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Molecular Screening for Potential Pathogenic Organisms in Some Six Major Representative Sources of Water in Saki

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Abstract: Water security and safety is of vital concern in Saki and the rest of the world. The aim of this was to screen major sources of water in Saki of potential pathogens of public health importance. Water samples of six different water sources were purposely collected aseptically from six different locations in Saki (well water, river water, rain water, borehole water, sachet water and bottled water); these were subjected to standard laboratory analysis. Four highly populated plates were sent for molecular characterization for Polymerase Chain Reaction and species sequencing for molecular identification. The highest total colony count was 70.0×10^{-5} from river water followed by rain water with the lowest of 0.1×10^5 CFU from borehole water. Molecular analysis identified amplification of isolates genes at 15000 base pair for river water and well water samples. The analysis showed that Klebsiella pneumonia (MN208186) and Enterobacter kobeii (KM593928) were the organisms found in rain water and river water respectively. The resulting nucleotide sequences was blasted at NCBI for pairwise comparison with existing data in the GenBank and their similarity was noted. The pathogenicity of the two isolates have been well-documented, making some of the water sources tested so unfit for human usage. Large-scale treatment of water sources are recommended before consumption viz-a-vis hygienic practices around water bodies.

Keywords: Fecal Coliform, Aseptically, Blasted, Enterobacter Kobeii, Water-borne Diseases, Well Water

1. Introduction

Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide [1, 2]. Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites that are transmitted by the faecal oral route [3, 4]. Waterborne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year [3, 5].

Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide [5, 6]. Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites that are transmitted by the faecal oral route [7, 8]. Waterborne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year [3, 4].

Water is essential for the survival of every form of life and the need for water is constantly increasing due to high rates of population growth and urbanization. However, the increased demands on water for drinking, domestic agricultural and industrial purposes are not commensurate with water availability thus, posing significant risks in maintaining acceptable water quality [6].

Water is of course, absolutely essential to life, not only life but all life, animal and plants. Most of the biochemical reaction that occur in metabolism and growth of living cells involved water and all takes places in water [9].

Most of our water supplies are from surface water which includes rivers, streams, lakes, oceans and seas and these water bodies are likely to be polluted with domestic and industrial as well as agricultural waste. As populations increase the problem become more serious and as such water can endanger the health and life of human beings because when polluted by fecal materials it becomes potential carriers of pathogenic organisms [10].

Man uses water not only for drinking purposes but also for bathing, washing, laundering, heating, air conditioner, agriculture, stock raising and gardens. Industrial process and cooling water power and stream power, fire protection, fishing, swimming and wild life propagation and navigation. Natural water contains not only natural floral but also microorganism from soil and possible for animals or sewage. Surface waters in streams or pools and stored water in lakes and large ponds vary considerably in microbial content. Water is broadly divided into three types viz surfaces water includes: streams, rivers, lakes, seas and oceans [11].

Most bacteria are mostly commonly found ordinarily in fresh water which: Pseudomonas, Archaebacter and Vibrio. Also present are the gram positive bacterial which are found in water include: Micrococcus and Archaebacterial and actinomycetes. Tap water as one of the water sources is mostly used domestically, it was observed that tap change sometimes the tap water will be clear, this would calls for load in order to be sure if its portability [12].

The underground water, well, borehole, many people have defined the underground water in different ways: underground water is non saturated that occurs where all pore in the soil or rock counting materials are saturated [7].

Bacterial contamination of drinking water is a major public health problem worldwide, because this water can be an important vehicle of diarrhea diseases, thus, the needs to evaluate the micro-biology quality (Bacterial contamination of drinking water is a major public health problem worldwide, because this water can be an important vehicle of diarrhea diseases, thus, the needs to evaluate the micro-biology quality [13]. Monitoring the micro-biology quality of drinking water is done through laboratories testing for the coliform group. The term total coliforms refers to a large group of Gram negative, rod-shaped bacterial that share several characteristics. The group includes thermo-tolerant coliforms which are of fecal origin, found in intestines of humans and warm-blooded animals, as well as the environmental species that are naturally found on plants, soil, and water. The thermo-tolerant coliforms are widely used as micro-biological parameters indicating fecal contamination while total coliform serves as a parameter to provide basic information on water quality [8]. The need assess the quality of water from some of these alternatives sources is imperatives because they have direct effects on the health of individuals. Molecular techniques are major tools for the analysis of microorganisms from food and other biological substances. The techniques provide ways to screen for a broad range of agents in a single test [14].

Rapid progress in the field of molecular biology has enabled the development of new methods for the identification of microorganisms. These techniques have proven to be particularly useful in the identification of microorganisms that are difficult to detect by phenotypic methods. Molecular biology methods can be used to confirm the presence of a specific microorganism in the tested material, as well as screen research. The most commonly used molecular methods include amplification of species-specific PCR products [9], PCR-based methods, and 16S rRNA/16S ribosomal DNA (rDNA) gene sequencing (currently considered the gold standard in identifying microorganism. The PCR technique was developed by [6] in the early 1980s. PCR is based on the amplification of DNA chains by repeatedly heating and cooling the sample in the presence of a polymerase and primers (i.e., short DNA fragments complementary to the sequence of the researched gene). Molecular biology methods are also used for the rapid identification of pathogens directly from clinical samples. Used PCR-based assays for the identification of Mycobacterium tuberculosis (MTB) and non-tuberculous mycobacteria (NTM). For this purpose they carried out the 16S rRNA gene sequencing by using 283 and 264 primers for PCR amplification and the Mbakt-14 primer for sequencing. Molecular-based detection gave concordant results for 97.7% and 97% of the specimens for MTB and NTM, respectively. PCR-based techniques also allowed for the explicit distinction of closely related bacteria, such as distinguishing Bacillus anthracis from other bacteria of the B. cereus group, a distinction that is not possible by any other method [15] developed a new multiplex real-time PCR assay to uniquely identify B. anthracis—a life-threatening pathogen. [16] Also developed a rapid pyrosequencing assay to distinguish *B. anthracis* from the *B. cereus* group.

2. Methods

2.1. Study Area

The study was collected in Saki, Saki town Oyo State, western Nigeria. It lies near the sources of the Ofiki river (the chief tributary of the Ogun river), about 40 miles (60km) from the Benin border. For the purpose of this study, the sampling locations were distributed over the four administrative units of Challenge, Wewe and Oke-Ogun as 1, 2 and 3 respectively.

The water sources were then categorized as ground waters sources, surface water sources and chlorinated water sources. Purpose samples or each water sources were drawn from the three administrative units.

2.1.1. Collection of Water Samples

During the collection of well water, tie a chord to a sterile bottle, lower it gently and collect the water sample, for river water remove the stopper then deep the sterile bottle to a depth of about 10ml directly opposes the current to collect the sample furthermore to collect pipe borne water sample, sterilize specimen bottle, crystal sterilize tap with alcohol and

allow water to fairly run for five minute then aseptically collect the water with the bottle and cork up, then bottled water and sachet water were purchase in the campus and lastly for Rain water, remove the stopper then collect the sample directly and cork up.

2.1.2. Materials Used

Syringe was used for collection of water samples, petri dishes was used for the culturing of the microorganisms, conical flasks was used for melting and storing the media, cotton wool was used for mopping up liquid, cleaning slides, and sterilizing the working bench of the laboratory, autoclave was used for sterilizing materials like culture media, conical flasks, incubator was used for incubating at different temperature, spirit lamp was used for sterilizing the atmosphere during the work processing, test tube was used for serial dilution, test tube rack was used for putting test tube rack, Weighing balance was used for weighing and lastly measuring cylinder was used for measuring liquid substance e. g water

2.2. Sterilization of Materials

Working benches was sterilize with methylated and cotton wool. All the material used like flask, petri dishes, measuring cylinder were washed thoroughly and allowed to dry and there after sterilize in oven at 180°C for one hour all the media were also sterilized in an autoclave at 120 c for 15mintes.

2.2.1. Preparation of Culture Media

The media used were prepared according to the manufacturer's specification. These media used include nutrient agar, MacConkey agar, eosin methylene agar and *Salmonella Shigella* agar.

2.2.2. Nutrient Agar

5.6g of nutrient agar was prepared to dissolve in 200ml of distilled water. The mixture was heated gently to dissolve the medium completely and then sterilized in an autoclave at 121°C for 15minutes.

2.2.3. MacConkey Agar

11g of MacConkey agar was prepared to dissolve in 200ml of distilled water. The mixtures was heated gently to dissolve the medium completely and then sterilized in an autoclave at 121°C for 15minutes.

2.2.4. Eosin Methylene Blue Agar

7.2g of MacConkey agar was prepared to dissolve in 200ml of distilled water. The mixtures was heated gently to dissolve the medium completely and then sterilized in an autoclave at 121°C for 15minutes.

2.2.5. Salmonella Shigella Agar

12.6g of Salmonella-Shigella agar was prepared to dissolve in 200ml of distilled water. The mixtures was heated gently to dissolve the medium completely and then sterilized in an autoclave at 121°C for 15minutes.

2.2.6. Isolation of Bacterial Species from Sources of Water

One gram (1g) of each water samples was homogenized in 9ml of sterile distilled water and vortexed for 10 minutes, then serial dilution of water were prepared. One millimeter (1.0ml) each of 10^3, 10^5 dilution were inoculated on sterile nutrient agar plates and MacConkey plate using pour plate method; the plates were incubated at 28+ 2°C before averted. The number of colonies in the surface of the plate were counted and expressed. Furthermore, counting and picking of colonies from plates 10^{-3} and 10^{-5} for the organism to survive. Pure culture of the bacteria where obtained by sub culturing on nutrient agar, MacConkey agar, eosin methylene blue agar and *Salmonella-Shigella* agar respectively.

2.3. Characterization of Bacterial Isolates

The selected bacteria were characterized by obtaining their colony morphologies like shape and gram staining.

2.3.1. Gram Staining Techniques

A small drop of distilled water was placed in a clean slide. The inoculating wire loop was sterilized by flaming it until it was red hot. The loop was allowed to cool and picked a small portion of the isolate and smeared in the drop of water on the slide. A thin smear was made and allow it to air dried after this, this smear was stained with 1% crystal violet for 60 seconds and washed with tap water, it was stained with gram iodine for 60 second and washed with water. It was then decolorized with 75% alcohol for 30 seconds and rinsed with tap water. The slide was counter stained with safranin for 30 seconds and washed off with tap water and air dried. The slides were observed under the microscope under oil immersion objective lens (×100). Gram negative appeared red while gram positive appear purple [15].

2.3.2. Biochemical Test

Several biochemical test were conducted for the pure isolates which are: A loopful of the isolate was placed on glass slide, a drop of 3% of hydrogen peroxides (H_2O_2) was added to the isolates, so production of gas bubbles of foaming indicated positive result while no bubbles show a negative result.

2.4. Molecular Identification (PCR) Techniques

2.4.1. Electrophoresis for DNA and PCR

Measure 1 g of agarose (for DNA); 2g of agarose for PCR, Mix agarose powder with 100 mL 1xTAE in a microwavable flask and Microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel then wait till agarose solution cool down to about 50°C (about when you can comfortably keep your hand on the flask), about 5 mins. Then add 10μL EZ vision DNA stain. EZ vision binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light. Pour the agarose into a gel tray with the well comb in place and Place newly poured gel at 4°C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

2.4.2. Loading Samples and Running an Agarose Gel

Add loading buffer to each of your DNA samples or PCR

products once it is solidified, place the agarose gel into the gel box (electrophoresis unit). Then Fill gel box with 1xTAE (or TBE) until the gel is covered. Carefully load a molecular weight ladder into the first lane of the gel and load your samples into the additional wells of the gel to run the gel at 80-150 V for about 1-1.5 hours then turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box to visualize DNA fragments or PCR product under UV trans illuminator.

2.4.3. PCR Mix Components

The PCR mix is made up of 12.5μL of Taq 2X Master Mix from New England Biolabs (M0270); 1μL each of 10μM forward and reverse primer; 2μL of DNA template and then made up with 8.5μL Nuclease free water.
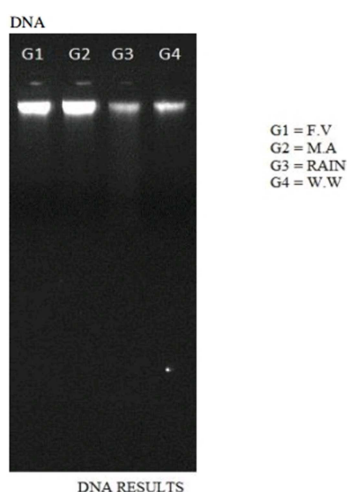
3. Result

Figure 1. *Bacterial DNA samples (G3, G4) loaded into the wells showing amplification.*
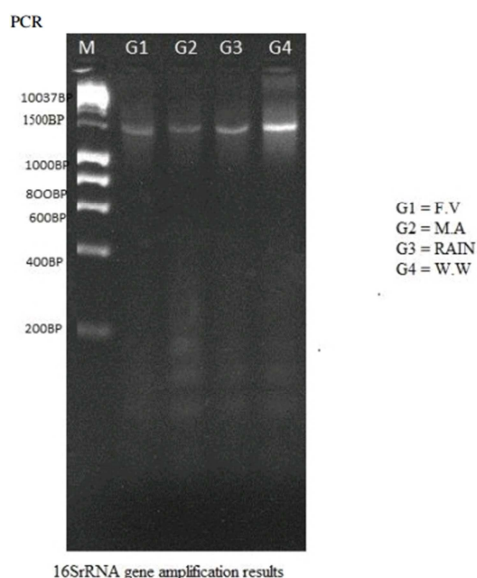

Figure 2. *1.0% Agarose gel electrophoresis showing molecular weight of bacteria DNA samples.*

Sample Code: Rain

Identified Organism: *Enterobacter kobeii* strain Sdv1B10% PAIRWISE IDENTITY: 72.95% NCBI ACCESSION NUMBER: KM593928 E Value: 0 Isolate Sequences:
GGGGTTAATCTATGGGATCATGGCTCAGGTGCGCTG GGAACCACCCCTTTAAAGTTT
GACGGCCTCAGGGGGGCTGGATGTCCCCTAAAAATT GTGGGGGGGGGGTATCTCCT
GTGAACACTATATATAACCCCGTATCGCCTCACAAC ACAAAAGAGGGGCCTTTTCGC
CTCTCGTGCTCTCATATGTCCCCATATGGTTATATAT AATGTGGGGTGAAGGCTCTCC
CCTGCGACAATATCCATCTGTGCTCTGAGAAGAACC CCCCCCACTGTGGCTGTGACA
CACTCCCCACACCCCCAGGGGACGCCACTGTGGAA AATTGTGCACTGTGGGCACAC
CTGTTACGCCCCTGTGCCGCGTGTAAAAAAAGCCTT CTCGTTGTGAAATATTTTTTCC
GCGGAGAAAGCGTTTTGATTATTATCCTCTCCGATA GTGACTTCCCCCACAAAAAA
CACCCGCTATATCTCTGCGCCCGCCCCCGCGATTATA CGGGGGGGCGCGCGTGTTTCT
CAGATTTAGTGCGCATAACGCACACGCGCGCTCTGT GTAGTCTCATATGAAATCCCC
CCGGGTCTCCCCGTGAAATCTGTTTTTAAACTGTGAC GCTATAGTCTCGTGTAGAGG
GGGGATAATTTCTCCGTGTGTCGCGGAAATGCGCATA GATCTGGGAGGAAAAATCA
CCGGGTGGGAAAGCGGCCCCCCCCTGGGGAAAAACA GTAGACTCTCGATGTGCAAAT
CGCGTGGGAGCACAAAACGAGTATATACCCCTGTGT ATTTCTTCCACCCCTATACAA
CGATCTCCCATGTGAGATGTTTGCGCCCTTTGGACGC GGGGTTTTCCGAGAGTATAA
CGGTTTTATATCTTACCCCCCCCTCGTGGGAGAACAGC CCCCCCACGAGTTTTTAACTC
CTCAAATGATATTTGTGCGACGGGGGCGCCACCCAC AAGAAGCGTGTGTGACACTC
GATGGAGTGTTATATATCTGCAGACGCGCACCGTGAG AAAATACCTCTATCACAAAT
CCTGTGGAAATTCACAGAAGAGTTTCTCT

Sample Code: Well Water Identified Organism: *Klebsiella pneumoniae* strain

201-b% PAIRWISE IDENTITY: 87.56% NCBI ACCESSION NUMBER: MN208186 E Value: 0 Isolate Sequences:
GGGGTATCATATGTCGATCATGGCTCAGGTGCGGTGG GATCACCCCCTAAAAGTTTG
ACGGCCTCGGGGGGGTGGGAACCCCCCTTAAAATTGT TGGAGGGGGATAACTACTGG
AAACAGTATCTAATACAGCATAACGCCGCACGACCC AAGTGGGGGACCTTCTCGCC
TCTTGCGATCTCATGTGCCCCGATGGGATTAGCTATTA GGTGGGGTAACGGCTCACC
TATGCGACAATCCCTATCTGGTCTGAGAGAATGACCC CCCCCACTGGAACTGAGACA
CGGTCCACACTCCCACGGGAGGCGCCACTGGGGAAT

ATTGCACAATGTGCGCAAGC
CCGATGCACCCCTGCCGCGTGTGTGAAGAAAGCCTT
CTGGTTGTAAAGCACTTTCTC
CGCGGGAGGGAGGCGATAAGGTTAATATCCTTGTCG
ATTGTCGTTACCCGCACAAA
AAACACCGGCTATATCTGTGCGACCACCCGCGGTAAT
ACGGGGGGTGCGAGCGTGA
ATCTGAATTACTGGGGCGTAAAACGCACACGGGCGG
TCTGTGAAGACGGATGTGAA
ATCCCCCGGGCTCTCCCCTGGGAACTCCGTTCTAAA
CTGGGGACGCTATAGTCTCTG
TAGAGGGGGGGAATAATTCCCCGGTGTGTCGCTGGA
AATGCGCAATAGATATGGGA
GGAAAAACCCCGGTGGGCGAAAGGCGGCCCCCCCC
TGGGACAAAAAACTCACGGC
CTCTCGATGCGAAAAACGGCGGGGGCAGCAAAAAA
AACGAATATATAATACCCCTG
  GGTTCTTCCCCCCGCCCGGTAAAACAGAATGATTC
GTACTTTTGTGAGGGTTTGTGG
CTCCCCTTTATGAGACGGTGGGTCTCTCCGGAAACAT
ATACACCCGTGTATAATCCC
ACCCCCGCCGCCTGCGGGAAGTACAGCGCCCCGCG
ACGAGTCTAAAACCTCCCAAA
ATGTATATATTGTGTACGGCGGGGGCTCCCCCCACAC
CAGACGGGCTTGCTGCAGAC
AGCGGTTGGGTTTTATATATTACTCTGACCGCACGCG
CGGAAAAATACTTTATCATG
CGTGGTGCTCTGTGCGCACATC

4. Discussion

The analysis examination carried out on the water sources
that serves for private and public water supply were intended
to assist in the determination of the quality of drinking water
in independence layout of Saki town. World Health
Organization [17, 18] have stipulated standards for water
meant for human consumption and the result of the present
investigation did not meet the standards except the tap water.
The total bacterial count of the water sources showed general
variation from different samples. The result shows that River
water sources has most poor quality in terms of
contamination with coliforms. The borehole water sources
showed that there are few content of coliform likewise bottle
water unlike other water sources [19]. The Isolation of
coliform in other sources of water is an indication of feacal
contamination of the water sources like the Well, River water,
Rain water and bottle water [21]. Water is one of the most
important vehicles for the transmission of infectious diseases
and fecal contamination of water sources particularly in
developing and underdeveloped countries has been well-
documented.

5. Conclusion

River water, well water, Rain water and bottled water has
been found to be unsafe for human consumption and for
industrial uses because of large number of bacterial that grew

on Agar plate incubated for 24 hours except being properly
treated before consumption to avoid outbreak of water
transmitting diseases. Disinfection such as boiling,
chlorination, using ultraviolet rays or ozonation is
recommended before consumption and use of water for
drinking and domestic purpose. This would minimize health
risk associated with other chemical consumption in the form
of firewood boiling water as well. Surface, ground and
chlorination urban water sources in Saki were contaminated
with bacteria and cysts to levels regarded as unsafe as per the
standards for portable water. This makes the water unsafe for
direct drinking and use in food processing.

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