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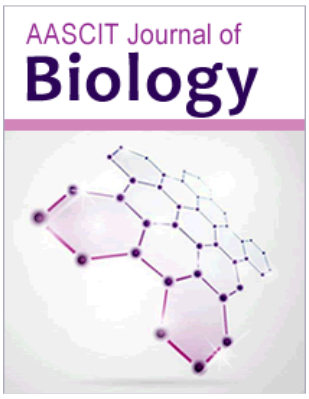

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Antibiotics Susceptibility Pattern of Escherichia coli Isolated from Well Water in Afikpo, South Eastern Nigeria

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Abstract
The aim of this study was to isolate and identify *E. coli* from well water sources in Afikpo and to determine their antibiotics resistance profiles. Water samples from ten different wells were analysed using standard microbiological and biochemical techniques. Results showed that the total bacterial counts and *E. coli* counts were all above limits set by World Health Organization. Antibiotics susceptibility studies were conducted using Kirby and Bauer disc diffusion method according to National Committee for clinical Laboratory Standards, (NCCLS). The result of antibiotic studies showed that all the *E. coli* isolates were 100% resistant to gentamicin and amoxicillin, and 70% resistant to chloramphenicol, while In contrast the isolates exhibited sensitivity to ciprofloxacin, Pefloxacin and tarivid. It is recommended that adequate awareness is created on water quality and sanitation together with construction of protected well to reduce the risk of water-borne diseases in Afikpo area.

1. Introduction

Water is one of the most important felt needs in public health and the availability of safe water dictates the quality of life since water is a basic requirement of life. About 1.2 billion people worldwide lack access to safe drinking water (Wilkes *et al.,* 2009). Sub-Saharan Africa accounts for over 1/3 of that number, and is lagging behind in progress towards the MDG target, with only 60% of the population using improved sources of drinking-water (WHO, 2010).

Dwindling municipal water supply leads to water and sanitation crisis. For domestic needs, people fall back on dubious water sources, many of which contain dangerous contaminants (Onyenekenwa, 2011). Water for their daily needs are majorly sourced from river, stream, well and ponds (Venter, 2001). Water intended for human consumption must be free from organism and from concentration of chemical substances that may be a hazard to health. The major health threat posed by drinking unsafe water is infectious diseases. It has been demonstrated vividly that water source are contaminated mostly by *E. coli* (Feachem, 2001).

E. coli the most commonly isolated organism found in well water find their way into the water through human faeces and sewage disposal from crowded communities (Kunin, 1993). *E. coli* and related bacteria constitute about 0.1% of gut flora, and faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease (Eckburg *et al.,* 2005). In addition to the wide spread cases of water-borne diseases resulting from the contamination of water sources, concerns have been raised when the

disease fail to be cured due to resistance to commonly prescribed antibiotics by the contaminating microorganisms originating from livestock faeces and human sewage(Prescott *et al.,* 2001). These days' antibiotic resistant bacteria species are ubiquitous in the environment and their negative impact has greatly increased (Mathews *et al.,* 2007). Improper antibiotic use and lack of awareness are considered as the most important factor for the emergence, selection and dissemination of antibiotic resistant bacteria species in the environment (Neu, 1992; Abera *et al*., 2013). Infection by antibiotic resistant strains of pathogenic bacteria can have severe health implications for then sickened individual including more virulent strains and less treatment options (Barza and Travers, 2003).

In view of the fact that good source of water such as pipe-borne water in Afikpo is in short supply or totally unavailable, majority of the inhabitants now depend on well water. Water from the well is often contaminated by surface or run-offs waters especially during rainy season and indiscriminate dumping of refuse around the wells. The study was designed to examine some wells in Afikpo for the presence *E. coli* which as used among other bacteria as indicators of water pollution.

2. Materials and Methods

2.1. Study Area

The study was conducted in Afikpo Local Government Area. Afikpo local government comprises of the following communities: Afikpo, Ozizza, Akpoha, Amasiri, Enohia, Ibii, and Itim. Afikpo city is the headquarters of Afikpo North Local government and is the second most popular and largest city in Ebonyi State. It lies on latitude 5.89°N and longitude 7.94°E and it has a population of 71,866. There are many wells locted in almost every compound within the communities.

2.2. Sample Location

A total of ten(10) wells were sampled within Afikpo Local Government. Three (3) of the wells were approximately 16 feet deep, while others were within the range of 10 and 12 feets. Five of the wells were at ground level, while the remaining five were raised above ground level. A brief description of these well water sources is presented in Table 1.

Table 1. *Description of Well Water Sources in Afikpo.*

Well Location	Dept(ft)	Well cover	Well opening
Mgbom	10	Uncovered	Raised
Amuro	11	Covered	Raised
Unwana	16	Covered	Raised
Amangballa	10	Covered	Unraised
Akpoha	16	Uncovered	Raised
Ozizza	12	Covered	Unraised
Ukpa	11	Covered	Unraised
Ndibe	9	Covered	Unraised
Kpogirikpo	12	Uncovered	Raised
Ngodo	16	Covered	Unraised

2.3. Sample Collection

Water samples were collected from ten different wells in the same geographical location with the use of sterile polypropylene sample bottles. A strong rope was attached to the neck of each sterile bottle, and gently released into the well. The opened bottle was allowed to sink below the water and was pulled up after observing there were no more bubbles from the bottle. The bottle was gently raised out of the well without allowing the bottles to touch the sides of the wells. The caps were carefully replaced. The bottles were labelled and the sample was transported to the Microbiology Laboratory of Akanu Ibiam Federal Polytechnic, Unwana immediately for microbiological analysis.

2.4. Microbiological Analysis

2.4.1. Isolation and Enumeration of *Escherichia coli*

About 100ml of the water samples was filtered through membrane filter with the aid of vacuum pump. The filter membrane was placed in the m-HPC agar plate. This was incubated using an incubator pre-set to 45.5 ± 5oC for 24 h.

2.4.2. Enumeration of Total Bacteria Count

The total bacteria counts were enumerated on nutrient agar plates by spread plate method using 0.1 ml of dilutions 10^{-1} to 10^{-7} of the bacterial suspensions. All inoculated plates were incubated for 24-48 hrs at room temperature. The bacterial colonies on the plates were counted and randomly picked and purified by sub-culturing unto fresh agar plates using the streak plate technique.

2.4.3. Identification and Characterization of Isolates

Identification of the isolates was based on their culture morphology, microscopic examination, carbohydrate fermentation, colonial morphology, Gram staining, Catalase, oxidase, motility, IMViC and citrate utilization tests. References were made to Bergey's Manual of determinative Bacteriology (1974) 8th Edition for the identification of bacteria.

2.5. Antibiotic Assay

The susceptibility of the *E. coli* were tested against a wide range of antimicrobial agents namely; Gentamicin(10µg), Chloramphenicol (10µg), Ciprofloxacin (5µg), Amoxicillin (10µg), pefloxacin (10µg), and Tarivid (10µg), using disc diffusion method of determining susceptibility (Bauer et al ., 1996). Standard inoculums of the overnight grown nutrient agar broth culture were spread on Mueller-Hinton agar plates using sterile swabs. The plates were dried at room temperature for 2 hrs before placing the antibiotic disc. The plates were incubated at 37^0C and the diameters of zone of inhibition were measured after 18 hrs. The interpretation of zone of inhibition was followed as recommended in the CLSI (2012).

3. Results

The microbial quality result of well water sources in Afikpo revealed that majority of the well water sources were contaminated with Bacteria. Total bacterial count ranged from 1.1 x 107 Cfu/mL to 9.0 x 10^7 Cfu/mL. The highest count was obtained in well water sampled from Amangballa area while the least count was obtained from water sampled from Ndibe area (Table 2). The occurrence of *E. coli* in the water samples obtained from the well also reveals that water from well in Amangballa has the highest count of *E. coli* followed by well water from Mgbom area, while the least E. coli count was obtained from Ndibe well water(Table 2)

Table 2. *Total bacteria and coliform loads in well water Sources in Afikpo.*

Well Location	Well Type	Total Bacteria count(Cfu/mL) x 10^7	*E. coli* Counts (Cfu/100mL)
Mgbom	Uncovered	6.4	54
Amuro	Covered	4.1	36
Unwana	Covered	1.9	12
Amangballa	Covered	9.0	62
Akpoha	Uncovered	3.1	18
Ozizza	Covered	2.4	16
Ukpa	Covered	3.2	20
Ndibe	Covered	1.1	10
Kpogirikpo	Uncovered	2.9	13
Ngodo	Covered	5.1	45

The result of the antibiogram of *E. coli* isolates was shown in Table 3. The result revealed that the isolates showed 100% resistance to Gentamicin and Amoxicillin, while it was 70% resistant to Chloramphenicol. On the other hand the isolate showed susceptibility to Ciprofloxacin, Pefloxacin and Ofloxacin at different degrees (Table 3).

Table 3. *Antibiogram of E. coli Isolates from well water Sources in Afikpo.*

Well Location	GEN	CHL	CPX	AMX	PEF	OFX
Mgbom	_	_	+		+	+
Amuro	_	_	+	_	+	+
Unwana	_	+	+	_	+	+
Amangballa	_	_	+	_	+	+
Akpoha	_	_	+	_	+	+
Ozizza	_	_	+	_	+	+
Ukpa	_	_	+	_	+	+
Ndibe	_	+	+	_	+	+
Kpogirikpo	_	_	+	_	+	+
Ngodo	_	_	+	_	+	+
*GRAND total (%)	100	70	0	100	0	0

Key: GEN: Gentamicin; CHL: Chloramphenicol; CPX: Ciprofloxacin; AMX: Amoxicillin; PEF: Pefloxacin; OFX: Tarivid,
*Grand total = % resistance of isolates to antibiotics
+ = Positive/ Susceptible; - = negative/ resistant

4. Discussion

The microbial quality of well water sources in Afikpo during the study period revealed that all the well water sources in Afikpo were not in compliance with WHO guidelines. The total bacteria counts (Table 2) of the water samples ranged from 1.1 x 10^7 Cfu/mL to 9.0 x 10^7 Cfu/mL with no sample having bacteria count within the limit of 100 Cfu/mL allowed for potable water (NSDWQ, 2007). Also, the result of the study revealed that all the well water had significant number of fecal coliform population above the WHO set limit of 0 coliform per 100 mL of drinking. The presence of *E. coli* in the water sample suggest faecal pollution, hence falls short of the standard of safe drinking water. The high fecal coliform counts obtained from the well water are not unlikely because the environment in which majority of the wells are located predisposed them to constant pollution from human and animal waste materials. For a well to be safe from contamination, it should be properly constructed, it should also be located some reasonable distance (100- 200 m) away from pit latrines, dumping grounds and bathrooms. However, it was observed that most of the wells examined were subject to various types of pollution from the environment. The pollution was made possible because majority of the wells sampled were not properly constructed, majority of them are not deep, have no well cover or the well opening were not properly raised above the ground, a situation that made it easy for the wells to be constantly polluted from surface runoff and seepage from contaminated ground water and waste waters. The result obtained from this work corroborates some other works that have been carried out in Nigeria and elsewhere. Oyetayo *et al.,* (2007) reported 58.3% prevalence of *E coli* in water obtained from Akure. Also, Adjuwon *et al.,* (2011) reported the presence of *E. coli* and *Aerobacter aerogenes* from well water in Ile- Ife. However, similar studies conducted in other developing parts of the world have reported the presence of bacteria especially coliforms from unprotected water bodies such as Well and River water sources. A similar study done in a rural community in Cameroon showed that almost all the water sources was contaminated with different types of bacteria including coliforms because they were obtained from unprotected sources(Abera *et al.,* 2013; Kuitcha *et al.,* 2010). Mengesha *et al.,* (2004) had also reported that 75% of unprotected well and spring water sources from North Gondar, Ethiopia, were contaminated by faecal coliforms, especially *E. coli.* The presence of this organism in the well water studied has an implication for public health.

The antibiotic susceptibility pattern of the *E. coli* isolates obtained from this work revealed all the isolates were 100% resistant to gentamicin and amoxicillin, and 70% resistant to chloramphenicol. In contrast the isolates exhibited sensitivity to ciprofloxacin, Pefloxacin and tarivid. Bacterial resistance to

antibiotics has become a global public health problem. In Nigeria like other developing nations, abuse of drugs such as indiscriminate use of antibiotics has become a common occurrence. Ayandiran *et al., (*2014) have detected bacterial resistance to more than one antibiotic from water and sediment of Oluwa River. Bello *et al.,* (2013) reported high level of multiple resistances among *E. coli* Isolates from well water in Akure in Ondo State. However, similar studies conducted in other parts of the world have reported high percentage of *E. coli* isolates being resistant to gentamicin, amoxicillin and chloramphenicol. A similar study made in the water supplies of rural Venda communities in South Africa showed that more than 95% of *E. coli* isolates were resistant to common antibiotics in use. Koesk *et al.*, 2014; Mydryk, 2012; Toth *et al.,* 2010; Cox & Wright, 2013 all have previously reported bacterial resistance against ampicillin, gentamicin, erythromycin, tetracycline and ciprofloxacin at different times. The reason why some of these *E. coli* isolates showed high level of resistance to the antimicrobial agent used is an indication that these antibiotics have been abused, hence the possibility they have acquired resistance. It has been reported that bacteria acquire resistance by horizontal gene transfer of mobile genetic element and that gross usage of the antibiotics influences the selection of existing resistance mechanisms (Stoke & Gillings, 2011).

5. Conclusion and Recommendation

The current research has revealed the sanitary condition and quality of well water in Afikpo. The high coliform density recorded in all the wells is an indication of the poor sanitary condition of the environment. The result obtained from the microbiological analysis showed coliform above the acceptable standard limits for drinking water by World Health Organisation. Majority of the wells were located in a very crowded location, some are also located near refuse dumps and pit toilets where they constantly receive doses of fecal materials. The research also discovered the occurrence of multiple antibiotic resistances *E. coli* from well water sources. In view of the above findings, effort should be made to educate the people on the importance of proper construction of well, good hygienic practices, adequate water protection mechanism and safe use of antibiotics. When all these are done, it will go a long way in eradicating the menace of water-borne associated illness in in the study area.

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