





# **Antibiotic Resistance Patterns of** *Salmonella spp* **from Clinical and Water Samples in Akure, Ondo State, Nigeria**

**A. K. Onifade<sup>1</sup> and O. I. Afolami<sup>1\*</sup>** 

*1 Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.*

## *Authors' contributions*

*This work was carried out in collaboration between both authors. Author AKO designed and supervised the study. Author OIA performed the statistical analysis, wrote the protocol, managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.*

## *Article Information*

DOI: 10.9734/AJRIMPS/2018/36277 *Editor(s):* (1) Dr. Aurora Martinez Romero, Professor, Department of Clinical Biochemistry, Juarez University, Durango, Mexico. (2) Dr. John Yahya I. Elshimali**,** Professor, Department of Pathology and Oncology, UCLA School of Medicine, Charles R. Drew University of Medicine and Science, California, USA. (3) Dr. Mario Ciampolini, Professor, (Rtd) Dept. of Pediatrics, University of Florence, Italy. *Reviewers:* (1) Sohad Mohamed Dorgham, Egypt. (2) Abhishek Kumar, Nitte University, India. (3) Onoriode Christian Eruteya, University of Port Harcourt, Nigeria. (4) Merih Kivanc, Anadolu University, Turkey. (5) Takashi Furukawa, Kitasato University, Japan. Complete Peer review History: http://www.sciencedomain.org/review-history/26316

> *Received 21 August 2017 Accepted 27 October 2017 Published 21 September 2018*

*Original Research Article*

## **ABSTRACT**

Investigations were carried out to determine the antibiotic resistance patterns of *Salmonella spp* isolated from clinical and water samples in Akure metropolis. Isolation of *Salmonella spp* was carried out using standard procedures and isolates were identified by various biochemical tests. Antibiotic sensitivity test was carried out on all the isolates of *Salmonella* identified against six antibiotics of standard concentrations (gentamycin, amoxicillin, ofloxacillin, tetracycline, ciprofloxacin and pefloxacin) using Kirby-Bauer test. A total of 61 strains of *Salmonella* from water samples and 79 strains of *Salmonella* from clinical samples were identified. Furthermore, a total of 20 multiple antibiotic resistant isolates (MDRIs) of *Salmonella spp* were screened from the water samples while 30 MDRIs of *Salmonella spp* were screened out from clinical samples respectively. A total of 30

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*\*Corresponding author: E-mail: afolamiolufemiifeoluwa@gmail.com;*

MDRIs of *Salmonella spp* were identified from clinical samples while 20 MDRIs of *Salmonella spp* were screened from water samples. High levels of antibiotic resistance were observed in MDRIs of *Salmonella spp* obtained. The results gave insights into the rising incidence of relapsing salmonellos is caused by MDRIs of *Salmonella* which was prevalent in the metropolis between July and October 2014 during which this research was conducted. Better antibiotic drug use control policies and public health education are encouraged for environmental health bio saftey of Metropolis.

*Keywords: Salmonella; multiple antibiotic resistances; kirby-bauer test; epidemiological surveillance; enteric relapsing salmonellosis; multiple-drug resistant isolates.*

## **1. INTRODUCTION**

*Salmonella species* are facultative anaerobes, gram-negative flagellated rod-shaped bacteria, about 0.4-0.6 μm in size [1,2]. The genus *Salmonella* is divided into six subspecies, which include *enterica, salamae, arizonae, diarizonae, houtenae* and *indica* [1-3]. Strains of *Salmonella*  are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann–White scheme; currently over 2500 serovars are recognized. The subspecies under *enterica* are responsible for several gastro-intestinal diseases; they include *typhi*, *typhimurium* and *paratyphi* A, B or C [1,4].

Salmonellosis is an infectious disease of humans and animals caused by organisms of the genus *Salmonella* [2-4]. Although primarily intestinal bacteria, *Salmonella* are present in the environment and may commonly be found in farm effluents, human sewage and in any material subject to faecal contamination [3-5]. Salmonellosis has been recognized in all countries, there are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide [5-7]. Salmonellosis can affect all ages, but the incidence and severity of disease is higher in young children, the elderly, and people who are immunocompromised or have debilitating diseases [6,7]. Many isolates are resistant to one or more antibiotics, and the choice of drugs should, if possible, be based on susceptibility testing [6-9].

*Salmonella* species are generally considered to be unable to ferment lactose and sucrose according to Bergey's Manual [2,4,5]. However, two separate species of *Salmonella* capable of fermenting lactose and sucrose have been reported [8,9,10]. The organism reported here again illustrates that certain strains of *Salmonella* are capable of fermenting lactose and sucrose rapidly and can resemble very a strain of *Citrobacter freundii* [9,10]. Comprehensive details of a close resemblance both

biochemically and serologically between *Salmonella tennessee* and *Citrobacter freundii* was described in the findings of Hamdan et al. [9], Anthony [10], Malkawi and Gharaibeh [11] Montville and Matthews [12]; the results of which were evidences that certain subspecies or serotypes of *Salmonella* can develop ability to degrade lactose due to a wide range of environmental or genetic factors such as mutation or natural selection to enable the organism survive outside its nutritional preferences [11,12].

Moreover, decades of indiscriminate use and abuse of antibiotics have resulted in increased development of antibiotic resistant *Salmonella spp* to different antibiotics, creating a major problem in treatment of salmonellosis and other enteric diseases [11-13]. There have been many cases of disease relapse and higher incidences in mortality of patients affected by salmonellosis as mortality rate can be as high as 20%, in the elderly; septicemia due to *Salmonella* has a 15% mortality rate [12-14]. Hence, it is therefore of great importance to conduct a study on the antibiotic resistant patterns of *Salmonella spp* in the Akure as this would help to ascertain the carrier rate of the resistant isolates from different sample sources, and also give a scientific insight into the prevalent relapsing salmonellosis resistant to antibiotic treatment in the Akure metropolis between July and October, 2014.

## **2. MATERIALS AND METHODS**

## **2.1 Study Area Description**

The Akure metropolis is found in Ondo State, Nigeria with coordinates 7°16' N 7°18' N/ 5°9' E 5°11' E [15]. It is located at the extreme southern region of Ondo State with an estimated population of about 750,954 inhabitants [15].

## **2.2 Sample Collection**

Two hundred samples were collected from both clinical and water sources in Akure between July and October, 2014. Out of these, a total of 80

human stool samples and 40 urine samples (clinical samples) were collected from seven different hospitals in all in the metropolis. The samples were collected by Medical Laboratory Scientists in the different hospitals, into labeled sterile universal bottles. A total of 80 water samples were collected into labeled sterile universal bottles from different water sources in different parts of Akure metropolis. All samples were collected into labeled sterile universal bottles using standard W.H.O guidelines, stored in ice packs and analyzed within 6hr of collection in the laboratory [16,17].

## **2.3 Ethical Considerations**

Ethical approval was obtained from the local health management authorities before clinical and water samples were obtained.

## **2.4 Sterilization of Materials and Media Preparation**

Glass wares were washed with detergents, rinsed and oven dried at 180°C for 2 h; forceps and wire loops were flamed to red-hot in a Bunsen flame, dipped in 70% ethanol [18]. Culture media, beakers, conical flasks, and other materials were autoclaved at 121°C for 15 minutes [18]. Incubators and inoculating<br>chambers were fumigated with 40% chambers were fumigated with formaldehyde, absolute ethanol and then irradiated with UV-lamp for 1 h [18]. Work benches were disinfected by cotton wool previously moistened with absolute ethanol [17,18]. Nutrient agar, *Salmonella* Shigella Agar and MacConkey agar were prepared with distilled water in separate conical flasks; the different mixtures which were further dissolved on a hot plate for 3 minutes and then sterilized by autoclaving at 121°C for 15 minutes [18].

## **2.5 Sample Preparation and Standardization of Inoculum**

The methods described by [Zvidzai et al. [2], Niemi et al. [4]; Hamdan et al. [9] and Mpenyana-Monyatsi et al. [1] were adopted in which sterile distilled water was used as diluents for the water samples, while sterile peptone water was used as diluents for stool and urine samples prior to analysis. A 1 ml of each stock was taken using a sterile syringe into 9ml of sterile distilled water or sterile peptone water for serial dilution procedure in sterile test tubes under aseptic conditions until four different dilutions were obtained. Hence, 1 ml of the dilution factors 3 and 4 were used to inoculate already prepared Nutrient Agar incubated for bacterial isolation at 37°C for 24 hours according to [16-18]. After the incubation time, the culture plates were observed for distinct colony forming units and thereafter, the fourth dilution factor was established as the standard for the isolation of the microbes due to easy numerical estimation of the colony forming units on the agar plates [Hamdan et al. [9] and Cheesebrough [18].

## **2.6 Sub-culturing, Characterization and Preservation of Isolates**

The methods described by [1,2,18] were adopted by subjecting the various obtained sub cultured distinct colonies to wide arrays of biochemical tests for characterization and identification. Gram staining technique, Catalase test, Motility test, Sugar fermentation (glucose, sucrose, lactose, mannitol and triple salt iron) tests, Methyl Red/VogesProskauer test, Oxidase test, Indole's test and Catalase test were carried out on the distinct isolates obtained after sub culturing [1,2,18]. The distinct biochemically characterized colonies were then further sub cultured on MacConkey Agar and Salmonella-Shigella Agar respectively; incubated at 37°C for 24 h [Fawole and Oso [19], Cheesebrough, [18]. Thereafter which the identity of the bacteria isolates was determined after their growth on these selective media. The identified pure isolates of *Salmonella spp* were preserved on Nutrient Agar Slants and stored at 4°C as described by Mpenyana-Monyatsi et al. [1], Zvidzai et al.[2], Niemi et al. [4], Cheesebrough [18], Fawole and Oso [19].

## **2.7 Antibiotic Sensitivity Test**

The Kirby-Bauer test, also known as disc diffusion method was used to determine the effect of standard antibiotics on bacterial isolates on Mueller Hinton agar; the agar was seeded with 18 hold pure broth cultures of *Salmonella* isolates for antibiotic sensitivity test as described in the methods of [Olutiola et al. [21]; Zanpantis and Hagravy, [20]; Cheesebrough, [18]. The discs were applied unto the seeded plates and incubated for 24 h at 37°C [18,20]. The bacterial isolates were tested against a wide range of standard antibiotic concentrations on antibiotic sensitivity discs namely: Gentamycin (10 µg) ofloxacillin (5 µg), amoxicillin (25 µg), ciprofloxacin (10 µg), tetracycline (30 µg), pefloxacin  $(5 \mu g)$  [18,20,21]. Thereafter, a ruler was used to measure the diameter of the clear zones of inhibition noticed on the plates and this

was noted as degree of antibiotic resistance as described in [20]. The isolates' zones of inhibition was classified into susceptible (17 mm and above) [18,20], intermediate (13 mm-17 mm) [18,20], and resistant (0-12 mm) [18,20] based on the specified standard of mean zone of inhibition for pathogenic gram negative bacilli [18,20,21].

## **2.8 Data Analysis**

Analyzed sample treatments were replicated thrice; data means obtained were subjected to a 2-way analysis of variance and treatment means were separated using Duncan's New Multiple Range test at  $P \le 0.05$  level of significance [1,8,9,19].

#### **3. RESULTS**

A total of 140 isolates of *Salmonella spp* were screened out from the 200 samples collected (Table 1 and 2); 79 *Salmonella spp* isolates from clinical samples (53 lactose fermenting *Salmonella spp* isolates and 26 non-lactose fermenting *Salmonella spp* isolates) and 61 *Salmonella spp* isolates from water samples (15 lactose fermenting *Salmonella spp* isolates and 26 non-lactose fermenting *Salmonella spp* isolates). *Salmonella spp* isolates from the sample sources analyzed were identified by various biochemical tests as represented in Table 3. The isolates zones of inhibition were subjected to statistical analysis using a 2-way ANOVA and means were separated by Duncan's New Multiple Range test at  $P \le 0.05$  levels of significance as represented in Figs. 1 and 2.

A procedure described in the findings of [Greene et al. [8] and Hamdan et al*.* [9] were used to evaluate Multiple antibiotic resistant isolates (MDRIs) from all the isolates of *Salmonella spp* obtained as isolates which are resistant to more than 3 of the 6 standard antibiotic used were screened out as MDRIs. Generally, the incidence of multiple antibiotic resistant isolates (MDRI) of *Salmonella spp* was slightly higher in clinical samples (38%: 30 out of 79 isolates) than in water samples (33%: 20 out of 61 isolates) as indicated in Tables 4 and 5. A total of 30 multiple drug resistant isolates (MDRIs) were screened out from clinical isolates of *Salmonella spp* while a total of 20 *Salmonella spp* isolates were MDRIs from water sources. The MDRIs have varying degrees of resistance to different antibiotics used. Ofloxacillin was discovered to be the most potent of the antibiotics to the multiple drug

resistant isolates from both clinical and water samples.

#### **Table 1. Distribution of isolates from water samples**



*Keys: LFSS—Lactose fermenting Salmonella spp, NLFSS--- Non-lactose fermenting Salmonella spp*

#### **Table 2. Distribution of isolates from clinical samples**



*Keys: LFSS—Lactose fermenting Salmonella spp, NLFSS--- Non-lactose fermenting Salmonella spp, AHAMU—apparently healthy adult male urine ages (18-45yrs), ADAMU apparently diseased adult female urine ages (18-45yrs), AHAFU—apparently healthy adult female urine ages (18- 45yrs), AHAMS—apparently healthy adult male stool ages (18-45yrs), ADAFU—apparently diseased adult female urine ages (18-45yrs), ADAMS—apparently diseased adult male stool ages (18-45yrs), AHAFS--- apparently healthy adult female stool ages (18-45yrs), AHMCS--- apparently healthy male children stool ages (6-17 yrs), AHFCS--- apparently healthy female children stool ages (6-17 yrs), ADAFS apparently diseased adult female stool ages (18-45yrs), ADMCS—apparently diseased male children stool, ADFCS apparently diseased female children stool ages (6-17 yrs), AHPWS—apparently healthy pregnant women stool ages (18-45yrs), ADPWS--- apparently diseased pregnant women* 

*stool ages (18-45yrs), AHagMS—apparently healthy aged male stool ages (55-80 yrs), ADagMS—apparently diseased aged male stool ages (55-80 yrs), AHagFS—apparently healthy aged female stool ages (55-80 yrs), ADagFS-- apparently diseased aged female stool*



#### **Table 3. Identification of** *Salmonella* **strains from clinical and water samples**

*Keys: NLFSWS—Non-lactose fermenting Salmonella spp from water samples, LFSWS—Lactose fermenting Salmonella spp in water samples, NLFSCS-- Non-lactose fermenting Salmonella spp in*  clinical samples, LFSCS-- Lactose fermenting Salmonella spp in clinical samples, -ve-Negative, +ve-Positive, Lac.- Lactose, Glu.- Glucose, Suc.- Sucrose, Mann.- Mannitol, TSI- Triple Salt Iron, O/C- Oxidase/ Catalase test, IND- Indole's test, MR/VP- Methyl red/ VogesProskauer, NA- Nutrient Agar, Mac. A.- MacConkey Agar, SSA-Salmonella-Shigella agar, N.I.- Number of isolates,

*K/NF- Alkaline slant/ No fermentation, K/H2S- Alkaline slant/ Hydrogen Sulphide produced, PCC—Pale coloured colonies, PCC+BC—Pale coloured colonies with black centers (indicating lactose fermenting isolates and H2S producers)*

#### **Table 4. The deduced antibiotic resistance patterns of MDRIs from clinical samples after estimation of their inhibition zones**



Key: SSADAMU-- Salmonella spp from apparently diseased adult male urine (ages 18-45 yrs), SSADAFU---Salmonella spp from apparently diseased adult female urine (ages 18-45 yrs), *SSADAMS--Salmonella spp from apparently diseased adult male stool (ages 18-45 yrs), SSADAFS---Salmonella spp from apparently diseased adult female stool (ages 18-45 yrs), SSADMCS--* Salmonella spp from apparently diseased male children stool (ages 6-17 yrs), SSADFCS---Salmonella spp from apparently diseased female children stool (ages 6-17 yrs), SSADPWS--Salmonella spp from apparently diseased pregnant women stool (ages 18-45 yrs), SSADagMS--Salmonella spp from apparently diseased aged male stool (ages 55-80 yrs), SSADagFS--Salmonella spp from *apparently diseased aged female stool (ages 55-80 yrs), MDRIs—Multiple antibiotic resistant isolates, R—resistant, S—susceptible, I—intermediate, OFL---Ofloxacillin, AMX----Amoxycillin, CPX--- Ciprofloxacin, TET---Tetracycline, PFX---Pefloxacin, , GEN---Gentamycin*

#### *Onifade and Afolami; AJRIMPS, 5(1): 1-10, 2018; Article no.AJRIMPS.36277*





*Key: SSWW— Salmonella sppfrom well water sample, SSTW--- Salmonella sppfrom tap water samples, SSAWW--- Salmonella sppfrom abattoir waste water samples, SSHDW-- Salmonella sppfrom household drinking water samples, SSDWW-- Salmonella sppfrom Drainage waste water samples, SSHWW--- Salmonella sppfrom hand washing water samples, SSPWW--- Salmonella sppfrom Plate washing water samples, SSRW--- Salmonella sppfrom River water samples, 1—resistant, 2—Susceptible, 3— Intermediate. Columns with short error bars have no significant difference at p≤0.05 level of significance*

*Onifade and Afolami; AJRIMPS, 5(1): 1-10, 2018; Article no.AJRIMPS.36277*



Error Bars: 95% CI

#### **Fig. 2. Statistical representation of the zones of inhibition of** *Salmonella* **isolates from clinical samples**

*Key: A—(SSAHAMU)--Salmonella spp from apparently healthy adult male urine ages (18-45 yrs), B—(SSADAMU)—Salmonella spp from apparently diseased adult female urine ages (18-45yrs), C—(SSAHAFU)—Salmonella spp from apparently healthy adult female urine ages (18-45yrs), D-(SSAHAMS)—Salmonella spp from apparently healthy adult male stool ages (18-45yrs), E-(SSADAFU)—Salmonella spp from apparently diseased adult female urine ages (18-45yrs), F-(SSADAMS)—Salmonella spp from apparently diseased adult male stool ages (18-45yrs), G-(SSAHAFS)---Salmonella spp from apparently healthy adult female stool ages (18-45yrs), H-(SSAHMCS)— Salmonella spp from apparently healthy male children stool ages (6-17 yrs), I-(SSAHFCS)— Salmonella spp from apparently healthy female children stool ages (6-17 yrs), J- (SSADAFS)—Salmonella spp from apparently diseased adult female stool ages (18-45yrs), K-(SSADMCS)—Salmonella spp from apparently diseased male children stool, L- (SSADFCS)—Salmonella spp from apparently diseased female children stool ages (6-17 yrs), M-(SSAHPWS)—Salmonella spp from apparently healthy pregnant women stool ages (18-45yrs), N-(SSADPWS)— Salmonella spp from apparently diseased pregnant women stool ages (18-45yrs), O-(SSAHagMS)—Salmonella spp from apparently healthy aged male stool ages (55-80 yrs), P-(SSADagMS—Salmonella spp from apparently diseased aged male stool ages (55-80 yrs), Q-(SSAHagFS)—Salmonella spp from apparently healthy aged female stool ages (55-80 yrs), R-(SSADagFS)— Salmonella spp from apparently diseased aged female stool,. Columns with short error bars have no significant difference at p≤0.05 level of significance*

<b>Codes of</b> <b>isolates</b>	<b>GEN</b>	OFL	AMX	<b>CPX</b>	TET	<b>PFX</b>	No. of MDRIS
SSAWW	1S. 1R	2S	2R	21	2I. 1R	1S. 1R	
<b>SSHDW</b>	1S.1R.1	2S. 1I	3R	1S. 2R	3R	31	
SSDWW	2S.1R.1	3S.1R.1	2S.2R.1I	5R	3I. 2R	2S. 3R	
<b>SSRW</b>	2S. 8R	6S.2R. 2I	4S. -6R	5S.2I. 3R	21.8R	4S.2I. 4R	10

**Table 5. The deduced antibiotic resistance patterns of** *Salmonella* **isolates from non-human samples**

*Key: SSAWW---Salmonella spp from abattoir waste water samples, SSHDW-- Salmonella spp from hand washing water*  samples, SSDWW--Salmonella spp from drainage waste water samples, SSRW--Salmonella spp from river water samples, *MDRIs—Multiple antibiotic resistant isolates, R—resistant, S—susceptible, I—intermediate, OFL---Ofloxacillin, AMX---- Amoxycillin, CPX---Ciprofloxacin, TET---Tetracycline, PFX---Pefloxacin, GEN---Gentamycin*

However, the lactose fermenting isolates were more generally resistant to used antibiotics than the non-lactose fermenting ones. The apparently<br>diseased adult males and females diseased adult males and females (ADAMS/ADFCS/ADMCS) were discovered to be chronic carriers of the lethal multiple antibiotic resistant isolates of *Salmonella spp* isolates obtained from the clinical sample sources, while river water and drainage waste water (RW/DWW) sources were the leading reservoirs for the multiple antibiotic resistant *Salmonella spp* isolates obtained from the water sample sources as indicated by Tables 4 and 5.

## **4. DISCUSSION**

Findings from the study have shown that, more lactose fermenting isolates are resident in human sources (clinical samples) than in non-human sources (water samples), this agrees with the findings of [Yoke-Kqueen et al. [22] and Greene et al. [8]; the high occurrence of the lactose fermenting *Salmonella* in human samples is possibly due to the increased exposure of human carriers of *Salmonella* isolates to lactose containing substrates of dairy products, resulting in nutritional adaptation of the isolates to the nutritional environment and hence ability to utilize and ferment lactose [5,9,22].

Subsequently, multiple antibiotic resistances were noticed in all the isolates ranging from each class of the human and non–human samples [8,9,23]. This is due to a variety of factors which includes indiscriminate use of drugs by human sample donors, faecal contamination of different water sources by human carriers of *Salmonella* where samples were collected from the metropolis, resulting in the introduction of drug resistant isolates of *Salmonella* to different water bodies. Thus, the spread of the multiple antibiotic resistant *Salmonella spp*. This was also implicated in a study carried out by Hamdan et al., Piu et al. and Zhao et al. [9,13,23].

In the findings of Zanpantis et al. Yoke-Kqueen et al. and Zhao et al. [20,22,23] pefloxacin and ofloxacillin were the choicest antibiotics for treatment of relapsing salmonellosis caused by multiple antibiotic resistant *Salmonella spp*; the results of this study affirm the susceptibility of antibiotic resistant isolates of *Salmonella* to pefloxacillin and ofloxacillin as these were the drugs with the highest susceptibility amongst the isolates tested for antibiotic sensitivity. Similarly, the zones of inhibition of isolates from water samples are generally lower than that of clinical samples; this indicates that high occurrence of MDRIs in water samples are connected to human faecal contamination of water sources in the metropolis, a correlation which was described in the findings of [1,2,10,22].

Furthermore, the prominent multiple antibiotic resistances noticed in all the *Salmonella* isolates obtained from the human sample sources gave a scientific insight into the reason for frequent relapses of salmonellosis amongst patients diagnosed across various health organizations in the metropolis between July and October, 2014. Similar findings were documented by Mpenya-Monyatsi et al., Zvidzai et al., Niemiet al. and Hamdan et al. [1,2,4,9] and multiple ranges of antibiotic combination therapies were strongly recommended.

## **5. CONCLUSION AND RECOMMENDA-TION**

The present study has given an insight into the different antibiotic susceptibility patterns of *Salmonella* isolates in the Akure metropolis; the incidence of multiple antibiotic resistant isolates and the high resistant rates of different isolates to standard antibiotics. The findings of this research also discovered that ofloxacillin and other antibiotic combination therapies are very effective against these multiple antibiotic

resistant isolates. Hence, adequate medical laboratory tests should be carried out on patients with suspected cases of typhoid fever or<br>salmonellosis before treatments are salmonellosis before treatments are recommended to them.

It is therefore recommended that public health education and awareness be given to peasants, artisans and patients about enteric diseases in Akure metropolis. Epidemiological surveillance should be mounted on food vending joints, major water sources and hospitals to reduce ingestion of contaminated food and water. Herd immunity of infants, aged and pregnant women should be implemented in primary health care centers to combat the lethal effects of the antibiotic resistant strains prevalent in the metropolis.

# **CONSENT**

It is not applicable.

# **ETHICAL APPROVAL**

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

# **REFERENCES**

- 1. Mpenyana-Monyatsi L, Onyango M, Momba M. Groundwater quality in south African rural community: A possible threat to public health. Policy Journal Environmental Study. 2012;21(5):1349- 1358.
- 2. Zvidzai C, Mukutirwa T, Mundembe R, Sithole-Niang I. Microbial community analysis of drinking water sources from rural areas of Zimbabwe. African Journal of Microbiological Research. 2007;1(6):100- 103.
- 3. Wright J, Gundry S, Conroy R. Household drinking water in developing countries: A systematic review of microbiological contamination between source and pointof-use. Tropical Medical Journal of International Health. 2004;9(1):106-117.
- 4. Niemi R, Mentu J, Siitonen A, Niemela S. Confirmation of *Escherichia coli* and its

destination from *Klebsiella spp*. by gas and indole formation at 44 and 44.5°C. Journal of Applied Microbiology. 2007;95:1242- 1249.

- 5. Boyen F, Haesebrouck F, Van Immerseel F, Pasmans F. Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis and control. Journal of Veterinary Microbiology. 2008; 130:11-22.
- 6. Byarugaba D. A view on antimicrobial resistance in developing countries and responsible risk factors. International Journal of Antimicrobial Agents. 2004;24: 105-110.
- 7. Gray J, Fedorka-Cray P. *Salmonella*: Foodborne diseases, San Diego: Academic Press. 2002;55-68.
- 8. Greene S, Stuart A, Medalla F, Whichard J, Hoekstra R, Chiller T. Distribution of multidrug-resistant human isolates of Mdr-Acssut *Salmonella typhimurium.* Newport, United States, 2003–2005. Foodborne Pathogen and Disease. 2008;5(5):669– 680.
- 9. Hamdan R, Musa N, Seong Wei L, Sarman A. Isolation and enumeration of coliform bacteria and *Salmonella spp*. from short necked clam East Coast, Malaysia. Internet Journal of Food Safety. 2008;10: 58-64.
- 10. Anthony BG. Emergence of lactose fermenting *Salmonella* in east Tennessee. Journal of Bacteriology. 1982;91:(4):1-3.
- 11. Malkawi H, Gharaibeh R. Rapid and simultaneous identification of two *Salmonella* enteric serotypes, Enteritidis and typhimurium from chicken and meat productsby multiplex PCR. Journal of Biotechnology. 2004;3(1):44-48.
- 12. Montville T, Matthews K. Food microbiology: An introduction (2<sup>nd</sup> ed.). United States of America: ASM Press, Washington. 2008;122-129.
- 13. Piu C, Wong W, Chai L. *Salmonella*: A food borne pathogen. International Food Research Journal. 2008;18:465-473.
- 14. Yousef A, Carlstrom C. *Salmonellos* is, a prevailing food borne infection. Food microbiology: A laboratory manual. New Jersey: John Wiley & Sons, Inc. 2003;167- 205.
- 15. National Bureau of Statistics (N.B.S.). Projected population of prominent Nigerian towns. 2011;337-341. Available:www.wikipedia.com
- 16. WHO (World Health Organization). Guidelines for Drinking Water Quality, Geneva, Switzerland, 1st edition. 2003;81-87.<br>17. WHO
- (World Health Organization). Drinking water quality of ground and surface water, Geneva, Switzerland. 2<sup>nd</sup> edition, 2010;121-134.
- 18. Cheesebrough M. District laboratory practice in tropical countries. Cambridge University Press, New York. 2010;157-164.
- 19. Fawole A, Oso O. The characterization of various bacteria pathogens from animal feed extracts in Abeokuta, Ogun State, Nigeria. Journal of Infection in Developing Countries. 2001;29(5):2-6.
- 20. Zanpantis E, Hagravy M. Modified antibiotic sensitivity of selected bacterial isolates using Kirby-Bauer test. University Press, New York. 2005;12-13.
- 21. Olutiola A, Musa B, Udoma H. Conventional and alternative methods for isolation and identification of bacteria from different sample sources: An overview. Handbook of Microbiological Laboratory Practice. 2001;11-12.
- 22. Yoke-Kqueen C, Learn-Han L, Noorzaleha S, Jiun-Horng S, Chai-Hoon K. Characterization of multiple-antimicrobial resistant *Salmonella enterica* isolated from indigenous vegetables and poultry in Malaysia*.* Letters in Applied Microbiology. 2007;46:318-324.
- 23. Zhao S, Datta R, Ayers S, Friedman S, White D. Antimicrobial-resistant Salmonella serovars isolated from imported foods. International Journal of Food Microbiology, 2003;84(1):87-89.

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