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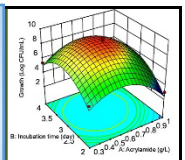

Evaluation of Bacteria Obtained from Private Well Water within Akungba-Akoko

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ABSTRACT

Availability of potable water is important for healthy living. Domestic water is usually supplied to homes through private wells, boreholes and public water companies. This study aimed at evaluating bacteria from private well water samples which serves as a major water source in the study area (Akungba-Akoko). Samples were subjected to total bacterial and coliform counts using nutrient agar and eosin-methylene blue (EMB) respectively. All isolates were identified based on their morphological and biochemical characteristics. This was followed by antibiotics sensitivity test (AST). The result showed that total bacterial count ranged from 4.0×10^3 CFU/mL to 22.5×10^3 CFU/mL while total coliform count ranged from 1.0×10^0 CFU/mL to 7×10^0 CFU/mL. Gram positive bacteria belonging to the following genera; *Bacillus, Corynebacterium, Enterococcus, Micrococcus,* and *Staphylococcus* were obtained, while Gram negative bacteria include; *Alcaligenes, Campylobacter, Citrobacter, Enterobacter, Escherichia, Klebsiella, Pseudomonas* and *Salmonella. Enterobacter* and *Pseudomonas* had the highest percentages of occurrence of 15%. *P. pseudomallei* had the highest sensitivity to ofloxacin (23 mm), followed by *P. fluorescens* (22 mm) and *C. freundii* (22 mm) while *B. subtilis* was susceptible to ciprofloxacin, erythromycin, levofloxacin. The result showed high level of bacterial contamination in all samples tested. The coliform count of all water samples exceeded the recommended level of zero. Hence, there is the need for proper wells water maintenance, control and improve hygienic practices by the households and individuals to help solve the risk of disease outbreak.

INTRODUCTION

Water supplied to public by water companies is safe to drink and does not pose health risk. Water of good drinking quality is of basic importance to human physiology as well as indispensable to man's continued existence. It is usually treated to make sure it is free from germs before supplying to homes [1]. Lack of safe drinking water is a threat to public health and well-being of the people and exposes them to risk of water borne diseases such as diarrhoea and dysentery as well as chemical intoxication [2, 3]. Water serves as a natural medium for the growth of microorganisms. Safe water is an indispensable resource that is becoming increasingly due to increase in world's population [4]. The growth of microorganisms in water depends on amount of available mineral nutrients and the dissolved oxygen. It has been estimated that between 1.1 and 2.6 billion people lack access to clean water and adequate sanitation respectively [2, 5]. In 1997, WHO reported about 40% deaths in developing countries occur due to infection from water related diseases [6, 7]. Well water is one of the common water supply sources and it is often unpolluted as a result of restricted movement of pollutants in the soil profile [7]. Many communities in Nigeria depend on well water as their major source of water supply [8].

Specific groups of microorganisms are used as indicator organisms to determine the level of pot-ability or level of purity. Similarly, there are some environmental factors that influence the distribution patterns of these organism are the coliform bacteria. The presence of *E. coli* in drinking water indicates presence of coliform contaminants [9]. When fecal coliforms (e.g., *E. coli*) are found in water sample, it indicates contamination by human or animal waste. *E. coli* can cause nausea, diarrhoea, and it is commonly associated with food poisoning. Fecal coliforms can be found in wells through direct discharge of waste from mammals and birds, agricultural and human sources [10]. This could also happen due cracks in the well or to nearness to wooded areas, pastures, feedlots, septic tanks, and sewage plants [11]. Also, in developing countries 1.1 billion people still defecate in

the open [12]. Determination of water quality involves mandatory microbiological parameters that include the presence of *E. coli, Clostridium perfringens, P. aeruginosa*, coliforms and related organisms grew at 22 °C and 37 °C. *E. coli, Bacillus* spp., *P. aeruginosa, Campylobacter jejuni, Vibrio cholerae, Salmonella typhi, Shigella* spp., *Yersinia enterolitica, Legionella* spp., *Aeromonas* spp. and *Mycobacterium* spp. are among bacterial pathogen found in tap water [6].

Water sanitation and hygiene interventions prevent intestinal parasitic infections, and these infections have synergistic effects with malnutrition [13]. Various studies have documented how access to safe water, sanitation and adequate hygiene can predict child growth and malnutrition [14, 15, 16]. Water sanitation facilities need to be accompanied by proper construction [17]. Maintenance and periodic replacement of existing services/facilities, and hygiene promotion are also necessary to achieve improvements [18].

MATERIALS AND METHODS

Sample collection

Six well water samples were collected randomly in Akungba-Akoko, Ondo State, Nigeria. Sterile bottles were tied with a strong string to a piece of metal of about 450 g. Bottles caps were aseptically removed and the weighted bottle was lowered into the well to a depth of about 10-20 cm. Each bottle was brought out and covered with a screw cap ensuring no air bubbles inside. Samples were immediately transported in an ice-pact container to the laboratory of Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko and sored at 4ºC throughout the study period.

Physicochemical parameters, bacterial and coliform counts

Temperature and pH of the well water samples was measured using a thermometer a pH meter respectively. Total bacterial count in water samples was estimated by adding 1 mL of each water sample (dilution factor of 10^{-3}) into 19 mL of molten nutrient agar, properly mixed and incubated at 37˚C for 24 h. Plates were examined and total colonies were counted using a colony counter. Coliform count was determined using pour plate technique in a medium containing Eosin Methylene Blue (EMB).

Identification of bacterial isolates

Cultural, morphological and biochemical characteristics of each isolate on agar media were determined [19, 20, 21]. The results were compared with those in Bergey's manual of determinative bacteriology [22].

Antibiotic sensitivity test

The agar plate was allowed to stand for 1 h before applying antibiotics sensitivity disc, each disc was applied aseptically and was lightly pressed down so the disk can make contact with the surface of agar. The plates were then incubated at 37ºC for 24 h. Result was observed by measuring the zone of inhibition of each disk as resistant, intermediate and susceptible [23].

RESULTS

Physical parameters such as pH, temperature, colour, and odour of samples were measured. The value of the samples indicated that the pH and temperature for all samples collected ranged from pH 6.9 to 8.0 and temperature 28 to 32ºC. All the water samples collected were clear except well water collected from Akungba (1 and 2) and all water samples were odourless (**Table 1**). After incubation for 24 h at 37 ºC, colonies formed on the nutrient agar plates were counted. IBK2 had the highest bacterial count while PS2 and IBK1 had the lowest bacterial count. For coliform count, colonies formed on EMB agar plates were counted after incubation for 24 h at 37 ºC (**Table 1**). A total of 20 bacterial species belonging to 13 genera were obtained, 14 were Gram-negative and 6 were Gram-positive bacteria (Tables 2 and 3). *Enterobacter* and *Pseudomonas* had the highest percentages of occurrence of 15% (**Table 2**). **Table 2** shows antibiotic susceptibility pattern. *P. pseudomallei* had the highest sensitivity to ofloxacin (23 mm), followed by *P. fluorescens* (22 mm) and *C. freundii* (22 mm). Similarly, *C. freundii* (22 mm) was sensitive to perfloxacin, *P. putida* was sensitive to septrin (trimethoprim/sulfamethoxazole) and *K. oxytoca* was sensitive to gentamycin (23 mm). Among Gram-positive bacteria, *B. subtilis* was susceptible to ciprofloxacin, erythromycin, levofloxacin and gentamycin while most of the isolates were resistant to amplicox, rifampicin, amoxacillin, streptomycin, norfloxacin, and chloramphenicol except *S. aureus*, and *M. luteus* was susceptible to only ciprofloxacin (21 mm). *E. faecalis* had intermediate activity against ciprofloxacin (20 mm), erythromycin (20 mm), levofloxacin (19 mm), and gentamycin (17 mm) (**Table** 3).

Table 1. Physical parameter, bacterial and coliform counts.

Sample code	pH	Tempe-rature (ºC)	Colour	Odour	Bacterial count (CFU/mL)	Coliform count (CFU/mL)
IBK1	7.0	32	Clear	Odourless	4.0×10^{-3}	2×10^{0}
IBK2	6.9	32	Clear	Odourless	22.5×10^{-3}	2×10^{0}
OK1	7.5	28	Clear	Odourless	7.5×10^{-3}	4×10^{0}
OK2	8.0	30	Clear	Odourless	16.5×10^{-3}	2×10^{0}
PS1	7.0	32	Turbid	Odourless	15.5×10^{-3}	1×10^{0}
PS2	7.6	30	Slightly turbid	Odourless	4.0×10^{-3}	7×10^{0}

KEY: IB1 = Well water from Ibaka quarters 1, IBK2 = Well water from Ibaka quarters 2, OK1 = Well water from Okusa quarters 1, OK2 = Well water from Okusa quarters 2, PS1= Well water from Akungba 1, PS2 = Well water from Akungba 2.

Table 2. Frequency of occurrence of bacterial genera.

S/N	Bacterial Genera	Frequency of Occurrence (%)
	Alcaligenes	5
	Bacillus	10
	Campylobacter	5
	Citrobacter	10
	Corynebacterium	5
	Enterobacter	15
	Enterococcus	5
	Escherichia	10
	Klebsiella	5
	Micrococcus	5
	Pseudomonas	15
	Salmonella	5
	Staphylococcus	5

Table 3. Antibiotic sensitivity test of Gram-negative bacteria.

Isolate	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN
Alcaligenes paradoxus	0	18	15	18	14	13	22	12	9	0
Campylobacter fetus	14	17	15	16	16	20	20	16	18	13
Citrobacter diversus	0	16	0	18	16	15	20	0	0	0
Citrobacter freundii	0	22	15	22	10	7	22	8	0	0
Enterobacter agglomerans	18	18	17	17	17	18	20	16	18	12
Enterobacter cloacae	16	18	19	15	18	15	15	16	18	0
Enterobacter dissolvens	17	20	15	20	15	19	19	15	17	18
Escherichia coli	0	8	16	12	16	20	20	15	12	6
Escherichia sp	0	18	0	18	18	15	18	18	18	0
Klebsiella oxytoca	15	14	0	12	23	16	12	12	0	0
Pseudomonas fluorescens	0	22	14	16	16	18	20	15	20	0
Pseudomonas pseudomallei	0	23	16	17	0	0	14	0	0	0
Pseudomonas putida	16	20	0	20	14	0	20	21	0	0
Salmonella sp	0	12	13	14	15	15	15	13	0	0

KEY: ≤14 = RESISTANT (R); 15-20 = INTERMIDIATE; 21≥ = SENSITIVE. CEP- ceporex, OFX- ofloxacin, NA- nalidixic acid, PEF- perfloxacin, CN- gentamycin, AU- augmentin, CPX- ciprofloxacin, SXT- trimethoprim/sulfamethoxazole, S- streptomycin, PN- ampicillin.

Table 4. Antibiotic sensitivity test of Gram-positive bacteria.

Isolate	APX	RD	AMX	S	NB	CH	CPX	E	LEV	CN
Bacillus subtilis	16	0	0	0	0	0	21	23	21	18
Bacillus sp	0	0	15	13	0	0	21	18	24	21
Corynebacterium sp	18	0	0	0	0	0	0	0	12	15
Enterococcus faecalis	0	0	0	0	0	0	20	20	20	20
Micrococcus luteus	0	0	16	17	17	14	21	18	0	0
Staphylococcus aureus	14	21	22	21	13	15	22	15	15	0

Key: ≤14 = Resistant (R); 15-20 = Intermediate; 21≥ = Sensitive. APX-ampiclox, RD- rifampicin, AMX- amoxacillin, S- streptomycin NB- norfloxacin, CH-chloramphenicol, CPX- ciprofloxacin, E- erythromycin, LEV- levofloxacin, CN- gentamycin.

DISCUSSION

Results of this study showed that well water samples were contaminated with different genera of Gram-negative and Gram-positive bacteria. A total of 20 bacterial species were obtained, out of which six (6) were Gram-negative and fourteen (14) were Gram-positive bacteria. Bacterial count ranged from 4.0×10^3 to 22.5×10^3 CFU/mL. This was higher than the recommended limits of < 500 CFU/mL and was consistent with the study of Ngwa and Chrysanthus [24] who reported a high total bacterial count that exceeded the standard limits. The high bacterial count could be an indication that the various well water sources are contaminated.

Coliform count of well water samples ranged from 1×10^0 to 7×10^0 CFU/mL. These values were high when compared with permissible maximum contaminant level goal for coliform count of zero (0) per 100 mL water sample [25]. This observation was similar to a report by Auta *et al.* [26] who reported high coliform count in all water analyzed. The high number of coliform could be due to inadequate maintenance of well water as many of the wells were not well managed. It can also be attributed to percolation of sewage into the ground water sources [7].

Groundwater, particularly private and public hand-dug wells supply drinking water for more than half of Nigerian population. It is clear that populations of people obtaining drinking contaminated water are at risk of waterborne diseases such as diarrhoea [26, 27]. *Pseudomonas, Bacillus, Staphylococcus* and *Alcaligenes* are of public health significance. This agreed with the report of Efuntoye and Apanpa [28], Onuoha [29]. *S. aureus* is known to produce enterotoxin [30] and also *Enterobacter* spp. isolated from the water samples were examples of coliform found in vegetation and soil which serves as sources through which the pathogens enters the water [31]. *Pseudomonas* spp. and *Enterobacter* spp. were predominant (15%). *Bacillus*, *Citrobacter* and *Escherichia* spp. (10%) were the second most predominant bacterial genera. The presence of *E. coli, K. oxytoca* and *Enterobacter* sp in well water indicated fecal coliform contamination. This corroborated a finding by Islam *et al.* [32] who reported *E. coli* contamination in dug well waters in rural areas of Bangladesh.

All the 20 isolates were screened for their antimicrobial susceptibility pattern. The disc contains ten different antibiotics. The result revealed that *E. coli, K. oxytoca* and *S. aureus* were resistant to more than three antibiotics. The number of antibiotics to which they were resistant ranged from ampiclox to gentamycin. The results correlate with previous study of Cardonha *et al*. [33] who isolated *E. coli* strains from water which were resistant to more than one antibiotic. Also, *E. coli* was slightly sensitive to gentamycin and ciprofloxacin, which corroborated the reports of Oyetayo *et al*. [34] who reported sensitivity against *E. coli* strains from well water in Ondo State, Nigeria. *K. oxytoca* in this study was highly sensitive to gentamycin which was at variance with a previous study by Dash *et al*. [35]. Also, this study has shown that wells water in the community studied harbor bacteria that are resistant to multiple antibiotics. The high prevalence of enteric bacteria in the water could be due to poor sanitation of members of the study area. The presence of bacteria that are resistant to antibiotic poses a serious health hazard especially since such organisms can serve as reservoir for antibiotic resistant genes that could be transferred to potentially pathogenic bacteria in the ecosystem.

Improper construction of wells, poor handling and unsanitary conditions such as; close to lavatories, broken sanitary sewers, garbage heaps are other likely cause of contamination. The microbiological analysis of well water is important to detect and evaluate pathogenic organism especially those causing diarrhea which constitute health hazard in water. This study serves as a guide to monitor and protect well water sources in the study area. Also, presences of some bacterial species indicated the presence of contaminants which may be linked to human activities in such an environment; these include surface run-off of the water. Therefore, other and related studies are needed to be carried out to understand the extent of water contamination and prevention.

CONCLUSION

Microbiological analysis of well water samples in the present study showed a high level of bacterial contamination. The coliform count exceeded acceptable limit which makes the water unfit for consumption and related use. Also, bacteria isolated from the various samples were mostly enteric organisms and potential pathogens of public health concern. There is the need for proper well water maintenance, environmental sanitation around the well areas and improved hygienic practices by every household and individual to help reduce the risk of disease outbreak. Therefore, it is recommended that well water in the study area should be well managed and treated properly before drinking. Enforcement of sanitary laws and further research should be done. Finally, appropriate agencies should embark on rapid enlightenment campaign to educate the populace living in Akungba-Akoko on the effects of consuming contaminated water and how to prevent contamination.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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