100cm away from latrine to avoid cross contamination, like in reservoir, taps should be checked periodically for leakage and corrosion.Also the tanks that stored water should also be checking for above mention reasons and to ensure the microbial load is within the acceptable limits.Generally, water treatment plant should be employed to minimize the microbial load and health risk for the student. Chlorination of water should at least be done.In case of infection or disease caused by Gram positive cocci should best be treated with ciprofloxacin and that caused by Gram Negative *E. coli* should best be treated with Ofloxacin as it is seen in the result above. Students that reside in the hostel are hereby recommended to be taken *Vernoniaamygdalina* and *Allium sativum*occasionally.

CONCLUSION

An evaluation of the bacteriological quality of drinking water in the present study confirmed the presence of *E.coli, Staph.aureus*and *Klebsiella spp*. The isolates were tested for antimbacterial activity test where all the isolates found to be susceptible to four antibiotics used comprising of Ofloxacin Ciprofloxacin, Gentamacin and Streptomycin 10ug respectively. However, the siolates were found to be relatively resistant to two of the antibiotics used comprising of Ampicillin and Nalixidic acid. It also found that Vernoniaamygdalina was sensitive against the isolates at a concentration of 35mg/ml, whereas Allium sativum exert antibacterial activity at a concentration of 25-35mg/ml.

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Table 4: Gram Staining and Biochemical Characterization of the Isolates

Isolates Code	Gram Staining	Morphology	Catalase Test	Coagulase Test	IndoleTest	Citrate Test	TRIPLE SUGAR IRON TSI					Probable Organism
							GLU	LAC	SUC	H_2S	Gas	
A1b	-	Rod	+	-	+	-	+	+	+	-	+	E. Coli
A2a	-	Rod	+	-	-	+	+	+	+	-	+	Klebsiellaspp
A2b	-	Rod	+	-	+	-	+	+	+	-	+	E. coli
A3a	+	Cocci cluster	+	+	+	-	+	+	+	-	-	*Staph. aureus*
A3b	+	Cocci cluster	+	+	+	-	+	+	+	-	-	*Staph. aureus*
A4b	-	Rod chain	+	+	-	+	+	+	+	-	+	Klebsiellaspp
A5a	+	Cocci cluster	+	+	+	-	+	+	+	-	-	*Staph. spp*
B1a	-	Rod chain	+	-	+	-	+	+	+	-	+	*E.coli*
B3a	-	Rod chain	+	+	+	-	+	+	+	-	+	*E.coli*
B4b	-	Rod chain	+	-	+	-	+	+	+	-	+	*E.coli*
C1a	-	Rod chain	+	-	-	+	+	+	+	-	+	Klebsiellaspp
C1c	-	Rod chain	+	-	-	+	+	+	+	-	+	Klebsiellaspp
C2a	+	Cocci cluster	+	+	-	-	+	+	+	-	-	*Staph. spp*
C3a	+	Cocci cluster	-	-	-	-	+	+	+	-	-	*Staph. spp*
C4b	+	Cocci cluster	-	-	-	-	+	+	+	-	-	*Staph. spp*
D1a	-	Rod chain	-	-	+	-	+	+	+	-	+	*E.coli*
D1b	-	Rod chain	-	-	+	-	+	+	+	-	+	*E.coli*
D2a	-	Rod chain	-	-	+	-	+	+	+	-	+	*E.coli*
D3a	-	Rod chain	+	-	+	-	+	+	+	-	+	*Eoli.c*
D3b	+	Cocci cluster	+	+	-	-	+	+	+	-	-	*Staph. spp*
D4	+	Cocci cluster	+	+	-	-	+	+	+	-	-	*Staph. spp*
D5a	-	Rod chain	-	-	+	-	+	+	+	-	+	*E.coli*
E1	-	Rod chain	-	-	+	-	+	+	+	-	+	*E.coli*
E5a	-	Rod chain	-	-	+	-	+	+	+	-	+	*E.coli*

Table 5: Antimicrobial Activity *Aliumsativum* and *Vernoniaamygdalina*extracts

S/N	CODE& ORGANISMS		PLANT EXTRACTS							
			Aliumsativum (mg)				*Vernoniaamygdalina (mg)*			
			20	**25**	**30**	**35**	**20**	**25**	**30**	**35**
25.	A1b	E.coli	4	6.5	8	10	2	3	3	6
26.	A2a	Klebsiellaspp	3.9	6	8	10	2	2.5	3	5
27.	A2b	E. coli	4	6.5	8	10	2	3	3	6
28.	A3a	*Staph. aureus*	4	4	5	6	3	3	4	7
29.	A3b	*Staph. aureus*	4	4	5	6	3	3	4	7
30.	A4b	Klebsiellaspp	3.9	6	8	10	2	2.5	3	5
31.	A5a	*Staph. spp*	4	4	5	6	3	3	4	7
32.	B1a	*E.coli*	4	6.5	8	10	2	3	3	6
33.	B3a	*E.coli*	4	6.5	8	10	2	3	3	6
34.	B4b	*E.coli*	4	6.5	8	10	2	3	3	6
35.	C1a	Klebsiellaspp	3.9	6	8	10	2	2.5	3	5
36.	C1c	Klebsiellaspp	3.9	6	8	10	2	2.5	3	5
37.	C2a	*Staph. spp*	4	4	5	6	3	3	4	7
38.	C3a	*Strep. spp*	4	4	5	6	3	3	4	7
39.	C4b	*Strep. spp*	4	4	5	6	3	3	4	7
40.	D1a	*E.coli*	4	6.5	8	10	2	3	3	6
41.	D1b	*E.coli*	4	6.5	8	10	2	3	3	6
42.	D2a	*E.coli*	4	6.5	8	10	2	3	3	6
43.	D3a	*Eoli.c*	4	6.5	8	10	2	3	3	6
44.	D3b	*Staph. spp*	4	4	5	6	3	3	4	7
45.	D4	*Staph. spp*	4	4	5	6	3	3	4	7
46.	D5a	*E.coli*	4	6.5	8	10	2	3	3	6
47.	E1	*E.coli*	4	6.5	8	10	2	3	3	6
48.	E5a	*E.coli*	4	6.5	8	10	2	3	3	6