



Prevalence and Antibiotic Resistance Profiles of Serotypes of *Shigella* Species isolated from Community Children in Odeda Local Government, Ogun State.

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Abstract

Shigellosis (bacillary dysentery) has been a menace that has eaten so deep into the bone marrow of children's health majorly in developing countries worldwide. The symptom ranges from mild diarrhoea to severe dysentery. The infection is of general health concern which is common in communities with inadequate and proper hygiene, lacking portable water. This study therefore addressed the preponderance and antibiotic resistance profiles of different serotypes of *Shigella* species gotten from stool samples of 248 school children among selected communities in Odeda Local Government Area of Ogun State, Nigeria. The stool samples were cultured in Glucose nutrient broth and later sub-cultured for *Shigella* species using MacConkey, Desoxycholate citrate agar, *Salmonella-Shigella* agar. The obtained bacterial isolates were phenotypically characterized using morphological and biochemical methods. Antibiotic susceptibility of isolates was performed by Kirby-Bauer's disc diffusion method while their Multiple Antibiotic Resistance index (MARI) was determined in addition to their hemolytic pattern on blood agar. Data obtained were methodically examined and analyzed using One-way Analysis of Variance (ANOVA). From the 248 stool samples cultured, 275 bacteria of different categories were isolated of which *Shigella spp* was isolated in 10 samples representing 4.03 % prevalence. The *Shigella* isolates revealed susceptibility of 80 % to Pefloxacin, Ciprofloxacin, Ofloxacin and 100 % resistance to Augmentin, Nitrofurantoin and Amoxycillin. High Multiple Antibiotic Resistance index greater than 0.2 in all the *Shigella* isolates while 4 out of ten isolates showed partial α -hemolytic reaction representing 40 % and the remaining 6 representing 60 % showed γ - hemolytic reaction. The low prevalence of *Shigella* isolates was not significant with sex, age group, social stratification, religion and educational level of the primary school learners ($P>0.05$). The need for urgent preventive measures, regulation of antibiotic usage and enforcement of safety hygiene is advocated.

Keywords: Antimicrobial Resistance, *Shigella spp*, Prevalence, Community Children, Public Health

1 Introduction

Diarrhoea can be defined as having loose or watery stools, at least 3 times a day or more frequently than normal for an individual [1, 2]. Most importantly, much cases of childhood diarrhoea are mild, less chronic cases can lead to a great fluid loss and dehydration which may unfortunately result in death or some other severe negative outcomes if the fluids are not replaced and replenished at the first sign

of diarrhoea [1], although local drinks such as fermented palm wine has shown antibacterial activity against diarrhoeagenic bacteria e.g. *Shigella dysenteriae*, therefore it can be seen as an alternative measure for the control of the diarrhoea produced by these organisms in the absence of antibiotics [3]. Shigellosis caused by different species of *Shigella spp* (*S. flexneri*, *S. dysenteriae*, *S. boydii* and *S. sonnei*) all belong to the *Enterobacteriaceae* family which is a crucial cause of worldwide mortality and morbidity in humans.

Bastos and Loureiro, [4] reported it to be a disease of general health importance in developing countries causing self-limited diarrhoea to severe dysentery and it is majorly a disease of impoverished, crowded and in-conducive communities that do not have access to good sanitation or

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portable drinking water [5]. Clinical intestinal manifestations such as extra-intestinal signs which include bacteremia or neurologic manifestations are always produced [6]. Barman *et al.*, [7] and Vinh *et al.*, [8] indicated also, that among the virulent *Shigella* serotypes, *S. sonnei* is frequently implicated for epidemic enteritis in developed countries while *S. boydii* is the most dominant strain in Low-middle income countries (LMIC) [9]. Although shigellosis is one of the severe gastroenteritis throughout the world, it has been found to co-infect human beings with other gastrointestinal pathogens such as *Entamoeba histolytica*, *Campylobacter spp*, *Salmonella*, *Enteropathogenic Escherichia coli* (EPEC), *Aeromonas hydrophyla* and *Bacillus cereus*.

Diarrhoea is a menace in Sub-Sahara Africa and the prevalence rate of diarrhoea in Nigeria stood at 18.8 %; resulting to 150,000 deaths annually amongst young children under the age of five as a result of poor sanitation and unhygienic practices. Diarrhoea ranked the second leading cause of children mortality under five, which is rampant due to poor sanitary practices [2]. *Shigella species* are highly infective, particularly *S. dysenteriae* known as the most virulent and have the ability to produce a potent cytotoxin known as “Shiga toxin” [10, 11]. *S. dysenteriae* type 1 cause severe and sometimes fatal disease, *Shigella* species recently have been reported to be the most identified agent of laboratory-acquired infections because of their high virulence and low infectious dose [12]. An enormous number of laboratory acquired infections have

been reported. Infection may be acquired through intake or accidental peritoneal inoculation or contact with contaminated fomites within community school [13-15].

A report about the progressive increase in antibiotic resistance among enteric pathogens in Low Middle Income Countries has been well documented [16], which might be as a result of geographical differences, different patterns of antibiotic usage and environmental factors [17]. In Nigeria, though cases of bacillary dysentery due to *Shigella* species have been presented, it is not comprehensive and significant enough; hence it is therefore imperative to investigate the prevalence rate and antibiotic susceptibility of *Shigella spp* in school children within Odeda L.G.A of Ogun state and also determine the Multiple Antibiotic Resistance index (MARI) of the *Shigella* isolates.

2 Methodology

2.1 Study Area/Site

This study was performed and carried out in selected public primary schools within Odeda L.G.A of Ogun state. The primary schools include

- I. Odeda L.G.A school, Bode Olude with coordinates
- II. Odeda L.G.A school, Ilupeju Agbaakin with coordinates
- III. Odeda L.G.A school I&II, Obantoko with coordinates
- IV. Methodist Primary school, Odeda with coordinates

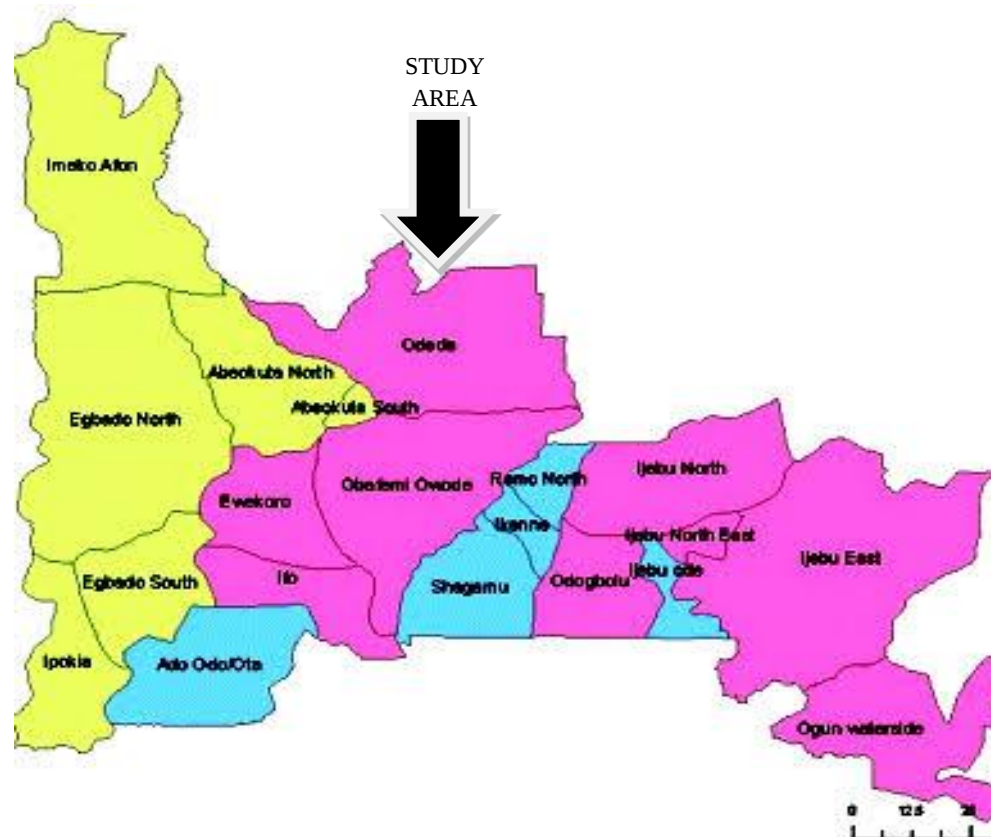


Figure 1: Map of Local Governments in Ogun state

2.2 Study Population

This included children, attending and schooling at different government owned primary schools in selected communities within Odeda L.G.A of Ogun State.

2.3 Inclusion criteria

Children currently registered and attending government owned primary schools within Odeda L.G.A.

2.4 Exclusion criteria

Children currently not registered and not attending government owned primary schools within Odeda L.G.A.

2.5 Ethical and Consent approval

Ethical approval was obtained from the Ethics Committee of the Ogun State Ministries of Health and Education, Science and Technology (Ref No: PL19/VOLIV/18). The Head teachers gave their consent after giving them the information about the study. Consent forms were signed by stakeholders, Parents and Guardians of pupils and the Head Teachers of the selected schools.

2.6 Sample size

Sample size was determined based on the population of pupils within selected communities. The following number of stool samples was collected from school children in the selected communities:

Bode olude- 33 samples
 Ilupeju Agbaakin- 38 samples
 Alabata- 33 samples
 Obantoko- 82 samples
 Odeda- 62 samples

2.7 Sample Collection

Sample collection entails the collection of 248 samples of stool which were collected from the pupils. The samples were gathered into transparent, big-mouthed and sterile bottles (Universal bottles). The biodata of the pupils such as name, age and sex of the pupils were properly labeled on the universal bottles. After collection, the stool samples were transported to the laboratory using a transport medium and processing was done within 2 hours of collection. The pupils' other biodata information was obtained by administering a semi-structured questionnaire.

2.8 Sterilization of materials

Proper sterilization of the Materials/Glass-ware to be used was done by washing, air-drying and sterilizing in a hot-air oven at 150°C for one hour. The media used were sterilized in an autoclave at 121°C for 15 minutes. The bench work areas were swabbed with alcohol before microbiological analysis was carried out in order to avoid contamination.

2.9 Characterization of the bacterial isolates

Bacterial isolates were identified and depicted morphologically using the cultural characteristics (size, shape, and edge, texture, elevation, degree of opacity, and color) of each colony and biochemically examined for further identification [18].

2.10 Antibiotics Susceptibility Test

Antibiotic sensitivity tests were carried out on all the *Shigella* isolates using disc diffusion technique [19, 20]. Mueller Hinton's agar prepared plates were inoculated with the standardized inoculums of the overnight pure bacteria culture. After inoculation, appropriate Gram negative antibiotic sensitivity discs were placed aseptically and incubated at 37°C for 24 h; the diameter of the zone of inhibition were measured after incubation and compared with zone diameter interpretative chart by Clinical and Laboratory Standard Institute [19].

Ten various antibiotics with different disc concentration such as Ceftriaxone (CRO) 30µg/disc, Cotrimoxazole (COT) 25µg/disc, Gentamycin (GEN) 10µg/disc, Tetracycline (TET) 30µg/disc, Ciprofloxacin (CPX) 10µg/disc, Augmentin (AUG) 30µg/disc, Amoxicillin (AMX) 25µg/disc, Ofloxacin (OFL) 5µg/disc, Pefloxacin (PFX) 5µg/disc, Nitrofurantoin (NIT) 20µg/disc were used for this study.

2.11 Multiple Antibiotic Resistance (MAR) Index Determination

The Multiple Antibiotic Resistances (MAR) Index was calculated for each isolate as shown in the equation below. MAR index is the number of antibiotic(s) to which the organism is resistant divided by the total number of antibiotics tested [21].

$$\text{MARI} = \frac{\text{Number of Antibiotic (s) to which isolate was resistant}}{\text{Total number of antibiotics tested}}$$

2.13 Data Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 for windows (SPSS, Chicago IL and USA) into simple percentiles and test for significance. The level of significance was considered as $P < 0.05$.

3 Results and Discussion

3.1 Percentage distribution of sample collection and other bacteria species

Table 1 revealed Stool samples collected from Primary school children in the three zones (Opeji, Ilugun and Obantoko) of Odeda L.G.A; Ogun State. Table 2 shows the Religion of primary pupils with Christian scoring the highest percentage of 53.2 %, Muslim having a percentage of 46 % and other religion having a percentage of 0.8 %. Of the 248 faecal samples cultured, 10 (4.03 %) *Shigella* species was isolated alongside other bacteria species. Other bacteria of medical importance isolated include *Escherichia coli* 126 (45.8 %), *Salmonella* species 41 (14.9 %), *Pseudomonas aeruginosa* 37 (13.5 %), *Enterobacter cloaca* 26 (9.5 %), *Proteus vulgaris* 19 (6.9 %) and *Citrobacter freundii* 16 (5.8 %) as displayed in Table 3.

3.2 Percentage distribution of gender of the pupils

Figure 1 revealed the distribution of pupils according to Gender, with female having the higher occurrence/percentage at 51% and Male at 49%.

Table 1: Locations of sample collection in Odeda Local Government

Locations	Number	Percentage
Opeji	104	41.9
Obantoko	82	33.1
Ilugun	62	25
Total	248	100

Table 2: Religion of the pupils

Religion	Number	Percentage
Christian	132	53.2
Islam	114	46
Others	2	0.8
Total	248	100.0

Table 3: Percentage distribution of other bacteria species isolated from the pupils

	Number	Percentage
<i>Escherichia coli</i>	126	45.8
<i>Salmonella species</i>	41	14.9
<i>Shigella species</i>	10	3.6
<i>Pseudomonas</i>	37	13.5
<i>Enterobacter cloaca</i>	26	9.5
<i>Proteus vulgaris</i>	19	6.9
<i>Citrobacter freundii</i>	16	5.8
Total	275	100.0

3.3 Percentage distribution of age group of the pupils

Figure 2 revealed the Age group distribution of the pupils with Age group 5-7 having the maximum percentage of 33%, followed by Age group 8-10 having a percentage of 32.2%, Age group above 10 having 21.8%, and Age group 2-4 having the least or minimum percentage of 12.9%.

3.4 Percentage distribution of educational level of the pupils

Figure 3 shows the percentage analysis of the educational level of the pupils with Primary pupils having the maximum percentage of 73.4 %, followed by Kindergarten (KG) pupils having a percentage of 22.2 %, while Nursery pupils had the least percentage of 4.4 %.

3.5 Biochemical delineation of *Shigella* species

Table 4 shows the biochemical characterization and delineation of *Shigella* species isolated from stool samples collected from the pupils. Five (5) isolates were confirmed to be *S. flexneri*, three (3) were given to be *S. boydii* and two (2) were *S. sonnei*. Plate 1 reveals the biochemical reaction of *Shigella* isolates on Triple Sugar Iron (TSI) agar having a Red slant (Alkaline) and Yellow butt (Acidic). No single isolates had black coloration which indicated that Hydrogen sulphide (H_2S) was not produced.

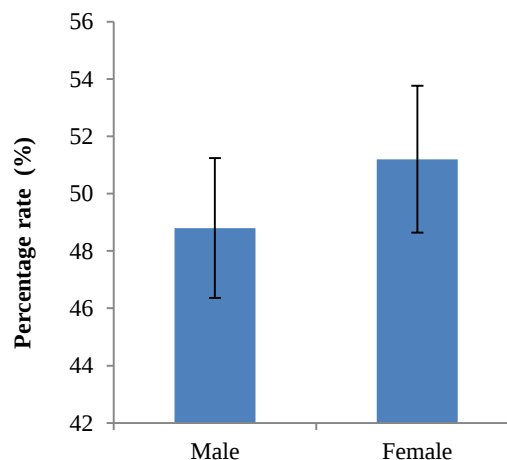


Figure 1: Distribution of pupils according to their gender.

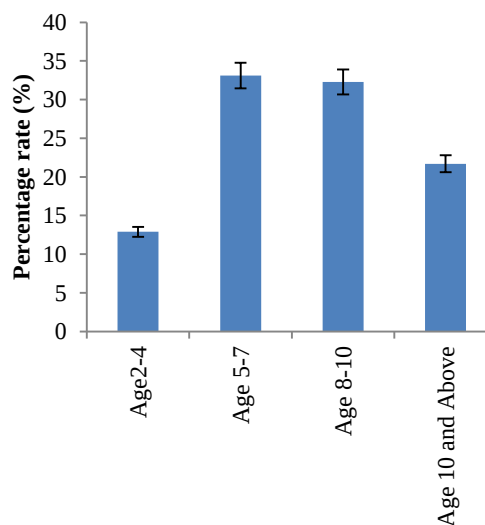


Figure 2: Age group distribution of the pupils

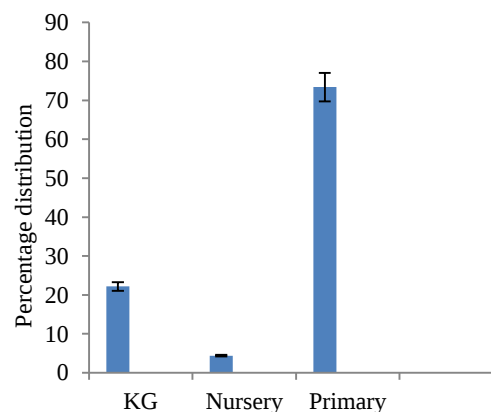


Figure 3: Percentage distribution of educational level of the pupils

3.6 Percentage occurrence of *Shigella* serotypes among the pupils

Table 5 reveals the distribution percentage of *Shigella* isolates applicable and obtained from the three zones of Odeda L.G.A with Opeji zone having the maximum

percentage of 50%. Table 6 shows the percentage occurrence of *Shigella* serotypes amongst the pupils with *Shigella boydii* accounting for 50 %, followed by *Shigella boydii* having 30 % and *Shigella sonnei* having 20 %. *S. dysenteriae* was not isolated.



Plate 1: *Shigella* isolates on Triple Sugar Iron (TSI) Agar

Table 4: Biochemical characterization of other bacteria species

Gram	Motility	Glucose	Lactose	Mannitol	Maltose	Indole	Methyl Red	Voges proskauer	Citrate	H ₂ S	Sucrose	Urea	Oxidase	Coagulase	Catalase	PROBABLE ISOLATE
GNB	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	<i>Citrobacter freundii</i>
GNB	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	<i>Enterobacter cloaca</i>
GNB	+	+	-	-	-	-	+	-	+	-	-	-	-	-	+	<i>Proteus vulgaris</i>
GNB	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	<i>Escherichia coli</i>
GNB	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	<i>Escherichia coli</i>
GNB	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	<i>Proteus mirabilis</i>
GNB	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	<i>Citrobacter freundii</i>
GNB	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	<i>Escherichia coli</i>
GNB	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	<i>Citrobacter freundii</i>
GNB	+	+	-	+	-	-	+	-	+	+	-	-	+	-	+	<i>Pseudomonas aeruginosa</i>
GNB	+	+	-	+	-	-	+	-	+	+	-	-	+	-	+	<i>Pseudomonas aeruginosa</i>
GNB	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	<i>Citrobacter freundii</i>
GNB	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	<i>Enterobacter cloaca</i>
GNB	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	<i>Salmonella arizonae</i>
GNB	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	<i>Escherichia coli</i>

KEY: + = Positive; - = Negative; GNB=Gram negative bacilli

Table 5: Percentage distribution of *Shigella* isolates according to the 3 zones of Odeda local government

Locations	Number n	Number of <i>Shigella</i> obtained	Percentage %
Opeji	104	5	50
Obantoko	82	2	20
Ilugun	62	3	30
Total	248	10	100

Table 6: Percentage occurrence of *Shigella* serotypes among the pupils

<i>Shigella</i> serotypes	Number	Percentage
<i>Shigella boydii</i>	3	30.0
<i>Shigella dysenteriae</i>	0	0.0
<i>Shigella boydii</i>	5	50.0
<i>Shigella sonnei</i>	2	20.0
Total	10	100.0

3.7 Resistance pattern of *Shigella* spp to different antibiotics

Table 7 shows the resistance pattern of *Shigella* spp to different antibiotics. Table 8 shows the Multiple Antibiotic Resistance (MAR) index of somatic antigenic positive *Shigella* strains in which second isolate has the maximum MAR index of 1.0, followed by the fifth, sixth and ninth isolates with MAR index of 0.7. The fourth and seventh isolates have the lowest MAR index of 0.5.

Table 9 shows the prevalence rate of *Shigella* isolates according to gender distribution. The highest percentage prevalence rate was observed among Male pupils (60 %) while female pupils had a percentage of 40 %.

Table 10 shows the prevalence rate of *Shigella* among the pupil according to their educational level. The highest percentage prevalence rate was observed among pupils at the Primary level (50 %). Plate 2 shows the antibiotic resistance pattern of the *Shigella* isolates on Mueller-Hinton agar while Plate 3 reveals α -hemolysis by the *Shigella* isolates on when cultured on Blood agar.

Table 7: Resistance pattern of Multi-drug resistant *Shigella* spp to different antibiotics (CLSI, 2014)

Isolates	Augmentin	Ceftriaxone	Nitrofurantoin	Gentamycin	Cotrimoxazole	Ofloxacin	Amoxycillin	Ciprofloxacin	Tetracycline	Pefloxacin
1	R	S	R	R	R	S	R	S	R	S
2	R	R	R	R	R	R	R	R	R	R
3	R	S	R	R	R	I	R	S	R	S
4	R	S	R	R	I	S	R	S	R	S
5	R	R	R	R	R	S	R	S	R	S
6	R	R	R	R	R	S	R	S	R	S
7	R	R	R	I	R	S	R	S	S	S
8	R	R	R	I	R	S	R	I	R	I
9	R	R	R	R	R	S	R	S	R	S
10	R	R	R	I	R	S	R	S	R	S

Table 8: MAR Index of *Shigella* isolates

Isolate	Resistance pattern	MAR Index
1	AUG, NIT, GEN, COT, AMX, TET	0.6
2	AUG, CRO, NIT, GEN, COT, OFL, AMX, CPX, OFL, TET, PFX	1.0
3	AUG, NIT, GEN, COT, AMX, TET	0.6
4	AUG, NIT, GEN, AMX, TET	0.5
5	AUG, CRO, NIT, GEN, COT, AMX, TET	0.7
6	AUG, CRO, NIT, GEN, COT, AMX, TET	0.7
7	AUG, CRO, NIT, COT, AMX	0.5
8	AUG, CRO, NIT, COT, AMX, TET	0.6
9	AUG, CRO, NIT, GEN, COT, AMX, TET	0.7
10	AUG, CRO, NIT, COT, AMX, TET	0.6

KEY: AUG= AUGMENTIN, CRO= CEFTRIAZONE, NIT= NITROFURANTOIN, GEN= GENTAMYCIN, COT= COTRIMOXAZOLE, OFL= OFLOXACIN, AMX= AMOXYCILLIN, CPX= CIPROFLOXACIN, TET= TETRACYCLINE, PFX= PEFLOXACIN, MAR=MULTIPLE ANTIBIOTIC RESISTANCE

Table 9: Prevalence rate of *Shigella* isolates according to gender distribution

Gender	Somatic antigenic positive <i>Shigella</i>		Somatic antigenic negative <i>Shigella</i>	
	Number	Percentage	Number	Percentage
Male	6	60	115	48.3
Female	4	40	123	51.7
Total	10	100	238	100

P>0.05

Table 10: Prevalence rate of *Shigella* among the pupil according to their educational level

Educational level	Somatic antigenic positive <i>Shigella</i>		Somatic antigenic negative <i>Shigella</i>	
	Number	Percentage	Number	Percentage
KG	2	20.0	53	22.2
Nursery	3	30.0	8	3.4
Primary	5	50.0	177	74.4
Total	10	100	238	100

P>0.05

Plate 2: Antibiotic resistance pattern of the *Shigella* isolatesPlate 3: α-haemolysis shown by the *Shigella* isolates on Blood agar

The isolation of *Shigella* species in this study indicates that the bacterium is one of the etiological agents implicated in diarrhoea in the area of study under focus. This corroborates studies by Moezardalan *et al.* [22], DeLappe *et al.* [23] and Fulla *et al.* [24] who reported and presented the growth of *Shigella* spp from stool samples of humans with diarrhoea on culture media; from the 248 stool samples examined, only ten (4.03 %) were confirmed to be positive for *Shigella* species. Which is lower than 4.5 % in Ethiopia as reported by Mengistu *et al.*, [25], similar to 4.04 % prevalence rate reported by Opintan *et al.*, [26] in Ghana and higher than 2.57 % reported by Gaurav *et al.*, [27] in India.

Investigation showed that the *Shigella* spp occurred at a lower degree in children above 10 years of age (1 among 10 cases) but with a much higher degree of occurrence between ages 5-7 years (4 among 10 cases). Sex distribution of infected persons harboring *Shigella* species in their stool samples revealed higher infection level in males accounting for 60 % than females having 40 %. This could be attributed to the enhanced activities of males that increases their environmental exposure than their female counterparts and it commensurate with the outcome obtained from the work done by Beyene and Tasew, [28] where they demonstrated the prevalence of the serious intestinal parasite, *Shigella* and *Salmonella* species amongst children with diarrhoea in Jimma Health Centre, Jimma; West Ethiopia, a cross sectional study in which male infants were more prone to the *Shigella* infection than their female counterparts.

Investigations has revealed that *Shigella dysenteriae* type 1 is the major cause of an enormous outbreaks of dysentery and that children less than 5 years of age are the most susceptible [6, 29]. According to this investigation, none of the strain of *Shigella dysenteriae* was isolated; this is in line with the study conducted at Jimma and Addis Ababa in 2006 [30]. A decreased frequency of occurrence

has been reported for this species in earlier studies in Nigeria [31, 32]. However earlier report had concluded that *S. dysenteriae* and *S. sonnei* are the predominant species in the tropics whereas *S. sonnei* is reported to be more predominant in the developed countries [33].

However the prevalence of *S. sonnei* in this particular study carried out was minimal (2 isolates), in contrast with some earlier reports [23, 34]. Dutta *et al.*, [35] revealed a modification in serotypes and antimicrobial resistance in *Shigella spp* in Kolkata between 1995 and 2000. *S. boydii* was reported to take the place *S. dysenteriae* and becoming the most prevalent serotype, followed by *S. sonnei* and *S. flexneri* in that position.

In the present study, there was a recovery of *S. boydii* (3 isolates), an outcome, which is in consonance with previous reports in Nigeria where there was minimal incidence of the serotypes [16]. The use and administration of antibiotics in the treatment of shigellosis is significant because there is evidence that antibiotics have the capability of bringing about a reasonable level of improvement in exhibited symptoms and also to reduce the course of the illness and by so doing, also reduce transmission rate of infection. A high degree of antibiotic resistance to *Shigella* will therefore make it very cumbersome to put the infection under control. Previously, various antimicrobial agents have shown their effectiveness in treating shigellosis, but treatment options available for this disease are becoming limited in an alarming way due to the continued rise of multidrug-resistant strains of *Shigella* as shown by Replogle *et al.* [36], Mclver *et al.* [37], Oh *et al.* [38], Lee *et al.* [39] and CDC [40].

This investigation showed high susceptible rate of the *Shigella* isolates to Ofloxacin (80 %) and Pefloxacin (80 %). This conclusion is in consonance with the result of the study done by [39] who revealed similar antibiotic susceptibility trend in Korea during the last two decades. Earlier reports have revealed that drug resistance in *Shigella spp* is an emerging global phenomenal challenge especially in developing countries where there is an increased level of drugs abuse in animals and humans [41-43], as shown by the antimicrobial resistance patterns acquired in this study, many of the antibiotics which were once the backbone of the treatment of shigellosis can no longer be depended on to give effective treatment as they have been found to be resistant to broad spectrum of antimicrobial agents against which they were tested. The high degree of resistance to amoxicillin, Nitrofurantoin, and Augmentin is suggestive of misuse of these antibiotics.

A high rate of resistance was also seen in ceftriaxone which is not in agreement to a study carried out in Gonder [44] where none of the isolates was resistant to ceftriaxone. The level of resistance to the most widely used antibiotics, tetracycline and amoxicillin was in consonance to studies conducted everywhere else in the world [45, 46]. Gentamicin resistant *Shigella* isolates were not found in previous studies from Addis Ababa [41, 47] in contradiction to this study as backed by the study done in Gonder [44]. This shows the emergence of gentamicin drug resistance *Shigella* isolates over a period of time. High level of resistance was also observed to Co-trimoxazole (76.5 %), which is in tune with the report from Gonder (73.4 %), [48] and 84.6 % [44] in contradiction to a study

from Awassa (56.0 %) [49]. This alarming increment in the resistance from those reports showed the exasperating menace of drug resistance by these microbes over time.

This may be due to inappropriate and unintended use of drugs [50, 51]. Iwalokun *et al.*, [32] also presented a high degree of resistance of *Shigella* isolates from diarrheic patients in Lagos, Nigeria to amoxicillin. Isenberger *et al.*, [52] had also reported that the resistance of *Shigella spp* to tetracycline was common. In India, investigations conducted by Pazhani *et al.* [53] between 2001 and 2004 showed all the *Shigella* isolates to be resistant to Tetracycline, a figure of 95 % was cited in the USA [54], 88 % in Brazil [55], and 80 % in Bangladesh [56]. Tetracycline and amoxicillin are widely used to treat shigellosis, but in this study, 90 % of the *Shigella* isolates were resistance to tetracycline and 100 % to amoxicillin and in such cases, Ciprofloxacin and Ofloxacin should serve as suitable alternative.

In contradiction to the outcome obtained in this study, high level of resistance to Ciprofloxacin have been recorded among *Shigella* strains in areas where this antibiotics is used in concentration [57-62] suggesting that *Shigella spp* in this study area are likely to develop resistance to ciprofloxacin since its use is not controlled to any significant extent. In other studies, overall resistance to Tetracycline, Amoxicillin and Pefloxacin was quite low [46]. The outcome of the recent study opined a reconsideration of the judicious utilization of some frequently antibiotics prescribed for the effective treatment of Shigellosis. The speedy management of shigellosis is therefore a significant economic and health challenge that should be combated due to global emergence of antibiotic resistant strains of *Shigella* [36, 37, and 39]. The high susceptibility of *Shigella* isolates to Ofloxacin and Pefloxacin as revealed in this study is a merit for practitioners in the management and treatment of shigellosis in the study area.

About 80 % of the isolates were multiple antibiotic resistant types, which is in consonance with the detection of multiple-resistant *Shigella* strains in developing world i.e. Africa [16], England and Wales [63], Asia [39]. There was an alarming widespread occurrence of drug resistance and Multiple Antibiotic Resistance indices (0.2 - 0.9) in the samples used for this study. MAR indexing is indicated as a good tool for risk assessment. It gives an idea of the number of bacteria displaying antibiotic resistance and the concomitant risk zone in a mundane susceptibility testing [64]. High MAR index values have been revealed to be indicative of environments with high endemic disease potential and risk sources where drugs are frequently used [65]. A MAR index value of ≥ 0.2 was reported in the drug resistant strains of *Shigella* used in this study, suggesting it to be in consonance with previous studies.

4 Conclusion

From this study, *Shigella spp* was isolated from pupils in all the age group, gender that was studied from the three zones of Odeda L.G.A. Majority of the *Shigella* isolated was reported to show resistance to Augmentin, Amoxicillin, Tetracycline, Gentamycin and other frequently utilized antibiotics in the three zones of Odeda L.G.A.

5 Recommendations

It is of utmost significance to pay quality attention to the distinctive use of antibiotics, health education of food handlers, maintenance of door fomites in the school, the validation of hygienic measures to minimize transmission, and the establishment of new efficient and effective drugs that can be safely utilized in combating Shigellosis. There should also be a Public Enlightenment Programme to create an urgent awareness in educating the general masses especially residents of rural communities on the adverse effects of this disease-causing bacteria.

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Declaration

No conflict of interests is declared for this study

References

1. UNICEF/WHO, Diarrhoea: *Why children are still dying and what can be done*. A Joint Publication of the United Nations Children's Fund (UNICEF) and the World Health Organization (WHO) 2009; pp. 1-40. 2006;55(9):247-249
2. Peter A.K., Umar U. Combating diarrhoea in Nigeria: the way forward. *Journal of Microbiology and Experimentation*. 2018; 6(4):191-197. DOI: 10.15406/jmen.2018.06.00213
3. Akinrotoye, K.P. Effects of fermented palm wine on some diarrhoeagenic bacteria. *Elite Research Journal of Biotechnology and Microbiology*, 2014; 2 (1): 4 – 14.
4. Bastos, F.C., and Loureiro, E. C. Antimicrobial Resistance of *Shigella* spp. isolated in the State of Pará, Brazil. *Rev. Soc. Bras. Med. Trop.*, 2011; 44: 607-610.
5. Huang, I. F., Chiu, C. H., Wang, M. H., Wu, C. Y., Hsieh, K. S., and Chiou, C. C. Outbreak of dysentery associated with Ceftriaxone-resistant *Shigella sonnei*: First report of Plasmid-Mediated CMY-2- Type Amp C β -Lactamase resistance in *S. sonnei*. *Journal of Clinical Microbiology*, 2005; 43, 2608-2612.
6. Ashkenazi, S. *Shigella* infections in children: new insights. *Seminar on Pediatric Infectious Diseases* 2004; 15: 246-252.
7. Barman, S., Saha, D. R., Ramamurthy, T., and Koley, H. Development of a new Guinea-pig model of Shigellosis. *FEMS Immunology Medical Microbiology* 2011; 62: 304-314.
8. Vinh, H., Anh, V. T., Anh, N. D., Campbell, J. I., Hoang, N. P., Nga, T. V., Nhu, N. T., Minh, P. V., Thuy, C. T., Duy, P. T., et al. A multi-center randomized trial to assess the efficacy of gatifloxacin versus ciprofloxacin for the treatment of shigellosis in Vietnamese children. *PLoS Neglected Tropical Diseases* 2011; 5: e1264.
9. Bangtrakulnonth, A., Vieira, A. R., Lo Fo Wong, D. M., Pornreongwong, S., Pulsrikarn, C., Sawanpanyalert, P., Hendriksen, R. S., and Aarestrup, F.M. *Shigella* from humans in Thailand during 1993 to 2006: Spatial-time trends in species and serotype distribution. *Foodborne Pathogen Disease* 2008; 5:773-784.
10. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office, 2003.
11. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013; pp. 1-32.
12. Peng J., Yang J., Jin Q. The molecular evolutionary history of *Shigella* spp. and enteroinvasive *Escherichia coli*. *Infection, Genetics and Evolution*. 2009; 9:147-52.
13. Singh, K. Laboratory-acquired infections. *Clinical Infectious Diseases*. 2009; 49:142-7.
14. Baron, E. J., and Miller J. M. Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. 2008; 3(60):241-6.
15. Akinrotoye Kehinde Peter., Mobolaji Oluwafunmilayo Bankole., and Stephen Oluwale Akinola. Occurrence of Pathogenic Bacteria on Public Surfaces within Community Schools in Abeokuta Environs, Ogun State. *Journal of Environmental Treatment Techniques*. 2018; 6(3):47-52.
16. Egah, D. Z., Banwat, E. B., Audu, E. S., Allanana, J. A., Danung, M. L., Damen, J. G and Badung, B. P. "Multiple drug resistant strains of *Shigella* isolated in Jos, central Nigeria". *Nigeria Postgraduate Medical Journal*. 2003; 10:154- 156.
17. Farshad, S., Sheikhi, R., Japoni, A. "Characterization of *Shigella* strains in Iran by plasmid profile analysis and PCR amplification of *ipa* genes". *Journal of Clinical Microbiology*. 2006; 44 (8):2879-83.
18. Cheesbrough, M. District Laboratory Practice in Tropical Countries: Part one (Second edition). Cambridge University Press, U.K. 2006, pp 143-157.
19. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 16th Information Supplement. Clinical and Laboratory Standards Institute, Wayne, PA. 2007.
20. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 20th informational supplement M100-S20. Clinical and Laboratory Standards Institute. 2014.
21. Akinjogunla, O. J., and Enabulele, I. O. Virulence Factors, Plasmid Profiling and Curing analysis of Multi-drug Resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* spp. isolated from Patients with Acute Otitis Media. *Journal of American Science*. 2010; 6:1022-1033.
22. Moezardalan, A. K., Zali, M. R., Soltan-Dallal, M. M., Hemami, M. R., and Salmanzadeh-Ahrabi, S. Prevalence and pattern of antimicrobial resistance of *Shigella* species among patients with acute diarrhoea in Karaj, Tehran, Iran. *Journal of Health Population Nutrition*. 2003; 21: 96-102.
23. DeLappe, N., O'Halloran, F., Fanning, S., Corbett-Feeney, G., Cheasty, T., Cormican, M. Antimicrobial resistance and genetic diversity of *Shigella sonnei*

- isolates from western Ireland, an area of low incidence of infection, *Journal of Clinical Microbiology*. 2003; 41: 1919-1924.
24. Fullá, N., Prado, V., Duran, C., Lagos, R., Levine, M.M. Surveillance for antimicrobial resistance profiles among *Shigella* species isolated from a semirural community in the northern administrative area of Santiago, Chile. *American Journal of Tropical Medicine Hygiene*. 2005; 72: 851-854
 25. Mengistu, G., Mulugeta, G., Lema, T., and Aseffa, A. Prevalence and Antimicrobial Susceptibility Patterns of *Salmonella* serovars and *Shigella* species. *Journal of Microbiology, Biochemistry Technology*. 2014; S2: 006.
 26. Opintan, J., Newman, M. J. Distribution of serogroups and serotypes of multiple drug resistant *Shigella* isolates. *Ghana Medical Journal*. 2007; 41: 8-29.
 27. Gaurav, A., Singh, S. P., Gill, J. P. S., Kumar, R., and Kumar, D. Isolation and identification of *Shigella* spp from human faecal samples collected from Pantnagar, India, *Vet world*. 2013; 6 (7):376-379.
 28. Beyene, G., and Tasew, H. Prevalence of Intestinal parasite, *Shigella* and *Salmonella* species among Diarrhoeal children in Jimma health center, Jimma southwest Ethiopia: a cross sectional study. *Annals of Clinical Microbiology Antimicrobial*. 2014; 13(10): 1-7.
 29. World Health Organization/United Nations Children's Fund. *Progress on Drinking Water and Sanitation: Special focus on sanitation*, UNICEF, New York. 2008.
 30. Beyene, G., Nair, S., Asrat, D., Mengistu, Y., and Engers, H, et al. Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. *Journal of Infection of Developing Countries*. 2011; 5: 23-33.
 31. Niemogha, M. T., Alabi, S. A., Uzoma, K. L., Odugbemi, T. O., Adegbola, R. O., and Coker, A. O. (2005). The incidence of *Salmonella*, *Shigella* and other enteric bacterial pathogens in stool specimens of diarrhoea patients, *Nigerian Medical Journal* 28: 70-74.
 32. Iwalokun, B.A., Gbenle, G.O., Smith S.I., Ogunledun, A., Akinside, K.A., and Omonigbehin, E.A. Epidemiology of shigellosis in Lagos, Nigeria: trends in antimicrobial resistance, *Journal of Health Population Nutrition*. 2001; 19(3):183-190.
 33. Preston, M. A. and Borczyk A. A. Genetic variability and molecular typing of *Shigella sonnei* strains isolated in Canada, *Journal of Clinical Microbiology*. 2004; 32: 1427-1430.
 34. Panhotra, B. R., Saxena, A. K., and AlMulhim, K. Emergence of nalidixic-acid resistance in *Shigella sonnei* isolated from patients having acute diarrhoeal disease: report from eastern province of Saudi Arabia, *Japan. Journal of Infectious Diseases*. 2004; 57: 116-118.
 35. Dutta, S., Rajendran, K., Roy, S., Chatterjee, A., Dutta, P., Nair, G.B., Bhattacharya, S.K., and Yoshida, S. I. Shifting serotypes, plasmid profile analysis and antimicrobial resistance pattern of *Shigellae* strains isolated from Kolkata, India during 1995-2000, *Epidemiology of Infection*. 2002; 129: 235-243.
 36. Replogle, M. L., Fleming, D. W and Cieslak, P. R. Emergence of antimicrobial-resistant shigellosis in Oregon. *Clinical Infectious Diseases*. 2000; 30: 515-519.
 37. McIver, C. J., White, P. A., Jones, L. A., Karagiannis, T., Harkness, J and Marriott, D. Epidemic strains of *Shigella sonnei* biotype g carrying integrons. *Journal of Clinical Microbiology*. 2002; 40: 1538-1540.
 38. Oh, J. Y., Yu, H. S., Kim, S. K., Seol, S. Y., Cho, D. T and Lee, J. C. Changes in patterns of antimicrobial susceptibility and integron carriage among *Shigella sonnei* isolates from southwestern Korea during epidemic periods. *Journal of Clinical Microbiology*. 2003; 41: 421-423
 39. Lee, J. C., Oh, J. Y., Kim, K. S., Jeong, Y. W., Cho, J. W and Park, J. C. Antimicrobial resistance of *Shigella sonnei* in Korea during the last two decades. *Acta Pathology Microbiology Immunology. Scand*. 2001; 109: 228-234.
 40. Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the United States. Available at www.cdc.gov/drugresistance/threat-report2013/pdf/ar-threats-2013-508.pdf, 2013.
 41. Reda, A. A., Seyoum, B., Yimam, J., Andualem, G., Fiseha, S., and Vandeweerd, J. M. Antibiotic Susceptibility of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia. *Journal of Infectious Diseases Immunology*. 2011; 3(8):134-139.
 42. Kasper, D. L., Fauci, A. S., Longo, D. L., Braunwald, E, Hauser, S. L, and Jameson, J. L. (Eds). *Harrison's Principles of Internal Medicine*. New York. The McGraw-Hill Companies. 2005; pp. 897-906.
 43. Sule, W. F., Adige, A. A., Abubakar, M. O., and Ojezele, M. O. Antimicrobial resistance of clinical isolate of *Salmonella typhi* in Ayingba, Kogi State, Nigeria. *Global Advance Research Journal of Microbiology*. 2012; 1(4):57-61.
 44. Tiruneh, M. Serodiversity and antimicrobial resistance pattern of *Shigella* isolates at Gondar University teaching hospital, Northwest Ethiopia. *Japan Journal of Infectious Diseases*. 2009; 62: 93-97.
 45. Subekti, D., Oyof, B. A., Tjaniadi, P., Corwin, A.L., Laraset, W., Putri, M., Simanjuntak, C. H., Punjabi, N. H., Taslim, J., and Setiawan, B. *Shigella* spp surveillance in Indonesia: the emergence and re-emergence of *Shigella dysenteriae*, *Emerging Infectious Diseases*. 2001; 7: 137-140.
 46. Voogd, C. E., Schot, C. S., Van Leeuwen, W. J and Van Klingeren, B. "Monitoring of antibiotic resistance in *Shigellae* isolated in the Netherlands". *Journal of Clinical Microbiology of Infectious Diseases*. 2002; 11: 164-165.
 47. Asrat, D. *Shigella* and *Salmonella* serogroups and their antibiotic susceptibility patterns in Ethiopia. *East Mediterranean Health Journal*. 2008; 14: 760-767.
 48. Yismaw, O, Negeri, C, and Kassu, A. A five-year antimicrobial resistance pattern observed in *Shigella* species isolated from stool samples in Gondar University Hospital, northwest Ethiopia. *Ethiopian Journal of Health Development*. 2009;
 49. Roma, B, Worku, S, Mariam, S. T, Langeland, N. Antimicrobial susceptibility pattern of *Shigella* isolates

- in Awassa. *Ethiopian Journal of Health Development*. 2000; 14:149-154.
50. Vu Nguyen, T, Le Van, P, Le Huy, C, Nguyen Gia, K, and Weintraub, A. Etiology and epidemiology of diarrhoea in children in Hanoi, Vietnam. *International Journal of Infectious Diseases*. 2006; 10: 298-308.
 51. Ecker, L., Olarte, L., Vilchez, G., Ochoa, T.J., Amemiya, I, *et al.* Physicians' responsibility for antibiotic use in infants from periurban Lima, Peru. *Rev Panam Salud Publica*. 2011; 30: 574-579.
 52. Isenberger, D. W., Hoge, C. W., Srijan, A., Pitarangsi, C., Vithayasai, N., Bodhidatta, L., Hickey, K. W., and Cain, P. D. Comparative antibiotic resistance of diarrhoeal pathogens from Vietnam and Thailand 1996-1999, *Emerging Infectious Diseases*. 2002; 8(2): 1- 4.
 53. Pazhani, G. P., Ramamurthy, T., Mitra, U., Bhattacharya, S. K and Niyogi, S. K. Species diversity and antimicrobial resistance of *Shigella* spp. isolated between 2001 and 2004 from hospitalised children with diarrhoea in Kolkata (Calcutta), India. *Epidemiology of Infection*. 2005; 133:1089-1095.
 54. Sivapalasingam, S., Nelson, J. M., Joyce, K., Hoekstra, M., Angulo, F. J., and Mintz, E. C. High prevalence of antimicrobial resistance among *Shigella* isolates in the United States tested by the National Antimicrobial Resistance Monitoring System from 1999 to 2002, *Antimicrobial Agents Chemotherapy*. 2006; 50: 49-54.
 55. Peirano, G., Souza, S. F., and Rodrigues, D. P., *Shigella* Study Group. Frequency of serovers and antimicrobial resistance in *Shigella* spp. from Brazil. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 2006; 101: 245-250
 56. Rahman, M., Shoma, S., Rashid, H., Siddique, A. K., Nair, G. B and Sack, D. A. Extended-spectrum β -lactamase-mediated third generation cephalosporin resistance in *Shigella* isolates in Bangladesh. *Journal of Antimicrobial Chemotherapy*. 2004; 10: 846-847
 57. Bhattacharya, S. K., Sarkar, K., and Nair G. B. Multidrug-resistant *Shigella dysenteriae* type 1 in south Asia. *Lancet Infectious Diseases*. 2003; 3: 755.
 58. Pazhani, G. P., Sarkar, B., Ramamurthy T., Bhattacharya S. K., Takeda Y and Niyogi S. K. Clonal multidrug-resistant *Shigella dysenteriae* type 1 strains associated with epidemic and sporadic dysenteries in eastern India. *Antimicrobial Agents Chemotherapy*. 2004; 48: 681-684.
 59. Taneja, N., Mohan, B., Khurana, S. and Sharma, M. Antimicrobial resistance in selected bacterial enteropathogens in north India. *Indian Journal of Medical Research*. 2004; 120: 39-43.
 60. Canada Communicable Disease Report (CCDR). Emergence of quinolone-resistant *Shigella dysenteriae* type 1 in Canada. *Canadian Communicable Disease Report*. 2005; 31:193-197.
 61. Enabulele, I. O., Yah, S. C., Yusuf, E. O and Eghafona, N. O. Emerging quinolones resistant transfer genes among gram-negative bacteria, isolated from faeces of HIV/AIDS patients attending some clinics and hospitals in the city of Benin, Edo State, Nigeria. *Online Journal of Health Allied Science*. 2006; 5: 1-9.
 62. Von Seidlein, L., Kim, D. R., Ali, M., Lee, H., Wang, X., Thiem, V. D., Canh do, G., Chaicumpa, W., Agtini, M. D., Hossain, A., *et al.* A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Med*. 2006; 3, e353.
 63. Cheasty, T., Skinner, J. A., Rowe, B., and Threlfall, E. J. "Increasing incidence of antibiotic resistance in *Shigella* from humans in England and Wales: Recommendations for therapy". *Microbial Drug Resistance*. 2008; 4: 57-60.
 64. Akhter, A., Imran, M., and Akhter, F. Determination of multiple antibiotic resistance patterns and indexing among metal tolerant β -lactamase-producing *Escherichia coli*. *African Journal of Microbiology Research*. 2014; 8(7):619-627.
 65. Hemen, J. T., Johnson, J. T., Ambo, E. E., Ekam, V. S., Odey, M. O., and Fila, W. A. Multi-antibiotic Resistance of some Gram negative bacterial isolates from poultry litters of selected farms in Benue State. *International Journal of Science and Technology*. 2012; 1(8): 543-547.