academicJournals

Vol. 9(37), pp. 2023-2036, 16 September, 2015
DOI: 10.5897/AJMR2015.7662
Article Number: 3184F3155584
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Drug resistance pattern of *Salmonella* and *Shigella* species isolated from selected hospitals in Anyigba, Kogi State, Nigeria

Helen Ojomachenwu Ocean, Emmanuel Aniebonam Eze, Chibuzor Nwadibe Eze and Matthew Chekwube Enebe*

Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

Received 8 July, 2015; Accepted 31 August, 2015

This work was designed to assess the status of *Salmonella* and *Shigella* infections in Anyigba, Kogi State, Nigeria with a view to making informed statements that may guide policy makers and public health experts in the containment efforts against these infections. *Salmonella* and *Shigella* species isolated from human faecal samples were examined for resistance to some antibiotic agents using disc diffusion methods. Drug resistant isolates were analyzed for presence of plasmids by basic curing and transferability methods. Age distribution of sources of resistant isolates showed that *Shigella* isolates from Youths (18 to 30 years) were more resistant to Ampicilin (66.67%) and Augmentin (33.33%) than isolates from Infants (6 months to 4 years) (3% Ampicillin and 0% Augmentin). *Salmonella* species isolated from Adults (31 and above) were more resistant to Augmentin (45%) and Ampicillin (40%), than isolates from Youths (18 to 31 years) (40% Ampicillin and 36.67% Augmentin). *Salmonella* isolated from Infants (6 months to 4 years) showed percentage resistance of 28.57% to Ampicillin and 14.29% to Augmentin while isolates from Children (5 to 17 years) showed percentage resistance of 25% to Ampicillin and 12.5% to Augmentin. *Salmonella* species showed higher percentage resistance to commonly used drugs than *Shigella* species. The result shows that 8% of *Shigella* species and 21.5% of *Salmonella* species were resistant to more than eight antibiotics with (MAR) index of between 0.2-0.9. Results of curing and agarose gel electrophoresis (AGE) presumptively showed that the resistance traits were plasmid borne. AGE showed plasmids of molecular weights 23.1 kb for both species of *Salmonella* and *Shigella* suggesting their possible exchange among them. This was substantiated by conjugative transfer of resistance determinants among few isolates. The results show convincing evidence of the presence of multidrug resistant *Salmonella* and *Shigella* spp. within the study population.

Key words: Hospital patients, *Salmonella* spp., *Shigella* spp., drug resistance, plasmids, resistance index.

INTRODUCTION

The phenomenon of drug resistance has been accelerating over the past three decades. As a consequence, humans face the real risk of a future without antibiotics. The implication of this is that life expectancy could fall due to people dying from diseases that are readily treatable today (Sandle, 2014). Very high

rates of resistance have been observed in bacteria that cause health - care associated and community acquired infections. Among these are strains of *Salmonella* and *Shigella* species (WHO, 2014).

Salmonella and *Shigella* infections therefore represent a major health problem worldwide, particularly in developing countries where they are recognized as the most frequent causes of morbidity and mortality (Yah, 2010; Mahbubur et al., 2007; Abdel et al., 2008). For example, it has been estimated that approximately 13 million cases of salmonellosis occur world-wide annually (Uma et al., 2010). Loss of life together with the high costs of local public health care system makes prevention and control a priority (Mahbubur et al., 2007; Yah et al., 2007a). The two pathogens have been associated with diarrhea but the severity of the diarrhea varies with the pathogens (Yah, 2010). *Salmonella* causes self-limiting gastroenteritis and the more severe forms of systemic typhoid fever. *Shigella* species are limited to the intestinal tract of humans and cause bacillary dysentery leading to watery or bloody diarrhea (Reda et al., 2011; Beyene and Tasew, 2014).

Generally *Shigella* causes bloody diarrhea while *Salmonella* induces non-bloody gastroenteritis (Yah, 2010). While most *Salmonella* strains cause gastroenteritis, *Shigella* species are a major cause of diarrhea and dysentery throughout the world (WHO, 2014). These bacteria are transmitted by ingestion of contaminated food or water, or through person-to-person contact. Shigellosis is primarily a disease of resource-poor crowded communities that do not have adequate sanitation or safe water (WHO, 2014). This is typical of the study area covered in this work.

Antibacterial drugs have proven to be effective in the management of *Salmonella* and *Shigella* infections and have been lifesaving. However, emerging resistance has been reported as a concern from some countries (Beyene and Tasew, 2014) while in many countries and hinter lands, there is paucity of information in this regard. Additionally, drug resistant *Salmonella* and *Shigella* are of global concern because they affect both developed and developing countries due to increased international travel (Yah, 2010; Dubois et al., 2007). For this and other obvious reasons, the gaps in surveillance data at national level raise the question as to whether or not representative local data are available to also inform treatment guidelines.

Examining the drug susceptibility patterns of pathogens is important towards tailoring treatment to the ever changing resistance patterns and distribution of pathogenic bacteria (Reda et al., 2011). This work was done in the light of this. It was therefore aimed at providing data on the drug resistance profiles of *Salmonella* and *Shigella* species isolated from Anyigba, a town in Kogi State of Nigeria. This is with a view to giving evidence based information to policy makers and public health workers. Our objectives were to carry out epidemiological survey of *Salmonella* and *Shigella* species from faecal samples, isolate and identify *Salmonella* and *Shigella* species from faecal samples, conduct antimicrobial susceptibility test on confirmed isolates and evaluate the transferability of the resistant traits.

MATERIALS AND METHODS

Study area and study population

The studies were carried out at different Hospitals in Anyigba, Kogi State, Nigeria on a population of Infants (6 months to 4 years), children (5 to 17 years), youths (18 to 30 years) and adults (31 and above).

Sample collection

Two hundred and fifty (250) faecal samples were collected aseptically from patients attending different Hospitals in Anyigba, Kogi State. Stool samples were collected from infants and children into sterile sample bottles using spatula. Youths and adults were given sterile bottles and spatula to collect the samples. This was after informed consents were obtained from participants or their parents or care givers.

Bacteria isolation

Samples were inoculated onto *Salmonella-Shigella* and MacConkey agar plates and incubated at 37°C for 24 h.

Identification

All isolates were Gram-stained, examined microscopically and tested for Triple Sugar Iron (TSI) utilization, catalase activities, indole production, motility test and urease production (Cheesbrough, 2004). CHROM agar ^{TM} was used to further confirm the identity of the isolates.

Drug susceptibility testing

The drug susceptibility of *Salmonella* and *Shigella* isolates was performed on Mueller-Hinton agar (LAB M^{TM}) plates by disc diffusion method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2012). The bacterial isolates were inoculated into Mueller-Hinton broth and incubated for 18 h at 37°C. The growth was standardized using

*Corresponding author. E-mail: enebematthew@yahoo.com. Tel: +2347034288686.

MacFarland turbidity standard. This was achieved by diluting the growth with normal saline until it matched the turbidity of 0.5 MacFarland standards.

About 0.1 ml of each bacterial isolate was seeded onto a separate Petri dish containing Mueller-Hinton agar and allowed to stand for about 5 min. The commercially available discs containing septrin (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), ciprofloxacin (10 µg), ampicillin (30 µg), augmentin (25 µg), gentamycin (10 µg), pefloxacin (10 µg), ofloxacin (30 µg) and streptomycin (30 µg) (Maxi Lab. Nig) were aseptically placed on the surfaces of the sensitivity agar plates and incubated at 37°C overnight. Zones of inhibition were measured in millimeters. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative table. The percentage resistance was calculated using the formula *PR=a/b×100, where 'PR'* was percentage resistance, '*a'* was the number of resistant isolates and '*b'* was the number of isolates tested with the drug.

Generally, organisms surrounded by a zone diameter between 0-13 mm were classified as resistant (CLSI, 2012; Akinjogunla and Enabulele, 2010).

Determination of multiple antibiotic resistance (MAR) index

Multiple antibiotic resistance indexing (MAR Index) was determined using the formula: MAR Index = x/y where x was the number of drugs to which test isolate displayed resistance and y is the total number of drugs to which the test organism has been evaluated for sensitivity (Akinjogunla and Enabulele, 2010).

Plasmid isolation and profiling

Selection of isolates for plasmid DNA isolation and profiling was based on drug resistance pattern of the test isolates. Emphasis was placed on the multidrug resistant (those resistant to three or more drugs) isolates and those completely susceptible to all or most of the test drugs.

Plasmid DNA was isolated by the alkaline lysis method as described previously by Kado and Liu (1981) and modified in both Lech and Brent (1987) and Mini-prep methods of Kraft et al. (1988) as follows: selected isolates were inoculated into Nutrient broth (10 ml each) and incubated for 24 h at 37°C. Each culture was subsequently decanted, leaving about 4 ml of the broth and sediments. From this, 1.5 ml was centrifuged in a microcentrifuge at 10,000/rpm for 5 min. The supernatant was decanted, leaving about 80 µl of the broth together with the pellet. This was vortexed to resuspend cells completely. After this, 300 µl of TENS lysis buffer was added, mixed for about 5 s or until the mixture became sticky. An aliquot, 150 µl of 3.0M Sodium acetate (pH 5) was added, vortexed for 4 s to mix completely and spinned for two minutes in the Microcentrifuge to pellet cell debris (Chromosomal DNA). The supernatant from this was transferred into fresh sterile Microcentrifuge (Eppendorf) tube and mixed with 0.9 ml of precooled 100% ethanol. This was spinned further to pellet plasmid DNA. The supernatant was discarded and pellet washed twice with 1ml of 70% ethanol.

The pellet was air dried for 5 min by spinning the tubes open in the centrifuge. Pellet was finally re-suspended in 20 µl of TENS buffer and stored in the refrigerator until needed for gel electrophoresis.

Agarose gel electrophoresis (AGE) of plasmid DNA

A 0.8% agarose was prepared using TBE running buffer. One hundred milliliters of this was melted completely over flame and cooled to about 50°C before 3 µl of ethidium bromide was added. After mixing, the gel was poured carefully (to avoid bubble formation) into the electrophoresis unit tray. This was allowed to gel (solution turned from translucent to opaque after about 18 min) and the comb was carefully removed.

Samples were prepared by adding 5 µl of loading buffer into fresh sterile microcentrifuge tube. In the first tube labeled "Standard" or 'Marker' (M), 4 µl of the DNA marker was added while 8 µl of each sample was added separately in the other tube(s). This was mixed by carefully pipetting the solution up and down (avoiding bubbles formation). After sample preparation, the gel carrier tray was placed in the gel electrophoresis box. Slowly, about 250 ml of running buffer (TBE) was poured into the box until the gel was submerged. This was checked for air bubbles. Subsequently, samples (10 µl each) were loaded (one sample per well) into the wells, with the standard in the central well. The gel was ran at 95 volts for about 1.25 h or until the bromophenol blue from the sample dye was near the end of the gel.

After running, the gel was removed from the electrophoresis box, allowed to cool on the bench and placed in a UV trans illuminator for visualization (using protective goggles) of plasmid bands. Photographs were taken.

Plasmid DNA curing

Curing the strains of plasmids was carried out using modified Yah et al. (2007a) method. *Salmonella* and *Shigella* cells were cured using 1 and 10% sodium dodecyl sulphate (SDS) and 25 µg/ml acridine orange.

Sodium dodecyl sulphate (SDS)

Nutrient broth was prepared and supplemented with 1 g of SDS in one batch of 99 ml and 10g of SDS in the second batch of 90 ml to achieve a final concentration of 1 and 10% ($^{w}/_{v}$) SDS respectively. They were then sterilized by autoclaving for 15 min at 121°C. Selected overnight cultures of isolates were standardized to 0.5 McFarland turbidity standards using sterile saline. From these, 0.1 ml of each culture was inoculated separately into SDS supplemented nutrient broth in test tubes and incubated at 37°C for 24 h. After incubation, cultures were standardized and spread on Mueller-Hinton agar and susceptibility testing was carried out as earlier described.

Use of acridine orange

Isolates of *Salmonella* and *Shigella* species were grown for 24 h at 37°C in sterile nutrient broth containing 25 µg/ml acridine orange final concentration. After 24 h, each broth tube was agitated to homogenize the content and loopful of the broth medium was then sub-cultured onto Mueller Hinton agar (MHA) plates and drug sensitivity testing was carried out as previously described. Absence of zone of inhibition on Mueller Hinton agar was suggestive of loss of plasmid-mediated resistance (plasmid cured) (Sheikh et al., 2003; Yah et al., 2007a; Akortha and Filgona, 2009; Akinjogunla and Enabulele, 2010 with slight modification).

Plasmid transfer

This was done by a broth culture conjugation method using donors and recipients as shown in Table 1. Overnight broth cultures of donor and recipient strains grown separately at 37°C were diluted

Table 1. Bacterial isolates used in gene transfer experiment.

Donor	Recipient
Salmonella sp.	*Escherichia coli*
Salmonella sp.	*Shigella* sp.
Shigella sp.	*Escherichia coli*
Shigella sp.	*Salmonella* sp.

to 0.5 McFarland turbidity standard and mixed in a sterile test tube at the ratio of 1 to 10 (donor to recipient) and incubated at 37°C for 14 h (Bartoloni et al., 2006).

After incubation, samples (0.1 ml) were spread onto the surfaces of Nutrient and MacConkey agar plates supplemented with nalidixic acid (30 µg/ml). As transduction and transformation controls, recipient isolates were also incubated with cell-free (Filtered with Millipore Type HC filters and confirmed to be cell free by Microscopic examination) donor cultures and incubated also for 14 h at 37°C. After incubation, transconjugants were recovered based on marker and biochemical characteristics and subjected to susceptibility testing as above.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the resistance of the two test organisms to the antimicrobial agents with 0.05 confidence limit.

RESULTS

A total of one hundred and fifteen (115) target bacteria were isolated from two hundred and fifty (250) human faecal samples collected from different hospitals in Anyigba, Kogi State, Nigeria. Results show that out of this total number of isolates (115), 50(43.48%) isolates were *Shigella* species while 65(56.52%) were *Salmonella* species (Table 2).

Out of the 50 (43.48%) isolates of *Shigella* species, 28 (56.00%) were from male patients while 22 (44.00%) were from female patients. Thirty three (50.77%) of the *Salmonella* species isolated were from male patients while 32 (49.23%) were from female (Tables 3 and 4). In general (that is irrespective of sex), 33 (66%) *Shigella* species were isolated from Infants (6 months to 4 years), 9(18%) from children (5 to 17 years), 3 (6%) from youths (18 to 30 years) and 5 (10%) from adults (31 and above) (Tables 2 and 3). For *Salmonella* species, 10 (15.38%) were isolated from infants (6 months to 4 years), 10 (15.384%) from children (5 to 17years), 25 (38.462%) from youths (18 to 30 years) and 20 (30.7696%) from Adults (31 and above) (Table 4).

Age distribution of sources of resistant isolates of *Shigella* species showed that *Shigella* isolates from youths were more resistant to ampicillin (66.67%) and augmentin (33.33%) than isolates from infants which showed percentage resistance of 3% for ampicillin and 0% for augmentin (Figure 1).

Similarly, *Salmonella* isolates from adults were more resistant to augmentin (45%) and ampicillin (40%) than isolates from youths which showed percentage resistance of 40% to ampicillin and 36.67% to augmentin. *Salmonella* isolated from infants showed percentage resistance of 28.57% to ampicillin and 14.29% to augmentin while isolates from children showed percentage resistance of 25% to ampicillin and 12.50% to augmentin. Isolates from children showed high percentage resistance to septrin (37.50%) while Isolates from Infants showed least percentage resistance to Septrin (14.29%) (Figure 2).

Sex distribution of sources of resistant isolates showed that *Shigella* species isolated from male youths were more resistant to ampicillin (100%) than those from female youths which were in turn more resistant to ampicillin (50%) (Figure 3).

Salmonella species isolated from male children showed high percentage resistance to Ampicillin (60%).Those from female children were 40% resistant to Ampicillin. Isolates from male adults, youths and children were 60% resistant to augumentin (Figure 4).

Salmonella species showed higher aggregate resistance to ampicillin (35.38%) and augmentin (35.38%) than *Shigella* species showed 8% resistance to both ampicillin and augmentin (Figures 5 and 6). ciprofloxacin (CPX) was statistically more effective ($p<0.05$) than AM, AU, PEF and SP antibiotics against strains of *Salmonella* tested. Similarly, SXT for example, was significantly more effective against *Shigella* spp than both AM and AU antibiotics.

All *Shigella* spp. evaluated for MAR indices showed 0.2 index values while the values were between 0.2 and 0.9 for species of *Salmonella* (Tables 5 and 6). Agarose gel electrophoresis (AGE) of isolated plasmid DNAs revealed the presence of plasmids which molecular weights clustered around 23.1 kb for both *Salmonella* and *Shigella* species (Table 7, Plates 1 and 2).

Drug resistance elimination with Acridine orange (AO) and sodium dodecyl sulphate (SDS) was conducted on multidrug resistant organisms. Resistance was eliminated in some organisms by both AO (25 µg/ml) and SDS (1 and 10% w/v concentrations). Seventeen (94.44%) of the 18 bacterial strains were not able to grow in the presence of 10% SDS. Eleven (11) of the 18 bacterial strains (61.11%) were not able to grow in the presence of 1% SDS and fourteen of the 18 bacterial strains (77.78%) were not able to grow in the presence of 25 µg/ml AO. There was loss of resistance in some of the drug resistant isolates as a result of the curing by acridine orange (25 µg/ml) and SDS (1 and 10%) (Tables 8 to 10).

Transfer of resistance traits was demonstrated from *Salmonella* sp. to *E. coli* and from *Salmonella* to *Shigella*

Table 2. Bacterial species isolated from 250 patients.

Number of human samples examined	Bacterial species isolated	Number of samples positive	Percentage (%) occurrence
250	*Shigella* species	50	43.48
	Salmonella species	65	56.52
Total		115	100.00

Table 3. Age and Sex Distribution of persons harbouring *Shigella* species in their faecal samples.

Age range	Number examined	Number infected	Percentage (%) infected	Number of males examined	Number of males infected	Percentage (%) infected	Number of females examined	Number of females infected	Percentage (%) infected
6 months - 4 years	64	33	66.00	38	19	67.86	26	14	63.64
5 - 17 years	28	9	18.00	12	5	17.86	16	4	18.18
18 - 30 years	36	3	6.00	16	2	7.14	20	1	4.55
31 - above	22	5	10.00	12	2	7.14	10	3	13.64
Total	150	50	100.00	78	28	100	72	22	100.00

but not from *Shigella* sp. to *E. coli* and from *Shigella* sp. to *Salmonella* sp. The percentage transfer of resistance was 100% from *Salmonella* sp. to *E. coli* stains and from *Salmonella* sp. to *Shigella* sp. (Table 11).

DISCUSSION

Drug resistance is a global crisis now threatening people's lives, livestock, and the economy (Anon., 2015). It has adverse impact on clinical outcomes and leads to higher costs due to consumption of health-care resources (WHO, 2014). This raises double-strength concern when it happens among organisms that are assuming endemic status in resource-poor areas like Anyigba. Enteric fever due to strains of *Salmonella* is, for example, a serious infection that poses problems for treatment due to drug resistance in many parts of the world. Previous reports have shown that drug resistance among *Salmonella* and *Shigella* are emerging global challenges especially in developing countries where there is an increased misuse of drugs in humans and animals (Reda et al., 2011; Kasper et al., 2005; Sule et al., 2012). One of the set objectives of this work was to carry out epidemiological survey of these two organisms among patients attending hospitals in Anyigba. The results show that a high percent (46%) of the patients do not only carry these pathogens but also carry multi drug resistant ones. This is higher than the overall prevalence of *Salmonella* occurrence (6.2%) obtained in Ethiopia by Beyene and Tasew (2014) but in tandem with the high resistance to Ampicillin they observed in their *Salmonella* and *Shigella* isolates. The isolation rate of *Salmonella* (56.52%) from human faecal samples in this study is similar to earlier work by Sule et al. (2012) who reported 60.0% incidence of *Salmonella typhi* infection. The relatively high incidence documented in the study was attributed to low sanitary condition of the town (Sule et al., 2012).

Shigella species were isolated more from age group 6 months to 4 years (66%), followed by the age group 5 to 17 years (18%) with the least from the age group of 18 to 30 years (10%). The

Table 4. Age and Sex distribution of persons harbouring *Salmonella* species in their faecal samples.

Age range	Number examined	Number infected	Percentage (%) infected	Number of males examined	Number of males infected	Percentage (%) infected	Number of females examined	Number of females infected	Percentage (%) infected
6 months - 4 years	10	7	10.77	5	3	9.10	5	4	12.50
5 - 17 years	10	8	12.31	5	4	12.12	5	4	12.50
18 - 30 years	50	30	46.15	30	16	48.48	20	14	43.75
31 - above	30	20	30.77	10	10	30.30	20	10	31.25
Total	100	65	100	50	33	100	50	32	100.00

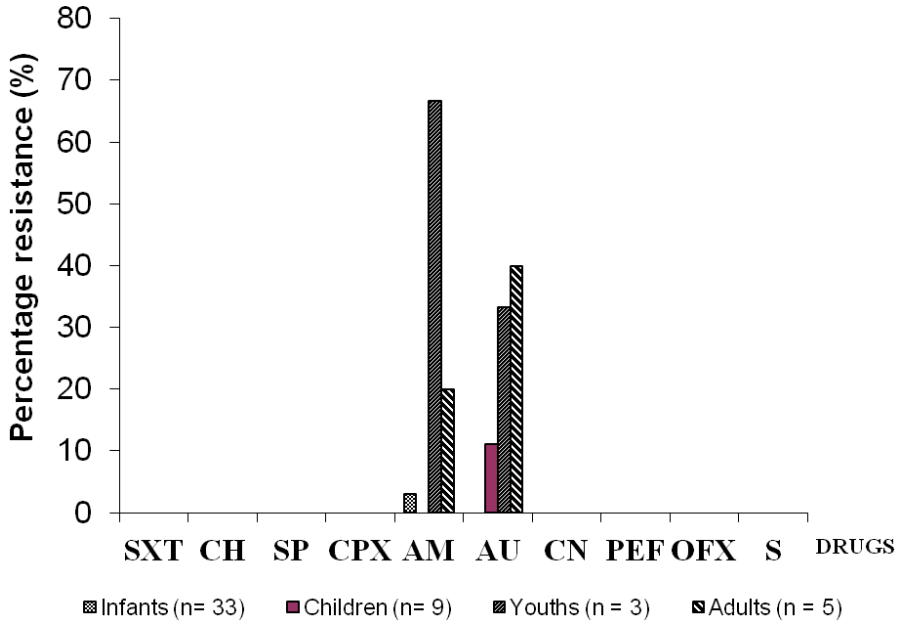

Figure 1. Drug resistance pattern of *Shigella* species isolated from infants (6 months - 4 years), children (5 - 7 years), youths (18 - 30 years) and adults (31 years - above). SXT= Septrin; CH= Chloramphenicol; SP= sparfloxacin; CPX= ciprofloxacin; AM= ampicillin; AU= augumentin; CN= gentamycin; PEF= pefloxacin; OFX= ofloxacin; S= streptomycin.

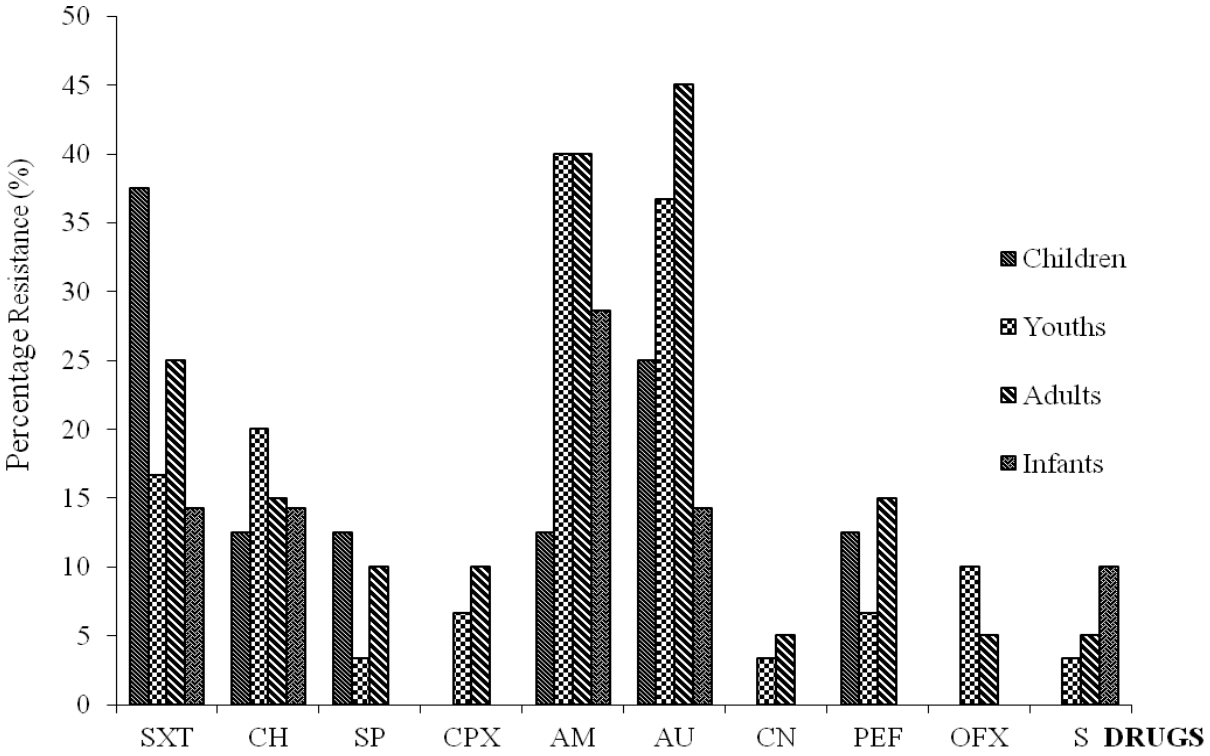

Figure 2. Drug resistance pattern of *Salmonella* species isolated from different age. SXT= Septrin; CH= chloramphenicol; SP= sparfloxacin; CPX= ciprofloxacin; AM= ampicillin; AU= augumentin; CN= gentamycin; PEF= pefloxacin; OFX= ofloxacin; S= streptomycin.

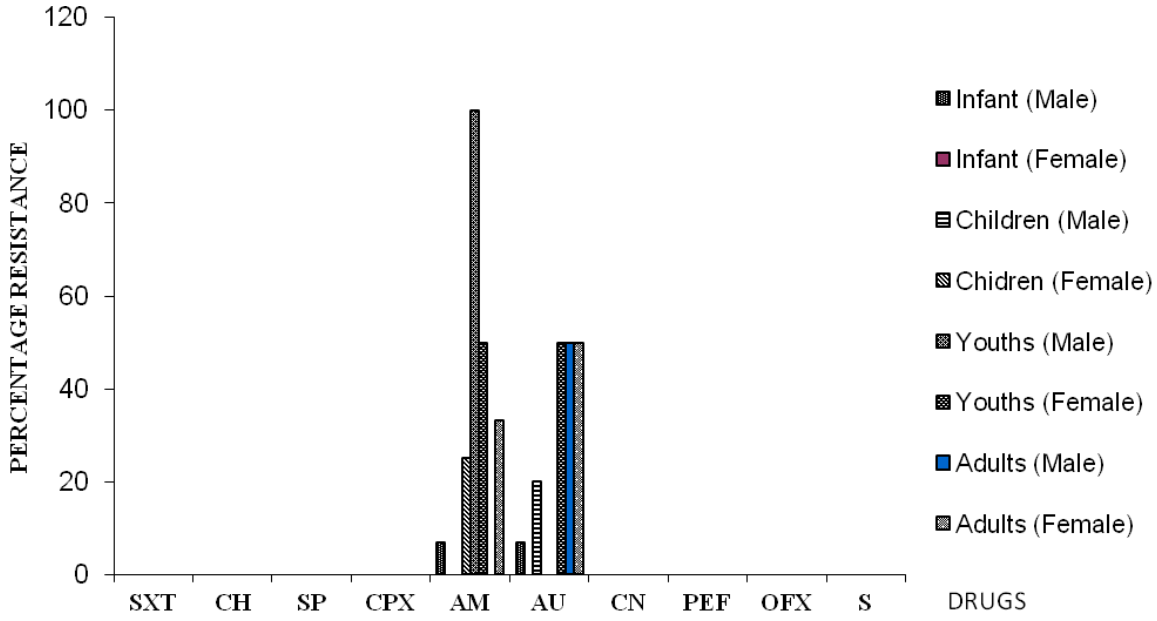

Figure 3. Drug resistance pattern of *Shigella* species isolated from different age. SXT= Septrin; CH= chloramphenicol; SP= sparfloxacin; CPX= ciprofloxacin; AM= ampicillin; AU= augumentin; CN= gentamycin; PEF= pefloxacin; OFX= ofloxacin; S= streptomycin.

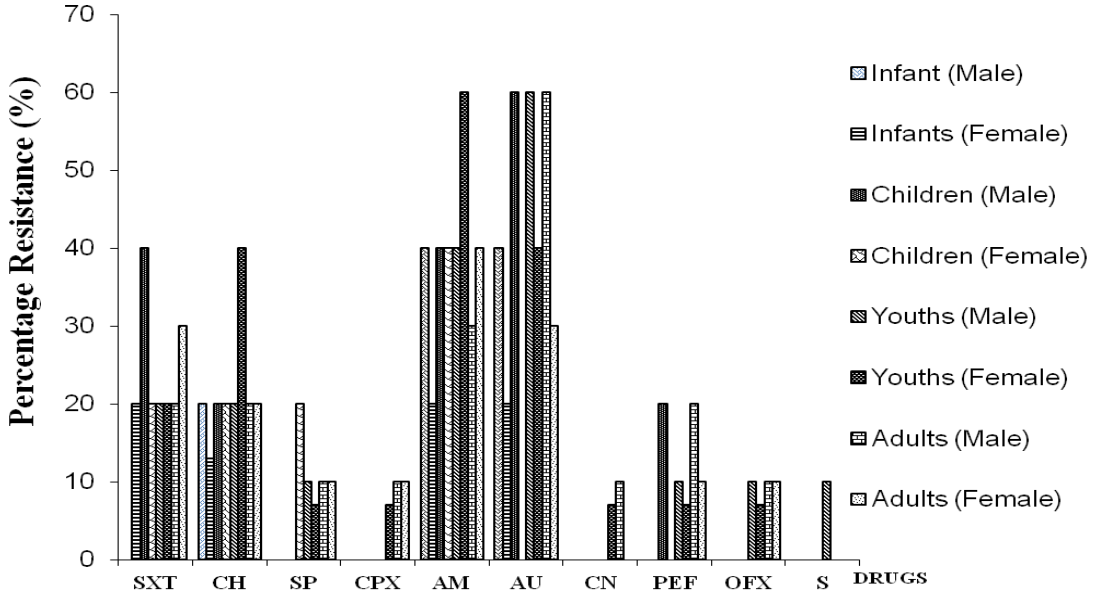

Figure 4. Drug resistance pattern of *Samonella* species isolated from males and females of different age groups. SXT= Septrin; CH= chloramphenicol; SP= sparfloxacin; CPX= ciprofloxacin; AM= ampicillin; AU= augumentin; CN= gentamycin; PEF= pefloxacin; OFX= ofloxacin; S= streptomycin.

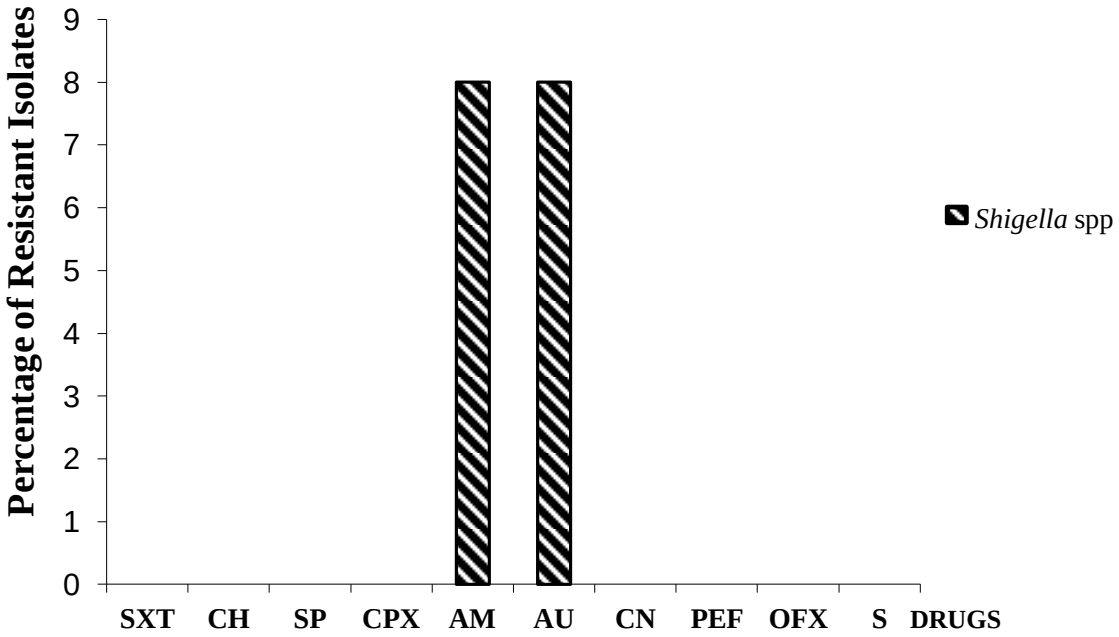

Figure 5. Aggregate Drug resistance pattern of *Shigella* species isolated from Human faecal samples SXT= Septrin; CH= chloramphenicol; SP= sparfloxacin; CPX= ciprofloxacin; AM= ampicillin; AU = augumentin; CN= gentamycin; PEF = pefloxacin; OFX= tarivid; S= streptomycin.

reason for the high incidence of *Shigella* species in age group 6 months to 4 years could be attributed to the fact that infants within this age group on their own cannot differentiate between what to eat and what not to eat.

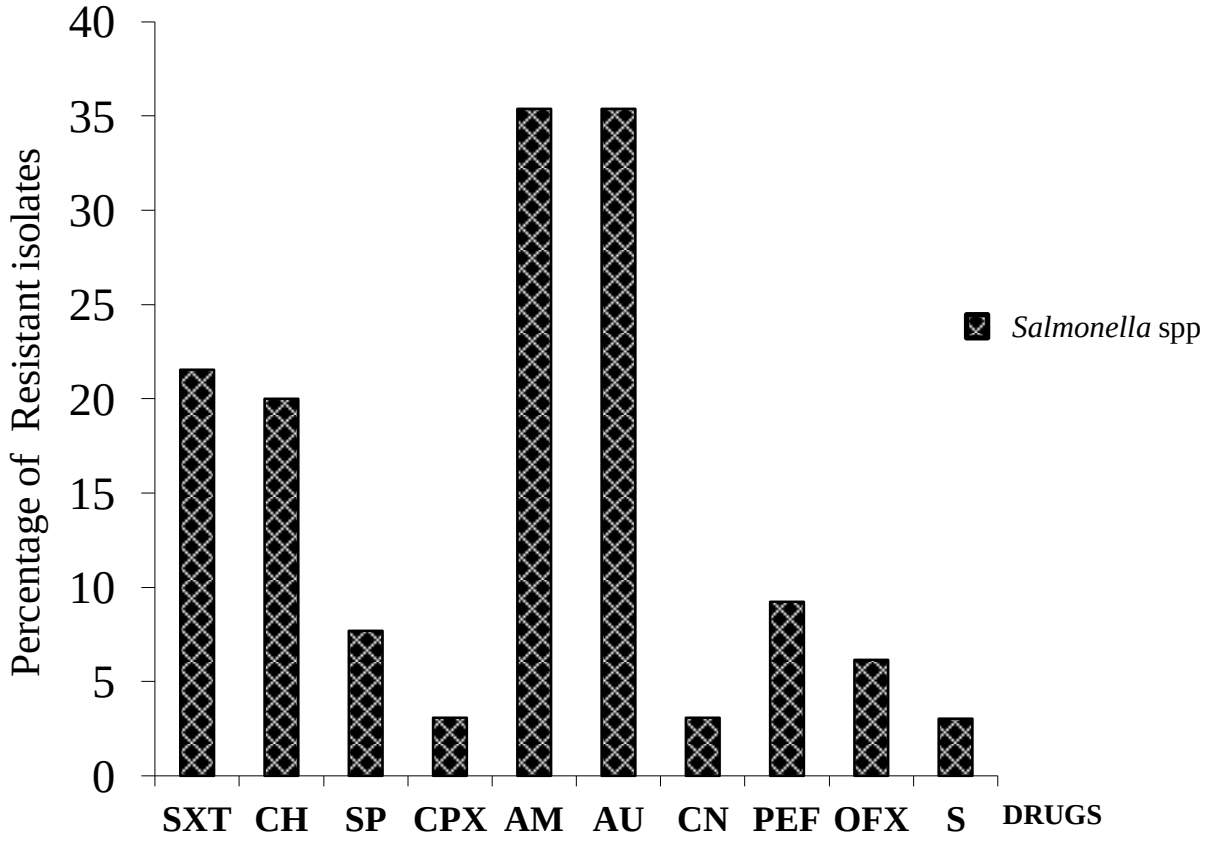

Figure 6. Aggregate Drug resistance pattern of *Salmonella* species isolated from Human faecal samples. SXT= Septrin; CH= chloramphenicol; SP= sparfloxacin; CPX= ciprofloxacin; AM= ampicillin; AU= augumentin; CN= gentamycin; PEF= pefloxacin; OFX= tarivid; S= streptomycin.

Table 5. Multiple Antibiotic Resistance Indices of *Shigella* species isolated from faecal samples.

Number of antibiotics resisted	Antibiotics resisted	Number of resistant isolates	Total Number of antibiotics tested	MAR index
2	AM, AU	1	10	0.2
2	AM, AU	1	10	0.2
2	AM, AU	1	10	0.2
2	AM, AU	1	10	0.2

AM = Ampicillin; AU = augumentin.

These infants have not learnt the rudiment of adherence to aseptic or hygienic practice and can barely express themselves (Sule et al., 2011). Sex distribution of persons harbouring *Shigella* species in their faecal samples showed higher occurrence in males than females. This could be attributed to the enhanced activities of males that increases their environmental exposure more than females and is in line with the result obtained from the work done by Beyene and Tasew, (2014) where they demonstrated the prevalence of intestinal parasite, *Shigella* and *Salmonella* species among diarrheal children in Jimma Health Centre, Jimma West Ethiopia, a cross sectional study in which male infants were more infected with *Shigella* than female ones.

Further analysis revealed that some of the isolates

Table 6. Multiple antibiotic resistance indices of *Salmonella* species isolated from faecal samples.

Number of antibiotics resisted	Antibiotics resisted	Number of resistant isolates	Total Number of antibiotic tested	MAR index
3	SXT, CH, AM	1	10	0.3
4	SXT, SP, AM, AU	1	10	0.4
9	SXT, SP, CH, CPX, SXT, CH, SP, AM, AU	1	10	0.9
5	SXT, CH, SP, AM, AU	1	10	0.5
3	AM, AU, PEF.	1	10	0.3
4	AM, AU, OFX, S	1	10	0.4
4	SXT, CH, AM, AU	1	10	0.4
8	SXT, SP, CH, CPX, AM, AU, PEF, OFX	1	10	0.8
5	SXT, AM, AU, PEF, OFX	1	10	0.5
4	SXT, CH, AM, AU	1	10	0.4
4	SXT, CH, AM, AU	1	10	0.4
3	SXT, AM, AU	1	10	0.3
2	AM, AU	1	10	0.2
4	SXT, CH, CPX, AM	1	10	0.4

SXT= Septrin; CH= Chloramphenicol; SP= Sparfloxacin; CPX= Ciprofloxacin; AM= Ampicillin; AU= Augumentin; CN= Gentamycin; PEF= Pefloxacin; OFX= Tarivid; S= Streptomycin.

Table 7. Distribution of plasmids in some species of *Salmonella* and *Shigella* isolates.

Bacteria isolates tested	Number of clones tested for presence of plasmids	Number of clones positive for plasmids	Percentage presence	Antibiotic resistance pattern	Molecular weight (kb)
Salmonella species	8	7	87.50	MDR and MDS	23.1
Shigella species	8	3	37.5	MDR and MDS	23.1

carried transferable multidrug resistance plasmids (Tables 7, 8, 9, 10 and 11; Plates 1 and 2) of high molecular weights. These have the semblances of the results obtained by Kim et al. (2004) in which plasmid presence and transfer of ESBL phenotype to *E. coli* was successful for all their 20 isolates. Also, as in ours, the electrophoresis of their plasmid DNA from wild-type *Shigella* spp. and the transconjugants showed the presence of plasmids ranging in size from 5.2 to 135Kb. Several studies have shown that bacterial plasmids can harbour different plasmid genes as well as the ability to transfer replica of these genes to other bacteria (Yah, 2010; Yah et al., 2007a). In this work, plasmids were effectively transferred from *Salmonella* to *E. coli* and from *Salmonella* to *Shigella* (across genus).

In addition to the detection of plasmid bands in Agarose gel electrophoresis, these conjugative plasmid transfers confirmed that the resistances observed are largely, if not totally, plasmid mediated. This has epidemiological and public health consequences considering that plasmid is a very important means of spreading interspecies resistance (Livermore, 2003).

There was a worrisome widespread occurrence of drug resistance and MAR indices (0.2 - 0.9) in our samples (Tables 5 and 6). MAR indexing is considered a good tool for risk assessment. It gives an idea of the number of bacteria showing antibiotic resistance and the consequent risk zone in a routine susceptibility testing (Akhter et al., 2014).

High MAR index values have been shown to be indicative of environments with high endemic disease potential and risk sources where drugs are used often (Hemen et al., 2012). MAR index values of ≥ 0.2 was observed in the drug resistant strains of *Salmonella* and *Shigella* used in this study, suggesting, as is consistent with earlier studies, exposure of the studied patients to bacteria and/or resistance traits from significantly contaminated sites or individuals (Hemen et al., 2012).

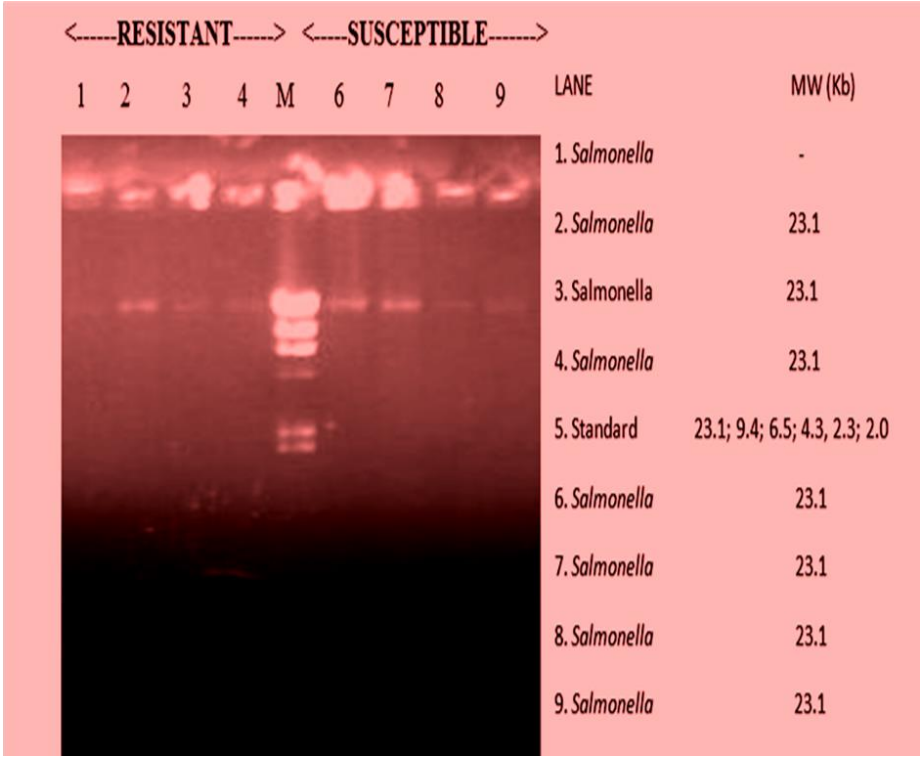

Plate 1. AGE of plasmid DNA from some MDR and MDS *Salmonella* species.

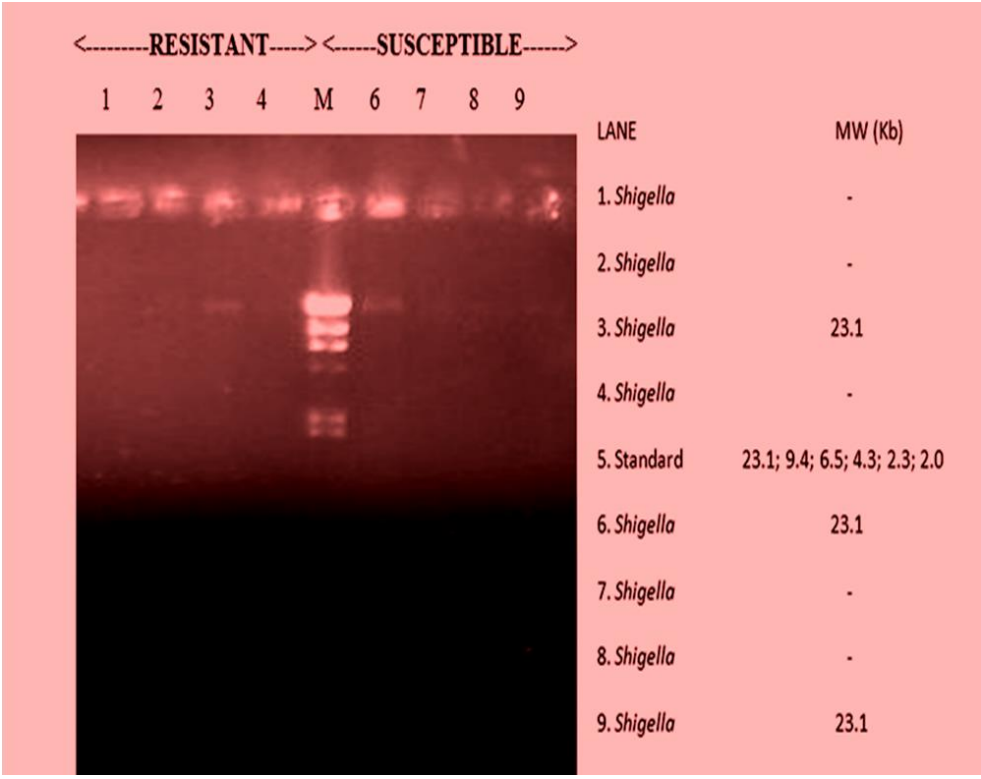

Plate 2. AGE of Plasmid DNA from some MDR and MDS *Shigella* species.

Table 8. Plasmid curing (10% SDS) of MDR *Salmonella* and *Shigella* isolates.

Test drug	Number of organisms resistant (pre-curing)	Number/percentage cured	Number/percentage resistant (post-curing)
Septrin	11	11 (100.00)	0 (00.00)
Chloramphenicol	8	8 (100.00)	0 (00.00)
Sparfloxacin	3	3 (100.00)	0 (00.00)
Ciprofloxacin	2	2 (100.00)	0 (00.00)
Ampicillin	18	17 (94.44)	1 (5.56)
Augmentin	16	14 (87.50)	2 (12.50)
Gentamycin	2	2 (100.00)	0 (00.00)
Pefloxacin	4	4 (100.00)	0 (00.00)
Tarivid	3	3 (100.00)	0 (00.00)
Streptomycin	2	2 (100.00)	0 (00.00)

Table 9. Plasmid curing (1% SDS of MDR *Salmonella* and *Shigella* isolates.

Test drug	Number of organisms resistant (pre-curing)	Number/percentage cured	Number/percentage resistant (post-curing)
Septrin	11	10 (90.09)	1 (9.91)
Chloramphenicol	8	7 (87.5)	1 (12.50)
Sparfloxacin	3	2 (66.67)	1 (33.33)
Ciprofloxacin	2	2 (100.00)	0 (00.00)
Ampicillin	18	11 (61.11)	38(38.89)
Augmentin	16	14 (87.50)	2 (12.50)
Gentamycin	2	2 (100.00)	0 (00.00)
Pefloxacin	4	3 (75.00)	1(25.00)
Tarivid	3	2 (66.67)	1(33.33)
Streptomycin	2	2 (100.00)	0 (00.00)

Table 10. Plasmid Curing of MDR *Salmonella* and *Shigella* isolates (25 µg /ml acridine orange).

Test drug	Number of organisms resistant (pre-curing)	Number/percentage cured	Number/percentage resistant (post-curing)
Septrin	11	10 (90.09)	1 (9.09)
Chloramphenicol	8	8 (100.00)	0 (00.00)
Sparfloxacin	3	2 (66.67)	1 (33.33)
Ciprofloxacin	2	2 (100.00)	0 (00.00)
Ampicillin	18	14 (77.78)	4 (22.22)
Augmentin	16	14 (87.50)	2 (12.50)
Gentamycin	2	2 (100.00)	0 (00.00)
Pefloxacin	4	4 (100.00)	0 (00.00)
Tarivid	3	3 (100.00)	0 (00.00)
Streptomycin	2	2(100.00)	0 (00.00)

This work is limited in scope by lack of adequate surveillance system. We therefore posit that more active surveillance and antibiotic stewardship for *Salmonella* and *Shigella* species infections are needed in Anyigba and by extension, Nigeria, to minimize the spread of these infections with the attendant public health

Table 11. Transfer of resistance traits among some species of *Salmonella, Shigella* and *E. coli.*

Donor		Recipient		
Bacteria	**Resistance pattern of donor**	**Recipient bacteria**	**Susceptibility pattern before transfer**	**Pattern after transfer**
Salmonella 103	Resistant to all antibiotics except S	*E. coli* 1	Sensitive to all antibiotics	Resistant to all antibiotics except S
		E. coli 2	Sensitive to all antibiotics	Resistant to all antibiotics except S
Shigella 4	Resistant to AM and AU	*E. coli* 3	Sensitive to all antibiotics	Sensitive to all antibiotics
		E. coli 4	Sensitive to all antibiotics	Sensitive to all antibiotics
Salmonella 86	Resistant to SXT, SP, AM, AU.	*Shigella* 20	Sensitive to all antibiotics	Resistant to all antibiotics
Shigella 16	Resistant to AM and AU.	*Salmonella* 91	Sensitive to all antibiotics	Sensitive to all antibiotics

consequences. This call is the desired aim of this study in line with earlier observation (Anon. 2015) showing that there are few public health issues of greater importance than drug resistance in terms of impact on society.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Abdel HH, Hawas S, EL-Daker M, El-Kady R (2008). Extended-spectrum Beta lactamase producing *Klebsiella pneumoniae*in neonatal intensive care unit. J. Perinat. 28:685-690.

Akhter A, Imran M, Akhter F (2014). Determination of multiple antibiotic resistance patterns and indexing among metal tolerant β-lactamase-producing *Escherichia coli.* Afr. J. Microbiol. Res. 8(7):619-627.

Akinjogunla OJ, Enabulele IO (2010). 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. J. Am. Sci. 6:1022-1033.

Akortha EE, Filgona JL (2009). Transfer of gentamycin genes among Enterobacteriaceae isolated from the out patients with urinary tract infections attending three hospitals in Mubi, Adama State. Sci. Res. Essays, 4(8):745-752.

Anon (2015). Nice Antimicrobial Stewardship: Right Drug, Dose, and Time? Editorial. Lancet 386: 717

Bartoloni A, Pallechi L, Benedetti M, Fernandez C, Vallejos Y, Guzman E, Liz A, Lucchetti C, Gamboa BH (2006). Multidrug - resistant commensal *Escherichiacoli* in Children in Peru and Bolivia. Emerg. Infect. Dis. 12(6):310-322.

Beyene G, Tasew H (2014). Prevalence of Intestinal parasite, *Shigella* and *Salmonella* species among Diarrheal children in Jimma health center, Jimma southwest Ethiopia: a cross sectional study. Ann. Clin. Microbio. Antimicrob. 13(10): 1-7.

Cheesebrough M (2004). District Laboratory Practice for Tropical Countries (part 2). Cambridge University Press, UK; pp. 180-197.

Clinical & Laboratory Standards Institute (CLSI) (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second informational supplement, M100 - S22. Clinical and Laboratory Standards Institute, Wyne, PA.

Dubois V, Parizano MP, Corinne A, Laure C, Bezian MC, Claudine Q (2007). High genetic stability of integrons in clinical isolates of *Shigella* spp. of worldwide origin. Antimicrob. Agents Chemother. 51(4):1333-1340.

Hemen JT, Johnson JT, Ambo EE, Ekam VS, Odey MO, Fila WA (2012). Multi-antibiotic Resistance of some Gram negative bacterial isolates from poultry litters of selected farms in Benue State. Inter. J. Sci.Technol. 1(8): 543-547.

Kado CT, Liu ST (1981): Rapid procedure for detection and isolation of small and large plasmids. J. Bacteriol. 145: 365-1373.

Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL (Eds). (2005). Harrison's Principles of Internal Medicine. New York. The McGraw-Hill Companies. pp. 897-906.

Kim S, Kim J, Kang Y, Park Y, Lee B (2004). Occurrence of Extended-Spectrum β-Lactamases in Members of the Genus *Shigella* in the Republic of Korea. J. Clin. Microbiol. 42(11):5264-5269.

Kraft R, Tardiff J, Krauter KS, Leinwand LA (1988). Using Miniprep Plasmid DNA for sequencing double stranded template with sequenase. Biotechniques 6:54-62.

Lech K, Brent R (1987). Miniprep of plasmid DNA, In Ausubel FM, Brent R, Kinston RE, Moore DD, Seiidman JG, Smith JA, Struchl K (ed). Current Protocols in Molecular Biology. John Wiley and Sons NY. pp. 161-184.

Livermore DM (2003) β-Lactamases in Laboratory and Clinical Resistance. Clin. Microbiol. Rev. 8:557-587.

Mahbubur R, Shereen S, Harunur R, Shams ELA, Baqui AH, Siddique AK, Nair GB, Sack DA (2007). Increasing spectrum in antimicrobial resistance of *Shigella*isolates in Bangladesh: Resistance to Azithromycin and Ceftriaxone and decreased susceptibility to ciprofloxacin. J. Health Pop. Nutr. 25(2):158-167.

Reda AA, Seyoum B, Yimam J, Andualem G, Fiseha S, Vandeweerd JM (2011). Antibiotic Susceptibility of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia. J. Infect. Dis. Immun. 3(8):134-139.

Sandle T (2014). Novel methods to Address Antimicrobial Resistance. SOJ. Microbiol. Infect. Dis. 2(1):1- 2.

Sheikh AR, Afsheen A, Sadia K, Abdu W (2003). Plasmid borne antibiotic resistance factors among indigenous *Klebsiella*. Pakistan J. 35(2):243-248.

Sule EI, Aliyu AM, Abdulaziz BM (2011). Isolation of diarrhoeagenic bacteria in Children attending some selected hospitals within Kaduna metropolis, Kaduna State, Nigeria. Cont. J. Appl. Sci. 6(1):1-6.

Sule WF, Adige, AA, Abubakar MO, Ojezele MO (2012). Antimicrobial resistance of clinical isolate of *Salmonella typhi* in Anyigba, Kogi State, Nigeria. Global Adv. Res. J. Microbiol. 1(4):57-61.

Uma B, Prabhakar K, Rajendran S, Sarayu YL (2010). Prevalence of extended spectrum beta lactamases in *Salmonella* species isolated from patients with acute gastroenteritis. Indian J. Gastroenterol. 29: 201-204.

World Health Organisation (WHO) (2014). Antimicrobial Resistance

Global Report on Surveillance. X1 - 28.
Yah SC (2010). Plasmid-Encoded Multidrug Resistance: A Case study of *Salmonella* and *Shigella* from enteric diarrhea sources among humans. Biol. Res. 43:141-148.
Yah SC, Eghafona NO, Oranusi S, Abouo AM (2007a). Widespread plasmid resistance genes among *Proteus* species in diabetic wounds of patients in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. Afr. J. Biotechnol. 6(15): 1757-1762.