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Evaluation of the resistance pattern of *Escherichia coli* and *Salmonella typhi* isolates from University of Nigeria Teaching Hospital (UNTH), Enugu State University Teaching Hospital (ESUTH) and National Orthopaedic Hospital Enugu (NOHE) in Enugu State, Nigeria.

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ABSTRACT

The resistance pattern of *Escherichia coli* and *Salmonella typhi* isolates from University of Nigeria Teaching Hospital (UNTH), Enugu State University Teaching Hospital (ESUTH) and National Orthopaedic Hospital Enugu (NOHE) in Enugu State, Nigeria were evaluated. The study was conducted over a period of one and half years (from June 2017 to January 2019) and four hundred and fifty (450) stool samples from Out-patients and In-patients Departments of the hospitals were examined using the laboratory sections of the hospitals as the sampling points. *Escherichia coli* showed high resistance to Clindamycin and susceptible to Augumentin while *Salmonella typhi* showed high resistance to Chloramphenicol and susceptible to Imipenem. *Escherichia coli* showed high resistance to Amoxycillin and susceptible to Imipenem while *Salmonella typhi* showed high resistance to Imipenem and susceptible to Augumentin. In conclusion, the results of antibiotic susceptibility tests in this study showed high level of resistance among these isolates especially to ampicillin (100%), amoxicillin (80%) and tetracycline (70%) making them completely unreliable in the management of *Escherichia coli* and *Salmonella* spp. associated diarrhea in the study area.

Keywords: Resistance pattern, *Escherichia coli*, *Salmonella typhi* and isolates.

INTRODUCTION

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives [1,2,3,4]. Many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat [5,6,7]. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [8,9]. The Centers for Disease Control and Prevention (CDC) has classified a number of bacteria as presenting urgent, serious, and concerning threats, many of which are already responsible for placing a substantial clinical and financial burden on the U.S. health care system, patients, and their families [10,11]. Indiscriminate use of antibiotics in our populace particularly in cities invariably leads to development of resistance to these drugs by micro-organisms [12,13,14,15,16]. Also the delays in referring patients to tertiary hospitals from primary and secondary health institutions contribute to a large extent in the development of resistance, which brings about complications in the ailments and difficulties in combating the diseases.

Aim and Objective of the study

The aim of this study was to evaluate the resistance pattern of *Escherichia coli* and *Salmonella typhi* isolates from University of

Nigeria Teaching Hospital (UNTH), Enugu State University Teaching Hospital (ESUTH) and National Orthopaedic Hospital Enugu (NOHE) in Enugu State, Nigeria.

MATERIALS AND METHODS

Study Area

Enugu, usually referred to as Enugu State to distinguish it from the city of Enugu is a state in Southeastern Nigeria, created in 1991 from part of the old Anambra State. Its capital and largest city is Enugu, from which the state derives its name. Enugu State is one of the states in eastern part of Nigeria located at the foot of the Udi Plateau. The State shares borders with Abia State and Imo State to the south, Ebonyi State to the east, Benue State to the Northeast, Kogi State to the Northwest and Anambra State to the West. The principal cities in the state are Enugu, Nsukka, Agbani and Awgu. It has a total population of 3,267,837 individuals according to 2006 census and population density of 460/km2 with coordinates of 60 30'N 70 30'E; total land area of about 7,161 km2. Economically, the state is predominantly rural and agrarian, with substantial proportion of its working population engaged in farming, although trading (18.8 %) and civil services (12.9 %) are also important. In the urban areas trading is the dominant occupation, followed by civil services. A small proportion of the population is also engaged in manufacturing activities, with the most pronounced, among them located in Enugu, Oji, Ohebedim and Nsukka. It has some tertiary health institutions and numerous private hospitals and clinics. In the state, there are seven District Hospitals in Enugu – Urban, Udi, Agbani, Awgu, Ikem, Enugu Ezike, and Nsukka and at least one health centre or cottage hospital in each of the 17 Local Government Area of the State [9]. The study areas were three tertiary health institutions in Enugu State. The study area covered the three senatorial zones (Enugu East, Enugu North and Enugu West) of the state and the hospitals serve as a major referral points in the state.

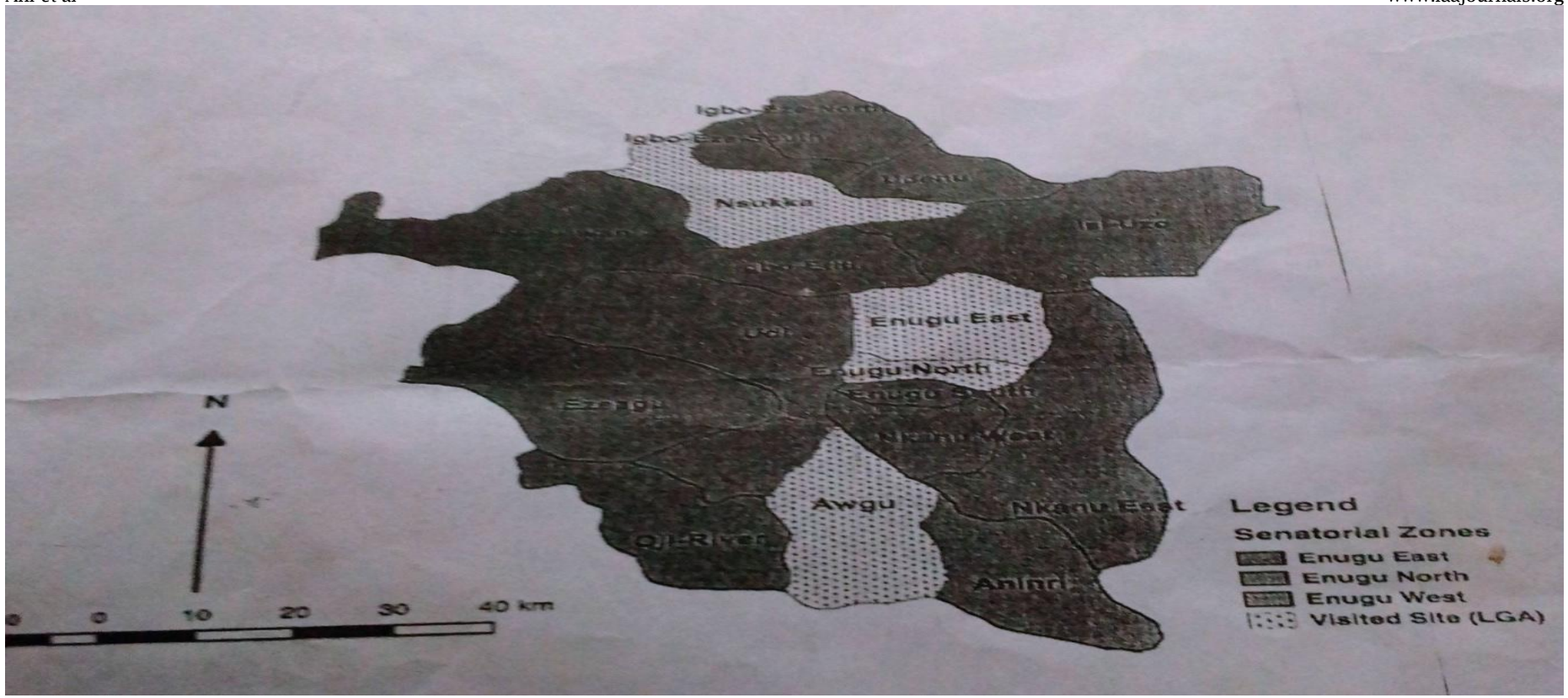


Figure 1: Map of Enugu State showing the three senatorial zones and the L.G.As of the study sites [5].

Study Population

The samples used were sourced from these tertiary hospitals in Enugu State. Parklane Teaching Hospital, National Orthopeadic Hospital and University of Nigeria Teaching Hospital.

Study Period

The study was conducted over a period of one and half years (from June 2017 to January 2019) and four hundred and fifty (450) stool samples from Out-patients and In-patients Departments of the hospitals were examined using the laboratory sections of the hospitals as the sampling points.

Ethical Clearance

Ethical clearance was granted by the Joint Committee on Human Research Publications and Ethics of the hospitals – vide: UNTH/CSA/329/VOL.5, ESUTHP/C-MAC/RA/034/Vol. II and NOHE S/313/IX/963.

Determination of Sample Size

The sample size was calculated using Daniel's standard size determination technique according to the following equation [2]. The formula $n = (Z_1 -\alpha)^2 (P (1-P)/d^3$ was used to calculate the sample size, where P is the estimated proportion and d is the desired precision (Suresh and Chandrashekara, 2012). The sample size was calculated using an approximate prevalence rate of 50%, confidence interval of 95 % and precision of 5 %. The value of $Z_1-\alpha$ at 5% level of significance was 1.96. Using the formula with provision for 10 % attrition, a sample size of 450 (150 from each hospital and from patients with enteric diseases) was used.

Sterilization of Glassware/Media/Equipment

All glass wares used were washed and sterilized in hot air oven at temperature of 160^{0}C for 1hr according to the standard method described by Yashmir (2007). Culture media were reconstituted and sterilized at 121^0 C for 15 mins using an autoclave. Metallic equipment (spatula, wire loop, Bunsen burner, etc) were sterilized under flame for 2-5 mins and the work bench was sterilized using 70 % ethanol.

Media Preparation

All media used include: MacConkey agar, Nutrient agar, Mueller Hinton agar, Nutrient broth, Cystine-lactose electrolyte-deficient agar (CLED) and *Salmonella Shigella* Agar.

Preparation of Peptone water

A 3.6 g of peptone water was weighed and dissolved in distilled water in a sterile flaks and shaken to enhance homogeneity after which was dispensed into sterile test tubes and covered with absorbent cotton wool wrapped with an aluminum foil. The neck of the conical flask was tied firmly with a masking tape, sterilized by autoclaving at 121^0 C for 15 min at 15 psi and allowed to cool to 45^{0}C before use [9].

Preparation of Nutrient Agar/Slants

The nutrient agar used was prepared by weighing and dissolving 14 g of nutrient agar powder in 500ml of distilled water in 1000ml conical flask. This was properly mixed and heated under flame to dissolve. Then it was sterilized by autoclaving at 121^{0}C for 15 min at 15psi and allowed to cool to 45^0 C before pouring 20ml and 8ml each into sterile Petri dishes and bijou bottles respectively. They were left on the bench to solidify and then incubated at 37^{0}C for 24 hr to ascertain sterility.

Preparation of Normal Saline Solution

Normal saline solution was prepared by dissolving 8.5 g of sodium chloride (Sigma-Aldrich, Co., USA) into sterile distilled water and made up to 1L, mixed well and sterilized by autoclaving at 121^0 C for 15 min and cooled at 45^0 C and the solution was ready for use.

Sample Collection, Transportation and Culturing

Clinical samples (450 stools) were collected from diarrhea patients (newly hospitalized patients and out-patient) both adults and children (0 – 51 and above) using sterile container (wide mouth plastic container with screw cap). The sterile containers were distributed to the patients and taken from them after they had put in their stools and transported under ice with sterilized container labeled with study number. These samples were stored in a refrigerator and processed within 1hr of collection. A loopful of the specimen was homogenized first with normal saline and inoculated into 5ml double strength nutrient broth and incubated for 24 h at 37^{0}C prior to sub-culturing unto solid agar (Nutrient, MacConkay and SS agar) plates to obtain discrete cultures. Pure cultures were obtained by sub-culturing discrete colonies in fresh agar plates.

Microbial Identification and Characterization

The bacteria isolates were identified using the morphological appearance (macroscopy) of their colonies, their Gram stain reaction (microscopy), and confirmatory biochemical and sugar fermentation tests.

Antibiotics Susceptibility Testing

Antibiotics susceptibility tests were performed following the Kirby Bauer disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards [7].

Standardization of Test Organisms

All bacterial isolates were standardized before use. A loopful of the test bacteria from the nutrient agar slant was inoculated into 5 ml nutrient broth in a sterile test tube and incubated. A loopful of the nutrient broth culture was then diluted with 5 ml sterile water to obtain microbial population of 10^5colony forming unit per milliliter (CFU/ml), equivalent to 0.5 McFarland standards by incubating at temperature of 37^0C for 3 hours.

Determination of Susceptibility/Resistance pattern of the test Organism

The standardized inocula were streaked onto Mueller Hinton agar plate and allowed to stay on the bench for 30 minutes, before the discs were placed on the inoculated plates and pressed firmly onto the agar plate for complete contact. The bacterial strains were tested against the following antibiotics (Cephalosporin) discs; Nitrofurantoin (NIT 100μg), Ceftazidine (Caz 30μg), Gentamicin (10μg), Clindamycin (300μg), Tetracycline (30μg), Amoxicillin (30μg), Augumentin (30μg), Pefloxacin (30μg), Clarithromycin (30μg),Chloramphenicol (10μg), Ofloxacin (10μg) and Imipenem (10μg). The plates were inverted and left on the working table for 30 minutes to allow for pre diffusion of antibiotics into the agar. The plates were incubated at 37^0C for 24 hours. The susceptibility of each isolate to each antibiotic was shown by a clear zone of growth inhibition and this was measured using a meter rule in millimeters and the diameter of the zones of inhibition interpreted using standard chart [10]. The susceptibility test was set in triplicate for all the isolates and the average recorded.

Statistical Analysis

Statistical analysis of all the data was done using ANOVA statistics at 0.05 level of significance.

RESULTS

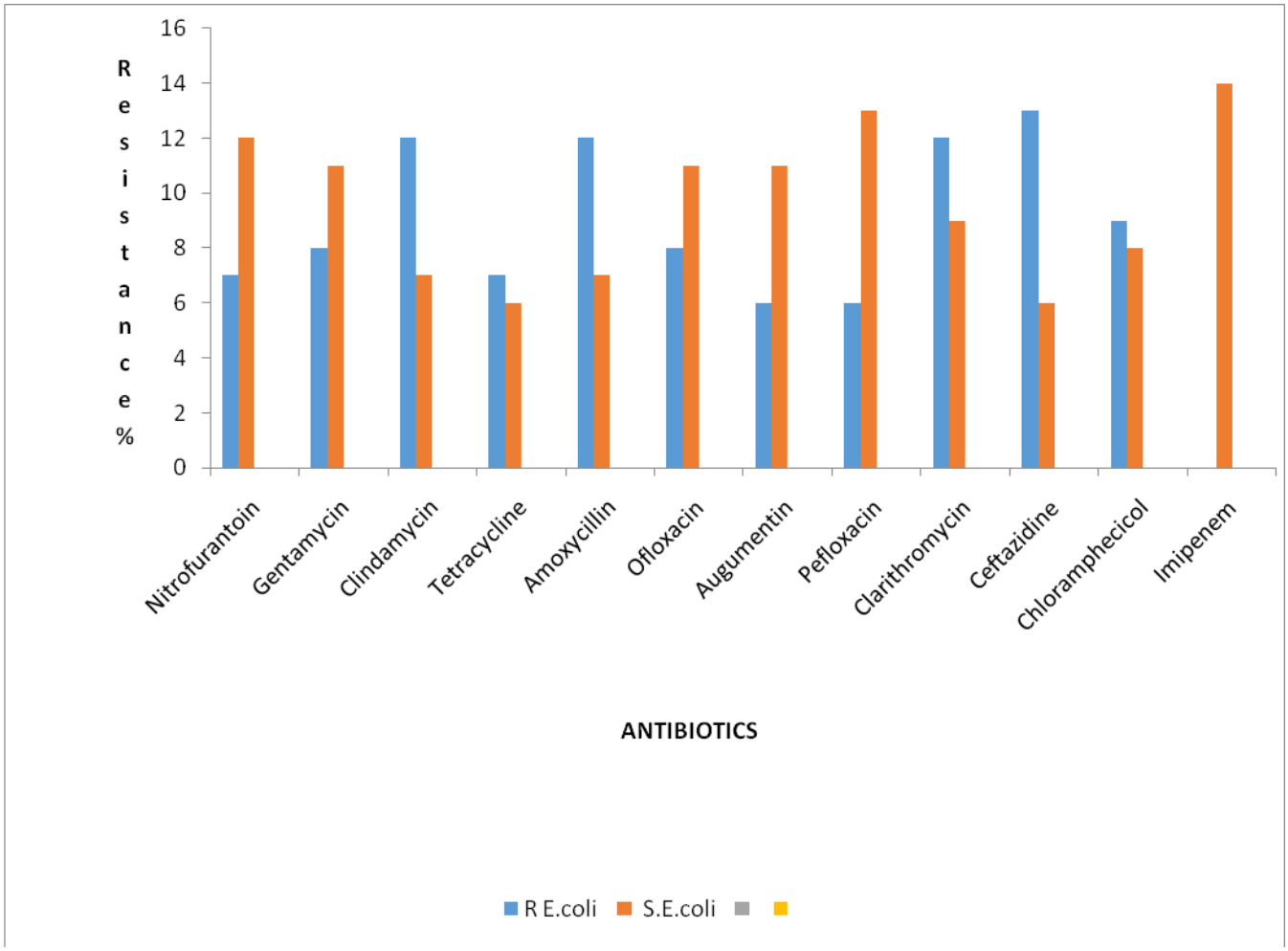
The result shown in Table 1 represents the summary of the cultural, microscopic, biochemical and sugar fermentation tests for the characterization of *Escherichia coli* and *Salmonella typhi* from stool sample.

Table 1: Identification and characterization of *Escherichia coli* and *Salmonella typhi* from Stool Samples of the Three Hospitals in Enugu State.

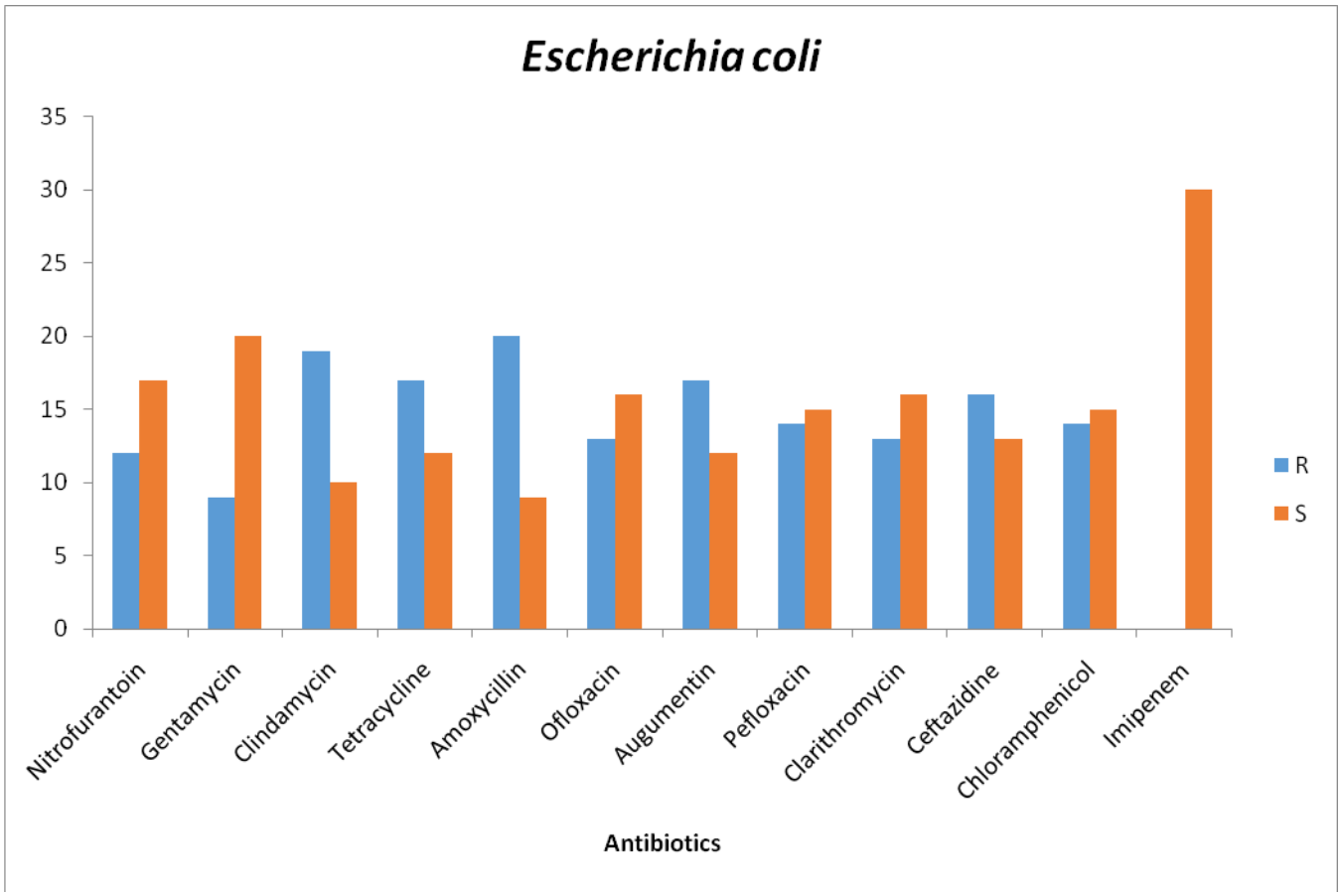
Colony morphology	Gram Stain		Biochemical Test								Sugar Fermentation				Suspected Organism
	Gram Reaction	Cell Morphology	Cat.	Oxi.	Cit.	M.R	VP	Ind.	Ure.	Mot	Glu.	Lac.	Mal.	Suc	
White, small, smooth, circular colonies	-	Rods	+	-	-	+	-	+	-	+	+	+	-	v	*Escherichia coli*
Pale, smooth, yellow, circular colonies	-	Rods	+	-	+	+	-	+	+	+	+	-	+	-	*Salmonella typhi*

Key: cat-catalase, oxi- oxidase, cit- citrate, M,R- methyl red, VP-voges proskaeur, ure- urease, Ind- Indole, Mot- Motility, Glu- Glucose, Lac-Lactose, Mal-Maltose and Suc-Sucrose.

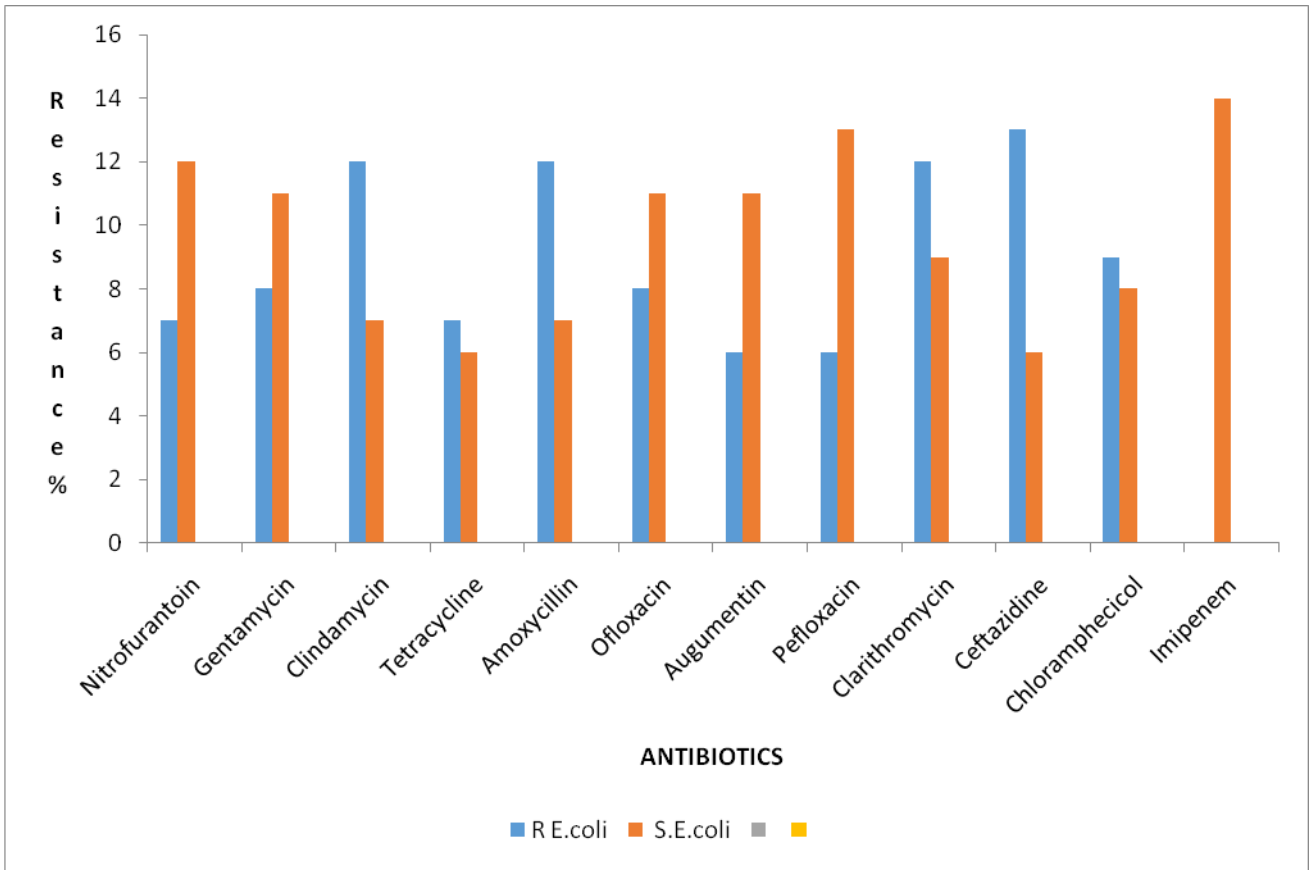
Overall resistance pattern of *Escherichia coli* and *Salmonella typhi* isolates from the various hospitals were represented graphically in Figures 2, 3 and 4.



Key: R= Resistant, S= Sensitive.
Fig 2: Pattern of resistance of *Escherichia coli* and *Salmonella typhi* isolates from ESUTH to antibiotics.
Escherichia coli showed high resistance to Clindamycin and susceptible to Augumentin while *Salmonella typhi* showed high resistance to Chloramphenicol and susceptible to Imipenem.



Key: R= Resistant, S= Sensitive.
Fig 3: Pattern of resistance of *Escherichia coli* isolates from UNTH to antibiotics.
Escherichia coli showed high resistance to Amoxycillin and susceptible to Imipenem.



Key: R= Resistant, S= Sensitive.
Fig 4: Pattern of resistance of *Escherichia coli* and *Salmonella typhi* isolates from NOHE to antibiotic.
Escherichia coli showed high resistance to Amoxycillin and susceptible to Imipenem while *Salmonella typhi* showed high resistance to Imipenem and susceptible to Augumentin.

DISCUSSION

This study indicated that *E. coli* isolates showed high resistance rate to clindamycin (Figure 2). This finding is in agreement with reports from Nigeria [12,13,14]. The antibiotics sensitivity pattern shows high sensitivity to ciprofloxacin and chloramphenicol which is similar to study conducted in Lagos, Nigeria by [15,16]. In addition, high sensitivity of Salmonella spp. isolates to chloramphenicol and ciprofloxacin observed in this study is similar to reports by other authors from Katsina, Nigeria [9]. These increases in resistance may be attributed to the widespread misuse of this drug, coupled with the fact that they are cheap and people can purchase these drugs from the open market in the study area without physician prescription. Increased and high susceptibility to chloramphenicol might be attributed to the cost of the antibiotics, it may be expensive compared to ampicillin, amoxicillin and tetracycline and cannot be easily afforded by majority of the people from the study area, therefore this may have contributed to the effectiveness of the chemotherapeutic agent in treatment of diarrhea caused by *Salmonella* spp. High resistance of bacteria (*E. coli* and *Salmonella* spp.) to beta lactam antibiotics observed in this study (Figure 4) is also in agreement with high resistance of bacteria to beta lactam antibiotics reported by [3] from non-clinical samples in a study conducted in Nigeria. In addition, occurrence of tetracycline and clarithromycin resistance among MDR bacteria (*E. coli* and *Salmonella* spp.) observed in

this study (Figure 3) is similar to tetracycline resistant MDR and aminoglycoside resistant MDR bacteria reported by [9,10] respectively from environmental samples in a study conducted in southwestern Nigeria. In Ghana [9] reported multidrug resistance in 30 of 58 isolates based on their resistance to three out of the five antibiotics tested. MDR *S. typhi* was also reported in a study by [11] in the Democratic Republic of Congo, in Togo [12] and on the Malawi–Mozambique border (2012). This might be explained by the fact that indiscriminate use of antibiotics may have contributed to vast emergence and spread of multidrug resistant bacteria from both clinical and non-clinical environment. With the vast increase of resistance to commonly used antibiotics associated with the studied bacteria, antidiarrheal agent could be considered as an effective alternative medication to antibiotics with respect to diarrhea diseases. However, susceptibility of bacteria isolates to chloramphenicol and fluoroquinolones (ciprofloxacin) observed in this study, coupled with its increased effectiveness in *in-vitro* antibiotic susceptibility profile reported by [6] in Ethiopia, [8] in Kenya, and [6] in Nigeria and [11] who worked on Antibiotic Susceptibility profile of *Escherichia coli* and *Salmonella* causing Diarrhoea from South Eastern Nigeria, among others showed that these chemotherapeutic agents could be effective in treatment of *Escherichia coli* and *Salmonella* species associated diarrhea in those regions.

CONCLUSION

In conclusion, the results of antibiotic susceptibility tests in this study showed high level of resistance among these isolates especially to ampicillin (100%), amoxicillin (80%) and tetracycline (70%) making them completely unreliable in the management of *Escherichia coli* and *Salmonella* spp. associated diarrhea in the study area. It was also observed that 15% of bacteria displayed multidrug resistant characteristics. Hence, comprehensive studies are needed for the determination of the molecular epidemiology of these resistant bacteria for public health surveillance.

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