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Antibiogram and Plasmid Profile of *Escherichia Coli* Isolates in Well Water In Akure, South Western Nigeria

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ABSTRACT: *Well water samples sourced from Akure, Ondo State, Nigeria were analyzed using standard and analytical methods to determine the level of divergence of Escherichia coli, in the well water, its antibiogram and the plasmid profile. A total of 400 well water samples were analyzed during the raining and dry season. The mean value of E .coli count obtained ranged from 1.0x10^{3}cfu/ml to 3.2x10^{3}cfu/ml. Highest E. coli count was obtained from the sample sourced from Isolo while lowest from FUTA environment. In all, E. coli were isolated from (76) well water sample representing 19% of the 400 sample sourced during the dry season and 108 representing 27% 400 well water sample collected during the raining season. Overall, of 184 E. coli isolates from different well water sourced from different location in Akure were tested against the selected antimicrobial agent. Fifty (50) representing 27.17% of the 184 E. coli isolates showed multiple resistances to (10) antimicrobial agents tested. However, of the ten antimicrobial agents tested, resistance was highest to AM (37) 20.1% follow by SXT (32) 17.4% and CH (31)16.85%. E. coli isolates obtained from Isolo community revealed the highest prevalence of resistance to antimicrobial agent (SXT, CH, S, SP, CPS, AM, AU, and CN). The antimicrobial agents tested were; Sulphamethoxazole (SXT) 30µg, Chloramphenicol (CH) 30µg, Sparfloxacin (SP) 10µg, Ciprofloxacin(CPX)10µg, Amoxcillin (AM)30µg, Augmentin (AU)30µg, Gentamicin (CN)10µg, Pefloxacin(PEF)30µg, Trivid(OFX)10µg, Streptomycin(S)30µg. Plasmid profile analysis of 50 E.coli isolates that showed multiple resistances by agarose gel electrophoresis showed a total of 48 different plasmid bands occurring in various combinations. The microbiological analysis of the well water sample used in this research did not meet the recommended limits and could pose a serious health risk to consumers.*

KEY WORDS: *Antibiogram, Antibiotic, E. coli, Plasmid, Well water,*

I. INTRODUCTION

About 70% of earth is made up of water. In spite of this, good quality water for human usage is still inadequate. About 1.2 billion people worldwide according to [1] lack access to safe drinking water. Almost 30% of population of people in developing country do not have access to good quality water. Dwindling municipal water supply leads to water and sanitation crisis. For their domestic needs, people fall back on dubious water sources, many of which contain dangerous contaminants [2]. Water for their daily needs are majorly sourced from river, stream, well and pond [3]. However, in most towns in south western Nigeria, people depend on well water for all domestic activities. These water sources are frequently exposed to microbial contamination from humans, animals and the environment [4,5]. Potential sources of these pathogens in water include wastewater effluents, combined sewer overflows, runoff from urban land, animal wastes, and municipal waste sludges disposed off on land or in water [6,7]A significant proportion of inhabitants of these communities are therefore exposed to water-borne diseases [8,9] which continues to grow with the increasing demands for potable water [10]. Inadequate supply of water free from pathogenic microorganisms has a significant and devastating impact on public health. Water intended for human consumption must therefore be free from microorganisms and chemical substances that constitute health hazards. The microorganisms most commonly used as indicator of microbial pollution, are *Escherichia coli* and the coliform group as a whole [11]. A well water is an excavation or structure created in the ground by digging, driving, boring or drilling to access water in underground aquifers. The well water is drawn by an electric submersible pump or a mechanical pump (eg from a water-pumping windmill. It can also be drawn up manually using containers, such as buckets, that are raised by hand. Although not essential, a storage tank with a pressure of 40–60 psi is usually added to the system (after the pump), so the pump does not need to operate constantly.

To reduce the electricity required to pump up the water, often, a cistern is also added along with a small second pump [12]. Wells can vary greatly in depth, water volume and water quality. Well water typically contains more minerals in solution than surface water and may require treatment to soften the water by removing minerals such as arsenic, iron and manganese contents.

A well is made by reaching groundwater in the water table. Groundwater is stored naturally below the Earth's surface. Most groundwater originates as rain or snow that seeps into the ground and collects. [13]
Escherichia coli are found as normal flora in the human intestine. *E. coli* and related bacteria constitute about 0.1% of gut flora, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease [14]. The organism is usually harmless in the intestinal lumen. Moreover, even the most robust members of our species may be susceptible to infection by one of several highly adapted *E. coli* clones which together have evolved the ability to cause a broad spectrum of human diseases. Infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or can disseminate throughout the body.
Water supply in Akure metropolis is mainly from wells. The water from the well is often contaminated by surface or runoff waters especially during rainy season and indiscriminate dumping of refuse around the wells. In view of this, this research is focusing on the Antibiogram and Plasmid Profile of *Escherichia Coli* which is the organism that is used as indicator of faecal pollution

II. MATERIALS AND METHODS

The water samples used in this research were sourced from four hundred (400) selected wells in Akure, southwestern Nigeria.

2.1 Sample collection

A total of four hundred well were sampled. Water was collected from the well using sterile bottle with tight covered. The water was collected by holding the bottle at the bottom while plunging the mouth into the water and covered immediately after collecting the water sample. The bottle was filled leaving about 30mm of empty space to allow mixing during laboratory analysis. Water samples were immediately transported to the laboratory and store at 4^0C prior analysis.

2.2 Isolation and identification of Escherichia coli

Isolation and identification of *Escherichia coli* were done using Eosine methylene blue agar (EMB). Individual colonies showing a green metallic sheen on EMB agar were further confirmed using biochemical tests [15,16]. The biochemical tests used to further differentiate *E. coli* from other feacal coliform bacteria were indole, methyl-red, voges-proskaher and citrate test [17].

2.3 Antibiotic sensitivity tests

The antibiotic sensitivity test of the *E. coli* isolates was determined using the disc diffusion method [18].

2.4 Plasmid Analysis

Plasmid profile of *E. coli* isolates were analysed by 0.8% agarose gel electrophoresis after staining with ethidium bromide and the DNA bands were visualised by UV-transilluminator.

III. RESULTS

The mean value of *E .coli* count obtained during the dry season ranged from $1.0x10^3$cfu/ml to $2.5x10^3$cfu/ml. Highest *E. coli* count was obtained from the sample sourced from Isolo $2.5x10^3$cfu/ml. While lowest was obtained from sample sourced from Ijoka. During the raining season, an increase was however noted on the *E. coli* count indicating that the water has been heavily polluted with faecal contamination, the values ranged from $3.0x10^3$cfu/ml to $3.2x10^3$cfu/ml. Highest *E.coli* count was obtained from Isolo ($3.2x10^3$cfu/ml) while lowest from FUTA metropolis. In all, *E. coli* were isolated from (76) well water sample representing 19% of the 400 sample sourced during the dry season and 108 representing 27% 400 well water sample collected during the raining season. Fig 1. This result is contrary to WHO recommendation of zero *E. coli* in 100ml water sample.

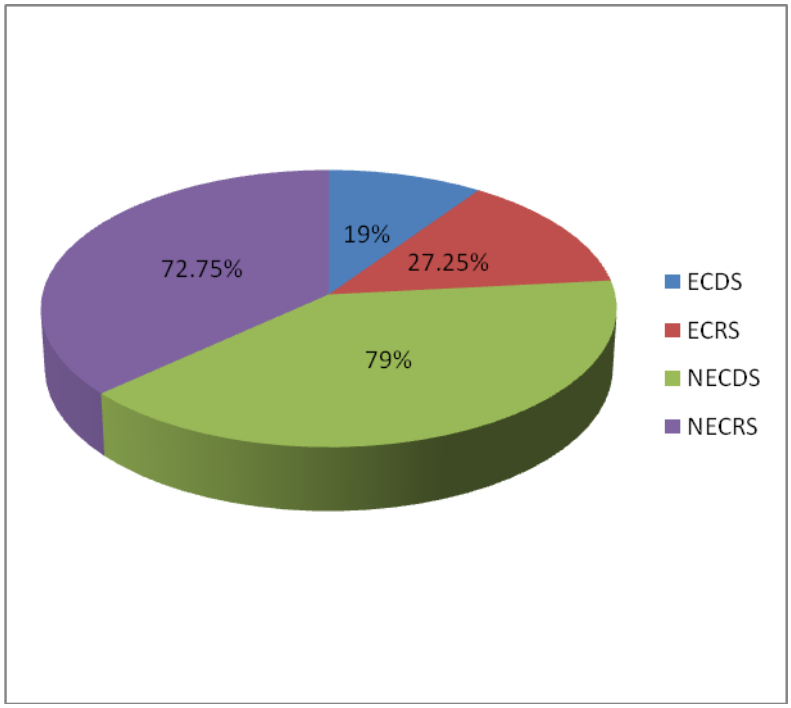

Fig1: *E. coli* count of well water samples collected in Akure metropolis at two different seasons of the year

ECDS= *E. coli* dry season
ECRS= *E. coli* raining season
NECDS= No *E. coli* Dry season
NECRS= No *E. coli* WHO raining season

Overall, of 184 *E. coli* isolates from different well water sourced from different location in Akure were tested against the selected antimicrobial agent. Fifty (50) representing 27.17% of the 184 *E. coli* isolates showed multiple resistant to (10) antimicrobial agents tested. During the dry season 17 representing 22.37% of the 76 isolate tested showed multiple resistant while 33 *E. coli* isolates representing 30.56% of the 108 isolate tested were resistance to many antibiotics during the raining season. However, 59 isolates representing 77.63% of the 76 isolates tested were sensitive during the dry season while 75 representing 63.4% of the 108 isolates tested were sensitive to one or more antibiotic agents tested during the raining season. Fig 2. The prevalence of resistance of the *E. coli* isolates to each antimicrobial agent tested during the dry season were; SXT (17) 9.2%; CH (13)7.06%; SP (9)4.89%; CPX (14)7.60%; AM (21) 11.4%; AU (8) 4.3%; PEF (5) 2.72%;CN (8) 4.3% OFX (4) 2.17%; S (18) 9.78%. During the raining season the prevalence were; SXT (32) 17.4%; CH (31)16.85%; SP (27)14.68%; CPX (23)12.5%; AM (37) 20.1%; CN 10.87%; AU (17) 9.2%; PEF (15) 8.15%; OFX (18) 9.78%; S (29) 15.76%. Fig 2.
However, of the ten antimicrobial agents tested, resistance was highest to AM (37) 20.1% follow by SXT (32) 17.4% and CH (31)16.85%. *E.coli* isolates obtained from Isolo community revealed the highest prevalence of resistance to antimicrobial agent (SXT, CH, S, SP, CPS, AM, AU, and CN). Isolates from Isolo metropolis showed the highest resistance pattern indicating the well water from this area were of poor microbiological quality. The antimicrobial agents tested were; Sulphamethoxazole(SXT)30µg, Chloranphenicol(CH)30µg, Sparfloxacin(SP)10µg, Ciprofloxacin(CPX)10µg, Amoxacillin(AM)30µg, Augmentin(AU)30µg, Gentamycin(CN)10µg, Pefloxacin(PEF)30µg, Trivid(OFX)10µg, Streptomycin(S)30µg. However, there is variation in the prevalence of the resistance of the *E. coli* isolates to the antimicrobial agent tested.

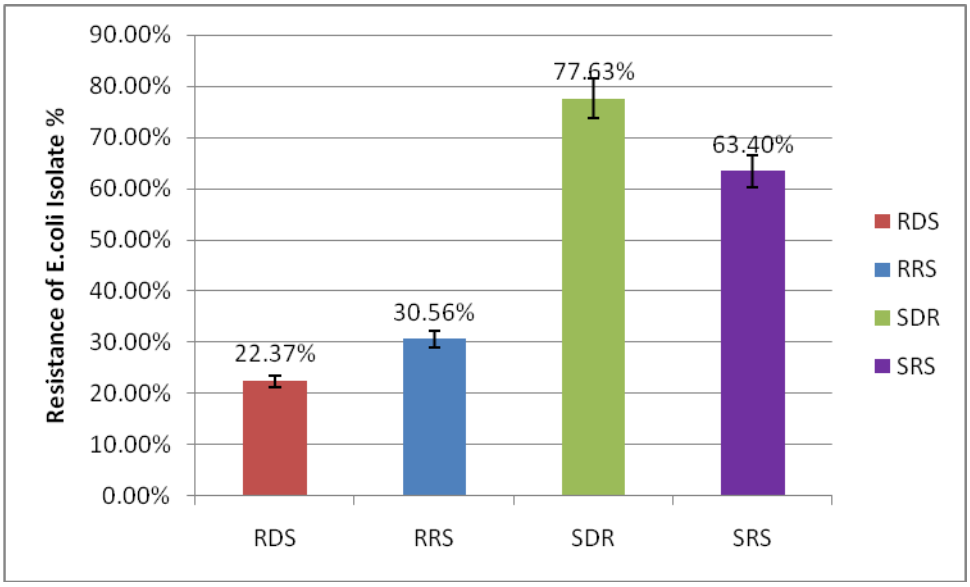

Fig 2: Resistance pattern of *E. coli* isolates of well samples collected in Akure metropolis at two different season of the year

RDS Resistant dry season
RRS Resistance raining season
SDS Sensitive dry season
SRS Sensitive raining season

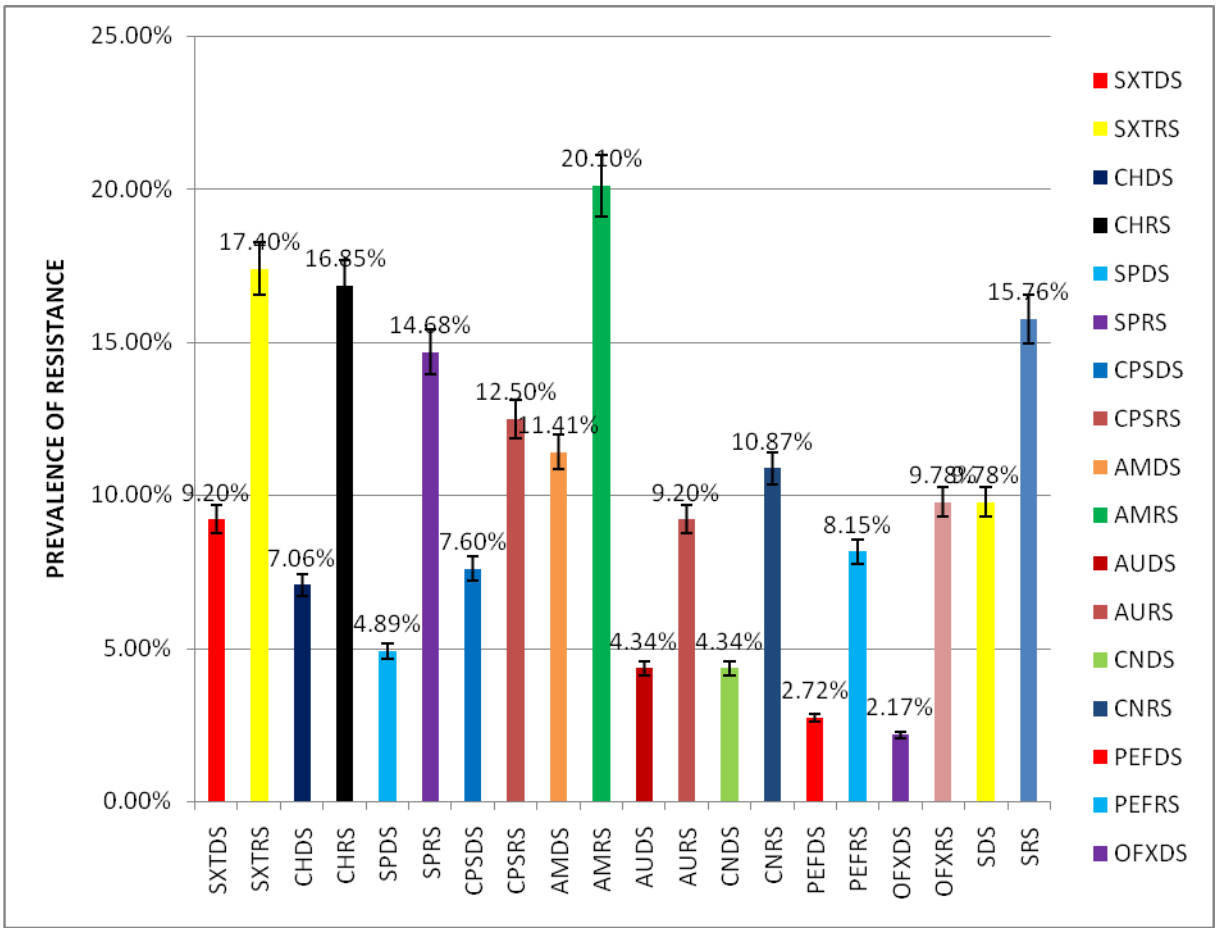

Fig 3: Prevalence of resistance of *E. coli* isolates from well water in Akure metropolis
SXTDS = Resistance to Sulphamethoxazole (SXT) dry season

SXTDS = Resistance to Sulphamethoxazole (SXT) raining season
CHDS = Resistance to Chloranphenicol (CH) dry season
CHDS = Resistance to Chloranphenicol (CH) raining season
SPDS = Resistance to Sparfloxacin (SP) dry season
SPRS = Resistance to Sparfloxacin raining season
CPSDS = Resistance to Ciprofloxacin(CPX) dry season
CPSRS=Resistance to Ciprofloxacin(CPX) raining season
AMDS =Resistance to Amoxacillin (AM) dry season
AMRS = Resistance to Amoxacilli(AM) raining season
OFXDS= Resistance to Trivid(OFX) dry season
OFXDS= Resistance to Trivid(OFX) raining season
SDS= Resistance to Streptomycin(S) dry season
SRS= Resistance to Streptomycin(S) dry season
AUDS= Resistance to Augmentin(AU) dry season
AURS= Resistance to Augmentin (AU) rain season
CNDS = Resistance to Gentamycin(CN) dry season
CNRS= Resistance to Gentamycin(CN) R S
PEFDS= Resistance to Pefloxacin(PEF) dry season
PEFRS= Resistance to Pefloxacin(PEF) R. season

Fig. 4 & 5 shows the plasmid profile of the *E. coli* isolates that shows multiple resistances to one or more antibiotics. Plasmid profile analysis of 50 *E.coli* isolates by agarose gel electrophoresis showed a total of 48 different plasmid bands occuring in various combinations.

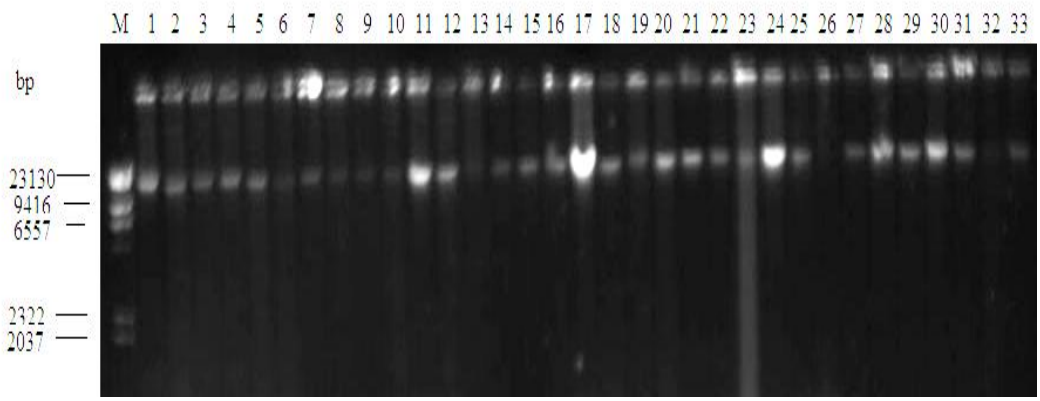

Fig. 4: plasmid profile of *E. coli* isolates obtained from well water during raining season analysed by 0.8% agarose gel electrophoresis after staining with ethidium bromide and the DNA bands were visualised by UV-transilluminator.

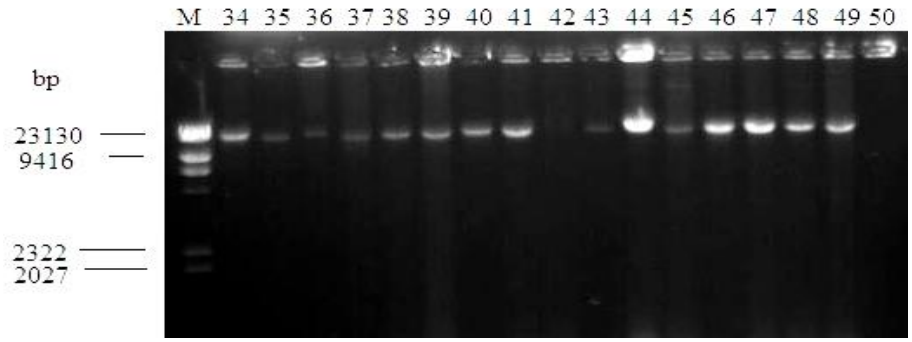

Fig. 5: plasmid profile of *E. coli* isolates obtained from well water during dry season analysed by 0.8% agarose gel electrophoresis after staining with ethidium bromide and the DNA bands were visualised by UV-transilluminator.

IV. DISCUSSION

The fact, that water is essential to all known forms of life cannot be over emphasized. The provision of clean drinking water and discharge of adequately treated wastewater is a fundamental requirement for human life [19]. man need water for industrial development, Irrigation to grow food, wash everyday item, sanitations needs, water makes up about 90% of cytoplasm, water is used as hydration to sustain health among others. However, inadequate safe drinking water, lack of proper sewage disposal system and drainage system and inadequate general environmental sanitation measures had resulted into a number of water diseases that claims millions of lives every year in developing countries [20]. Major factors affecting the microbial quality of surface water and underground waters are sewage disposal treatment surface runoff water, seepage from nearby sewage or septic tank [21]. Most wells are often highly contaminated were there are high concentration of physical and chemical parameter above the acceptable standard [22].

The results of this research revealed the *E. coli* count, the plasmid profile and the antibiotic sensitivity pattern of the *E. coli* isolates of the well water samples in Akure metropolis. This is to ascertain whether or not the well water is microbiologically safe for human consumption and other domestic use. According to the result obtained in this study it was revealed that of the four hundred samples well water sourced during the dry season, (76) well water sample representing 19% of the 400 sample sourced contain *E. coli* while 108 representing 27% 400 well water sample collected during the raining season contain *E. coli*. this corroborated the findings of [23]who reported 28.72% of *E.coli* occurrence in rain water in Ondo state. An indication that seasonal variation greatly has effect on the microbiological quality of the well water. The presence of this organism in the well water studied has an implication for public health. Species of this organism have been associated with human intestinal diseases [24]. However, this result is contrary or against WHO recommendation of zero *E coli* in 100ml water sample [25].

Highest recovery of *E. coli* from Isolo metropolis during the raining season is an indication that the water has been subjected to faecal contamination which may occur as a result of poor sanitation, closeness of the well to a pit latrine, poor sewage disposal systems, surface runoff and seepage from contaminated ground water and waste water [26]. [27,28,29,30]stated that water sources such as well and river serve as natural habitat for pathogenic *E. coli* strains that possess virulence factors that could cause gastrointestinal diseases. The virulence strains of *E. coli* are categorically divided into enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggretative *E. coli* (EAEC), enterohaemorrhagic *E. coli (*EHEC) and necrotoxigenic *E. coli* (NTEC) [31]. The antibiotic sensitivity patterns of the *E. coli* isolates obtained in this research reveals that larger percentage of the *E. coli* was resistant to one or more of the antimicrobial agent tested. [32] reported more than 50% isolates of vibro species to showed resistance against five commonly used antibiotics : ampicillin, ceftadizime, erythromycin, chloramphenicol, cefuroxime. The findings in the present study reveal high percentage of *E. coli* isolates being resistant to Amoxacillin (AM), Sulphamethoxazole (SXT), and Chloranphenicol (CH). [33] reported multiple resistance to antibiotic by strains of *E.coli*. [34] reported *E.coli* isolates to be resistant to antibiotic such as Amoxacillin (AM), Sulphamethoxazole (SXT) and chloramphenicol. The fact that some of these *E. coli* isolates show high level of resistance to some of the antimicrobial agents used is an indication that theses antibiotics have been abused or often used for the treatment of bacterial infection hence, the possibility of building resistance against the antimicrobial agent. [35] reported multi resistant pattern of the *E.coli* isolates of urinary tract infection.

The results from this research revealed that of all the *E. coli* isolates, isolates from well water obtained from Isolo revealed the highest level of resistance to one or more antimicrobial agent. This may also be attributed to the transfer of resistance gene (plasmid) from one organism to another since plasmids are easily acquired by organisms. The high microbial load of the well water in this metropolis may be a significant factor in transferring resistance gene from one organism to another. Resistance pattern may demonstrate multiple resistances to many antimicrobial agents and could have therapeutic consequences. The detection of many *E. coli* isolates resistant patterns was not unexpected. Resistance to antimicrobial agents is most common in areas with high usage of antibiotics such as hospitals [36]. The observed rare bacterial resistance to Trivid, Aumentin and pefloxacin has been attributed to the restricted use of the drug. [37]. The low toxicity of some of the antibiotics use in this research has resulted in the overuse in the medical community, hence the observed increased resistance. Plasmid profile analysis of 50 *E.coli* isolates by agarose gel electrophoresis showed a total of 48 different plasmid bands occuring in various combinations. [38] found 25 different plasmid bands in 63 *E. coli* isolates. The distribution of different plasmids among these isolates appeared to have been at random. The plasmid profiles were compared with reference DNA molecular weight marker (Hind III digest of Lambda DNA). After electrophoresis, the band size was estimated by careful eyes estimation. There was little interrelationship between the plasmid profile pattern

V. CONCLUSION

The microbiological analysis of the well water sample used in this research did not meet the recommended limits and could pose a serious health risk to consumers if used. This emphasizes the urgent need of Government intervention in the provision of safe water supply and provision of proper sanitation facilities for people living in Akure metropolis.

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