Prevalent And Antibiotic Resistance Patterns Of Shigella Species Isolated From Blood Samples Of Hospitalized Patients In Kano, North-West, Nigeria.

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Authors' contributions
This work was carried out in collaboration between all authors. Author A.M. conceived and designed the study, wrote the protocol, and wrote the draft of the manuscript.

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Abstract—Aim: The aims of the study were to determine the prevalent species of Shigella and the antibiotic resistance patterns of Shigella isolates recovered from the blood samples of hospitalized patients in Kano metropolis.

Study design: The study is a descriptive cross-sectional study.

Place and duration of study: One milliliter of venous blood was collected from each patient with some or all clinical features of Shigellosis that sign a consent form and transfer into EDTA bottles. If daily is unavoidable blood samples were stored at 4 ^{0}C. Samples were analyzed at the both laboratories of the authors. This work was carried out between May, 2012 and March, 2014.

Methodology: The blood specimens were cultured in thioglycollate broth and sub-cultured onto deoxycholate citrate agar (DCA), Salmonella-Shigella agar (SSA) and brilliant Green agar (BGA) followed by confirmation of presumptive colonies using different biochemical tests and analytical profile index 20E. Serologic identification of Shigella was performed by slide agglutination test using polyvalent O and H Shigella antisera. Antibiotic susceptibility studies were performed by the disc diffusion method using ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid and Trimethoprim-sulfamethoxazole.

Results: Although, the relationship between different age groups was not significantly associated ($P < 0.05$), patients under age bracket of 21-30 years were found to be more susceptible to Shigella infections with 13 representing 2.6% followed in that order by 11-20 years (6), , ≤10 - years (4) 31-40 years (3) and >40 years (2) age groups, representing 1.2%, 0.8%, 0.6% and 0.4% respectively. The result of in vitro – antibiotic susceptibility testing of Shigella isolates using disc diffusion test demonstrated that most of the isolates (2.2%) was significantly ($P >0.05$) resistant to ampicillin followed by nalidixic acid, chloramphenicol, and cotrimozaxole in decreasing order. However, ciprofloxacin remained effective against all the Shigella isolates

Conclusion: The frequency of Shigella infections was highest among 21-30 year age group lowest in ≥40 year age group. However the rates of infection among all the six (6) age groups were not significantly associated. The prevalent rate of Shigella infections was significantly higher ($P > 0.05$) in males than the females' patients. However, Shigella flexneri was the most common among patients followed by Shigella dysenteriae, Shigella boydii and Shigella sonnei. in decreasing order. Ciprofloxacine remained effective against all the Shigella isolates tested. 37 isolates were found resistant to 1 of 3 first-line anti-Shigella antibiotics (ampicillin, cotrimoxazole and chloramphenicol).

Keywords—Shigella species; blood samples; multiple antibiotic resistance; Kano-Nigeria.

1. INTRODUCTION

Shigella is named after Kiyoshi Shiga, who was the first scientist to isolate Shigella dysenteriae type 1 in the year 1896 during a large epidemic of dysentery in Japan.[1] Shigellosis is a collective description for infectious diseases caused by members of the bacterial genus Shigella, whose transmission occurs mostly via the faecal–oral route. Most Shigella outbreaks are associated with over-crowding, poor personal hygiene and abysmal conditions in daycare centres, nursing homes, custodial institutions, cruise ships, aboriginal reservations and refugee camps[2] with contaminated food or water serving as the vehicle for infection. The infectious dose of Shigella spp. is low, with 10–100 bacteria/mL sufficient to cause the disease.[3] In developed countries, shigellosis

transmission has also been linked to oro-genital and oro-anal sexual contact between men[4] and more recently with underlying human immunodeficiency virus[5]. Shigellosis is endemic throughout the world and it is among the most common pathogens associated with bacterial diarrhoeal diseases.6 It is particularly common in developing countries where affected populations are immunologically compromised due to poor nutrition and background infections, leading to high morbidity attributed to shigellosis.[1,4,7] Although epidemic Shigella dysentery is the most serious manifestation of Shigella infection in developing countries, the majority of Shigella infections are due to endemic shigellosis. Epidemiological reports have shown that shigellosis is responsible for approximately 165 million cases annually, of which 163 million (98.8%) are in developing countries and 1.5 million in the industrialized countries.[7] It has also been estimated that between 600,000 and 1.1 million (mean 850,000) people die annually from Shigella infection[7–8] and nearly 580,000 cases of shigellosis are reported among travellers from industrialized nations travelling to developing countries.[9] The genus Shigella comprises four subgroups that historically have been considered species, and these four Shigella spp. are recognized as pathogenic to humans. Subgroup A is referred to as S. dysenteriae, subgroup B as S. flexneri, subgroup C as S. boydii and subgroup D as S. sonnei.[10] Both S. sonnei and S. boydii are usually associated with mild illness of short duration in which the stool may be watery or bloody.[11] S. flexneri is generally more severe, lasts longer, causes dysentery more frequently and is the principal cause of endemic shigellosis in many developing countries.[12] Of all serotypes of Shigellae, particularly type 1 S. dysenteriae, attracts special attention for its endemic and epidemic-causing potential as well as its high attack rate, high case-fatality rate, and various complications.[13]

There are rampant indiscriminate prescriptions of antibiotics for the treatment of Shigella infection and sales of expired, fakes and substandard antibiotics [14,15]. This has been an important problem in the treatment and susceptibility of Shigella species to antibiotics. Antibiotics use increases the spread of antibiotics resistant bacteria. This is called 'selective pressure'[16,17]. As bacteria become resistant to increasing number of antibiotics, the remaining antibiotics are used more often; increasing the selective pressure of Shigella and therefore, making them more resistant [18,19,20]. In Nigeria most typhoid endemic areas, culture facilities are often available but have not been used for the routine laboratory diagnosis of enteric fever and blood cultures may be negative in patients who have taken antibiotics prior to the test [21].

However, majority of antibiotic resistance studies on Shigella in the study area are based on the use of isolates obtained from stool samples of patients with Shigella infection admitted into hospitals. Recent studies indicate that antibiotic resistance genes are a growing problem indicating the need to pay closer attention on the isolation of Shigella in blood and community acquisition of multiple antibiotic resistance in Shigella [22,23].

2. MATERIALS AND METHODS

2.1 Hospitals

The six most patronized hospitals were randomly selected including one Teaching Hospital (Aminu Kano Teaching Hospital), three specialist hospitals (Murtala Mohammed Specialist, Mohammed Abdullahi Wase Specialist and Sir Sunusi Specialist Hospital), one General Hospital (Sheik Waziru Gidado General Hospital) and one Private Hospital (Khadijat Memorial Private Hospital). All are situated within Kano metropolis. The selected hospitals are reference hospitals in the state where people from various parts of the state and neighboring states of various occupations attend. They gave more than 70% of health care delivery in the state at large.

2.2 Patients and Specimens

Patients (in and out) who patronized the six selected hospitals with some or all clinical symptoms of Shigella infections (i.e. vomiting, diarrhoea, headache, abdominal pain, body ache, breathlessness, weight lost, constipation and anaemia) recruited to sign the consent form were used for the study.

Any patient (in and out) who brought his blood specimen to the laboratory reception of one of the six selected hospitals for widel test, malarial test and other related blood tests recruited to sign the consent form were used for the study.

Blood (1ml) collected from each patient diagnosed positive for Shigellosis was used as sample for the study.

2.3 Collection and Handling of Specimens

One milliliter of venous blood was obtained using sterile syringe from an antecubital vein of each patient recruited for the study and dispensed immediately into 10ml thioglycollate broth. Sterile bijou bottle that contained blood and 10ml thioglycollate broth was then labeled with specimen number, type of medium and date of dispensing [18].

2.4 Isolation and identification of Shigellae

2.4.1; Presumptive isolation of Shigella

One milliliter of venous blood specimen was dispensed into 10ml thioglycollate broth and sub-cultured onto SSA, BGA and DCA agar everyday and incubated aerobically at $37^{o}C$ for 7 days [22]. The cultured plates, SSA, BGA and DCA agar were examined for the presence of typical colonies of Shigella based on cultural and morphological characteristics, that is, transparent colonies with black centre on SSA and pink colonies surrounded by a red medium on BGA, and small red translucent and or

dome-shaped colonies, which may have central black spot due to hydrogen sulphide production [18].

Bacterial isolates obtained were further sub-cultured by stabbing into nutrient agar slants and stored at 4°C after aerobic incubation 37°C for 24 hours for subsequent analysis.

2.4.2; Purification of isolates

Presumptive culture of *Shigella* stored in nutrient agar slant was sub-cultured onto SSA aerobic incubation 37°C for 24 hours to observe for the colonial characteristics of *Shigella* and isolation of pure culture for subsequent biochemical characterizations.

2.4.3; Biochemical characterization of *Shigella*

Isolation and identification of organisms were carried out as described by ISO [24], Habtamu *et al.* [16], and OIE [25]. A 24 h pure culture of each isolate was used to determine their gram stain reaction. The following biochemical tests were carried out: Indole test, triple sugar iron test, citrate test, methyl-red test, Voges-Proskauer test, lysine decarboxylase test, ornithine decarboxylase test, urease test, sugar (trehalose, sucrose, inositol, glucose, dulcitol, maltose, mannitol, melibiose, salicin, rhamnose and arabinose) fermentation test and motility test. Isolates were further characterized using commercially available identification system-Analytical Profile Index (API) 20 E test kit (Biomerieux, France) following the manufacturer's guideline.

2.5 Sero-typing of the isolates

Serological identifications of presumptive *Shigella* were performed by slide agglutination test. Presumptive isolates of *Shigella* obtained from the series of biochemical tests were screened serologically with *Shigella flexneri, Shigella dysenteriae*, *Shigella boydii* and *Shigella sonnei* antisera.

An agglutination test was performed on a clean glass slide. The slide was divided into sections with a wax pencil and one small drop of physiological saline was placed in each test section on the slide. By using a sterile inoculating loop a portion of growth from the surface of TSI agar was removed and emulsified in each drop of physiological saline on the slide. It was then mixed thoroughly to create a moderately milky suspension. A bent inoculating loop was used to pick a small drop of antiserum and transferred to one of the suspensions; the second suspension served as the control (usually approximately equal volume of antiserum and growth suspension was mixed). The suspension and antiserum were mixed very well and then the slide was tilted back and forth to observe for auto-agglutination (agglutination is more visible if the slides is observed under a bright light and over a black background) [26].

If clumping appeared within 30 to 60 seconds the reaction is positive, the saline suspension (control) was examined carefully to ensure that it is even and does not show clumping resulting from auto agglutination. If auto-agglutination occurs, the culture is termed "rough" and cannot be serotype. When positive agglutination reaction was obtained in one of the antisera, the *Shigella* species *Shigella flexneri*, *Shigella dysenteriae*, *Shigella boydii* or *Shigella sonnei*. subgroup was confirmed, and no further testing with antisera needed to be conducted [26].

2.6 Test of antibiotic sensitivity of the *Shigella* isolates.

In-vitro susceptibility of *Shigella* isolates to various routine antibiotics was tested by the standard disc diffusion technique [1].

2.6.1 Standardization of inoculum

This was done as described by CLSI [27]. Pure culture of identified *Shigella* isolate (s) from an 18-hour plate culture was selected. Sterile wire loop was used to pick 3 colonies of each *Shigella* serotype and emulsified in 5 ml of sterile normal saline. The tube containing the bacterial suspension was inserted into a sensititre nephelometer (TREK Diagnostic systems, UK) after calibration. Adjustment was made with extra inoculum or diluents, if necessary, until 0.5 McFarland standards were obtained. Fifty microliter of the broth was further transferred into 5 ml of Mueller-Hinton broth (Oxoid, UK) in a tube [27].

2.6.2 Inoculation of test plates

Optimally, within 10 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the standardized suspension in Mueller-Hinton broth. The dried surface of a 20 ml Mueller-Hinton agar plate in a 100 mm disposable plate (STERILIN, UK) was inoculated by streaking with the cotton swab over the entire sterile agar surface. The inoculated plates were air dried at 37ºC to allow for any excess surface moisture to be absorbed before applying the antibiotic discs [27].

2.6.3 Application of discs to inoculated agar plates

All positive cultures of *Shigella* species isolated from blood samples were tested *in vitro* for susceptibility to different antibiotic by agar diffusion technique as described by Kirby-Bauer [28] and WHO [29]. This was carried out according to WHO protocol [30]. The susceptibility testing of *Shigella* isolates were carried out using Mueller Hinton agar and were tested *in vitro* for susceptibility to five different antibiotics; ampicillin (25µg), chloramphenicol (30µg), ciprofloxacin (25µg), nalidixic acid (30µg) and Trimethoprim-sulfamethoxazole (30µg) [31].

The inoculated plates were air dried under aseptic condition to eliminate the liquid on the surface of medium, sterile forceps was used to place the antibiotic discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was inverted and incubated at 35°C for 18 hours. Meter ruler was

then used on the underside of plate to measure the diameter of each zone of inhibition in millimeter. Zone diameter for ATCC 25922 was compared with NCCLS Published Limits; Interpretative chart was then used to interpret the zone sizes of Inhibition [31].

Results were recorded as susceptible, intermediate susceptible or resistant based on the zones size of each antibacterial disc used [30, 31].

2.9 Statistical analysis of results

Statistical Package for Social Science (SPSS) version 14 was used [32]. Descriptive statistics were used to categorical (frequency percentages) variables. Chi-square test analysis was use to determined association between the resistant rate of *Shigella* isolates and antibiotics activities.

3. RESULTS

3.1 Bacterial isolation.

Of the five hundred blood specimens sampled from six selected hospitals studied, total of ninety (90) bacterial isolates and thirty nine (39) *Shigella* positive specimens were recorded: 110 were collected from Murtala Mohammed Specialist Hospital, 100 from Aminu Kano Teaching Hospital, 90 from Mohammed Abdullahi Wase Specialist Hospital, 80 from Sir

Sunusi Specialist Hospital, 60 from Sheik Waziru Gidado General Hospital and 60 from Khadijat Memorial Private Hospital.

3.2 *Shigella* identification by biochemical characterization.

Out of ninety (90) bacterial isolates obtained from six selected hospitals studied, Sixty (60) presumptive *Shigella* isolates were obtained from various biochemical characterization and identification test.

3.3 Sero-typing of the *Shigella* isolates.

Twenty eight (28) *Shigella* isolates were obtained after serologic identifications of presumptive *Shigella* isolates were performed by slide agglutination test.

3.4 The distribution of *Shigella* infections in relation to age and sex

Although, the relationship between different age groups was not significantly associated ($P < 0.05$), patients under age bracket of 21-30 years were found to be more susceptible to *Shigella* infections with 13 representing 2.6% followed in that order by 11-20 years (6), ≤10 years (4) 31-40 years (3) and >40 years (2) age groups, representing 1.2%, 0.8%, 0.6% and 0.4% respectively (Table 1).

Table 1: The distribution of *Shigella* infections in relation to age among hospitalized patients in Kano metropolis, Nigeria.

Age group (Years)	No. examined	No. (%) infected
≤10	46	4(0.8)
11-20	135	6(1.2)
21-30	172	13(2.6)
31-40	80	3(0.6)
≥40	67	2(0.4)
Total	**500**	**28(5.6)**

__KEY:__ ≥ = Greater than or equal to; ≤ = Less than or equal to; % = Percentage of total

number of specimen tested (500).

When the 104 infected patients where assed in relation to sex, the prevalence rate of *Shigella* infections was significantly higher ($P > 0.05$) in males than the females patients with 68 (13.6%) and 36 (7.2%) respectively (Table 2).

Table 2: The distribution of *Shigella* infections in relation to sex among hospitalized patients in Kano metropolis, Nigeria.

Sex	No. infected (%)	No. not infected (%)	Total (%)
Males	18(3.6)	304(60.8)	322(64.4)
Females	10(2.0)	188(37.6)	198(35.6)
Total	**28(5.6)**	**492(98.4)**	**500(100)**

Fisher's exact test two sides P value = 0.0081

Fisher's exact test two sides P value = 0.0081

<u>**KEY:**</u> *NO. = Number; % = Percentage of total number of specimens tested (500).*

3.5: The prevalence serovers of *Shigella*

From Figure 1 out of 104 presumptive *Shigella* isolates obtained in this study, *Shigella flexneri* was the predominant with 14(2.8%) followed by *Shigella dysenteriae* 8(1.6%), *Shigella boydii* 4(0.8%) and *Shigella sonnie* was the least prevalent serovar with 2(0.4%) prevalence rate. However, the relationship between each *Shigella* species obtained was also statistically significant ($P > 0.05$).
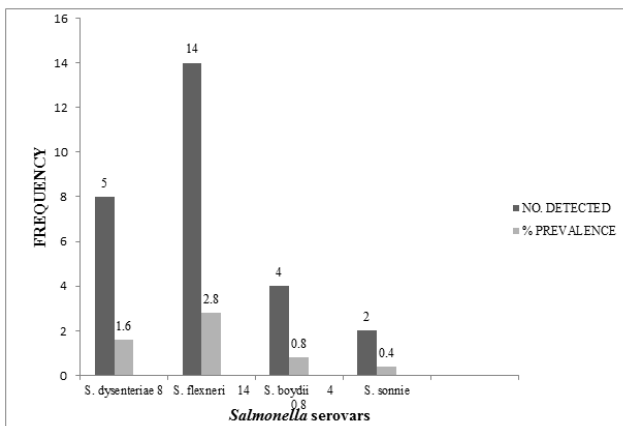

Figure 1: The prevalence serovers of *Shigella* from blood specimens of hospitalized patients in Kano metropolis, Nigeria.

t= 3.059 P value = 0.0377

<u>**KEY:**</u> *S. = Shigella; No. = Number; % = Percentage of total number of Shigella isolated*

(104).

3.6 Antibiotic sensitivity of the *Shigella* isolates.

Out of 28 *Shigella* species isolated, 5 were triple, 12 double, and 8 single-antibiotic resistant phenotypes. twenty (71.4%) of the isolates displayed resistant to at least one or two antibiotics and five (17.9%) displayed resistant to three of antibiotics tested. However, the remaining two (7.1%) isolates did not display resistance to any of the five (5) antibiotics tested (Table 3).

Similarly, 5 isolates were found resistant to 1 of 3 first-line anti-*Shigella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol). In addition, 12 isolates were found resistant to 2 of 3 first-line anti-*Shigella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol), none of the isolates were resistant to all three first-line anti-*Shigella* antibiotics (multi-antibiotics resistant Shigellae) (Table 3).

Table 3: Multiple antibiotic resistance patterns of *Shigella* species isolated from blood specimens of hospitalized patients in Kano metropolis, Nigeria.

Single Resistance types	Number with type	Multiple antibiotic resistance patterns/phenotypes		
		Number of antibiotics	Number of isolates with pattern	Resistance patterns/phenotypes
AMP.	8	2	7	AMP., NA.
			3	AMP., COT.
			2	CH., NA.
		3	4	AMP., CH., NA.
			1	AMP., COT., NA.

KEY: *AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole; NA= Nalidixic Acid*

The result of *in vitro* -antibiotic susceptibility testing demonstrated that of the five (5) antibiotics tested, most of the 28 isolates (2.2%) was significantly ($P > 0.05$) resistant to ampicillin. They were also resistant to nalidixic acid, chloramphenicol, and cotrimozaxole in decreasing order with 1.8% and 1.0%, resistance rate respectively. Ciprofloxacin remained effective against all the *Shigella* isolates tested.

Table 4: Disc diffusion test on *Shigella* species isolated from blood samples of hospitalized patients in Kano metropolis, Nigeria.

Antibiotic	Disc potency (µg)	No. (%) of Resistant Isolates n= 28
AMP	25	11(2.2)
CH	30	5(1.0)
COT	25	3(0.6)
CPX	30	0(0.0)
NA	30	9(1.8)

$X^2 = 11.03$ *P value = 0.001*

No. = Number; n= Number of isolates tested; S = Shigella; % = Percent; µg = microgram;
AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole;
NA = Nalidixic Acid; % = Percentage of total number of each Shigella species tested.

4. DISCUSSION

In the present study, the highest infections were recorded from 21-30 years age groups and least infection was recorded from patients with ≥40 year age group. However the rate of infection among all the six (6) age groups was not significantly associated. In this study, male were more infected than their female counter part in the same age groups. In addition, the rates of infections between males and females patients were statistically significant ($P > 0.05$). This

work is in consonance with the findings of Adkins and Santiago [33]; Abdullahi [34] and Mashi [35].

The highest incidence in males of 21-30 years age group patients recorded in this study is probably because most of the males in the study area were more prone to contaminations than their female counter part of the same age group. Males usually eat and drink outdoors and they do not recognize the state of the food or drink they eat and the nature of the environment in which the food and drink are prepared [35]. It was further observed that the high prevalence of *Shigella* infection in males of 21-30 years age group is connected with water exposure by these individuals in their community, who are supposed to be demographically and economically active and productive individuals in the communities, because they are more exposed to occupational hazard of farming, related water contact activities, contaminated environment, food and drink than children and elderly persons (Adkins and Santiago, 2006). Similarly, sharing of public toilet in school, market, bus stop by male would probably increase the level of infection among the male [34].

However, the low community awareness level of males of this age on the routes of *Shigella* infection may increase the transmission potential of the disease since individuals would not care to protect them or take other cautions when exposure becomes necessary. It was further observed that even among informed males awareness level does not play important role in discouraging water activities, and practices have become necessary as they are the major means of subsistence; in term of irrigation, agriculture, gardening, outdoors business and other occupational hazards [36,37].

Females are less likely to be found eating, drinking and defaecating outdoors. This is because of culture and religious inclination. Moreover, females are less exposed to occupational hazard of farming, related water contact activities, contaminated environment, contaminated food and drink than their males' counterpart [36]. It was observed that males were responsible for 98% of activities involving contamination and water exposure [35].

On the other hand, adults (≥40 year age group) appreciate the quality of health they have, they are wise, matured and therefore, protect and maintain most of their valuable physical health. In addition, the adults do not usually predispose themselves to various contaminated areas than youth [35]. Furthermore, the presence of little or no infection in older individuals may be connected with the less water contact activities thus reducing the risk of contracting infection. Another inference is the possibility of developed immunity by the older individuals who might have contracted the disease in their young age. Therefore, it can be concluded that such insusceptibility could reflect on age dependent acquired immunity [38].

Varius studies have been conducted by many researchers in different parts of the world establishing significance of *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei* in the causation of shigellosis. The above four species of *Shigella* were also encountered in the present study among the *Shigella* species isolated *Shigella flexneri* was the most common among patients followed by *Shigella dysenteriae, Shigella boydii* and *Shigella sonnei*. This agrees with the work of Zubairu (2002), Iwalokun et QO01) as well as Ngozi Onyenekwe (2003). The infective dose often to one hundred (10-100) bacilli only needed to initiate infection makes exposed patients easily infected (Gallies, 2004; Stephen, 2004)

In addition, in the study area many other reports indicated that multiple antibiotic resistances (MDRs) to *Shigella flexneri* were among the most frequent Shigellae which are responsible for high infection among carriers and high opportunistic infections among AIDS patients. There is also diagnostic dilemma in the issue of chronic carriers, in the study area, the *Schistosoma haematobium* is common, and it was reported that a chronic urinary carrier state is resulted from localization of typhoid bacilli in schistosomiasis, the *Schistosoma-Shigella* complex [41]. The Patient will become a variant that has chronic foci of infection but whom *Shigella* is shed intravascularly rather than in stool or urine [41]. Similarly, as few as 10^3 mean infective dose of *Shigella flexneri* organisms is required to produce clinical or subclinical infection in humans but perhaps 10^5 -10^8 Shigellae is required for other Shigellae in African countries. Furthermore, waterborne disease outbreaks are often unreported or under reported in developing countries because of the lack of systematic studies. Presently and, indeed, in the future Kano may not be an exception in this regard [34].

In this study, most of the *Shigella* isolates from patients in Kano were significantly (X^2 11.03, *P* value = 0.001) resistant to ampicillin (*P*< 0.05) with 11 resistant strains representing 2.2% of the *Shigella* isolates, followed by nalidixic acid with 9 resistant strains representing 18.0%. Ciprofloxacin remained effective against all the *Shigella* isolates tested. However, the relationship between *Shigella* species and antibiotics tested was not statistically significant (*p* > 0.05). Twenty (71.4%) of the isolates displayed resistant to at least one or two antibiotics and five (17.9%) displayed resistant to three of antibiotics tested.

The highest significant resistance (*P* < 0.05) of *Shigella* isolates to ampicillin and nalidixic acid could probably be due to the usage of antibiotics in the study area which is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine [44, 13]. However, the rate of development of resistance appears to have accelerated in the past decade and today multiple resistant *Shigella* constitutes a global

problem [47]. It has been observed that antibiotic susceptibility of *Shigella* isolates is not constant but dynamic and varies with time and environment. This therefore demands the need for periodic screening of common pathogens for their antibiotic susceptibility profiles in different communities. There is strong evidence that the use of antibiotics can lead to the emergence and dissemination of resistant Shigellae, which can then be passed onto people via food or through direct contact with animals. During recent years the wide spread use of antibiotics in the field of veterinary medicine have resulted in the development of increasing number of bacterial strains possessing resistance to many antibiotics. The property of multiple antibiotics resistance could be transferred through conjugation from resistant strains of Shigellae, to another by means of plasmid, which occur in cytoplasm of the donor bacterium and multiply independently of the chromosomal DNA. Thus a new bacterium with resistance factor emerges that is resistant to one or more antibiotics. In another instant the high resistance of *Shigella* isolates to commonly used antibacterial drugs is probably due to some factors ranging from the use of fake antibiotics, abuse and misuse of those antibiotics found commonly in circulation among the general populace and health resources centers [47, 46].

Abdullahi [36] reported that, acquired antibiotics resistance is a growing worldwide problem due to the increasing use of antibiotics in humans, animals, and agriculture. In developing countries the situation is particularly serious for the following reasons: In many countries, antibiotics can be obtained outside of recognized treatment centres, and taken without medical authorization or supervision. This leads to inappropriate use of antibiotics and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibiotic, results in an incomplete course being purchased, sufficient only to alleviate symptoms. Patients are not sufficiently informed about antibiotics and their use [18,36]. Problems also arise when antibiotics sold in local markets are sub-standard or expired antibiotics. Guidelines regarding the selection of antibiotics, correct prescription, and information about antibiotic resistance and how to minimize its spread are not communicated to those purchasing the antimicrobials. Antibiotics are often prescribed when they are not needed or for self-limiting infections, e.g. diarrhoeal disease and viral respiratory infections [18, 36].

Other overlapping problems are worsening the situation regarding typhoid fever and other *Shigella* infections within Africa: the failure to control the spread of the *Shigella* species involved, due to unclean water, poor sanitation, malnutrition, the failure to control resistant organisms and resistance genes so that, when infections occur, they produce more adverse consequences. It is perhaps obvious, if unaddressed, that poor and displaced persons in Africa are least likely to be able to access potable water, safe sanitation, and other factors to prevent faecal-oral infection and that public health facilities need to be strengthened to protect the poor [48].

Broad spectrum antibiotics are frequently used prophylactically, e.g. ampicillin. Laboratory facilities for accurate diagnosis and isolation of pathogens are often not available, resulting in an overuse and inappropriate use of antibiotics [49]. Many countries do not have effective surveillance of important antibiotic-resistant bacteria. Training and facilities for performing standardized antibiotics sensitivity tests are often lacking. Developing countries are often unable to afford costly second-line antibiotics to treat infections due to resistant organisms. This results in prolonged illness with longer periods of infectivity and to the further spread of resistant strains [18].

5. CONCLUSION

In addition, the frequency of *Shigella* infections was highest among 21-30 year age group lowest in ≥40 year age group. However the rates of infection among all the six (6) age groups were not significantly associated. The prevalent rate of *Shigella* infections was significantly higher ($P > 0.05$) in males than the females patients with 18 (3.6%) and 10 (2.0%) respectively.

From the results of many studies conducted in different parts of the world, the *Shigella* species that are frequently isolated in blood specimens of patients were also encountered in the present study. The study revealed that among the *Shigella* species isolated, *Shigella flexneri* was the predominate among patients followed by *Shigella dysenteriae*, *Shigella boydii* and *Shigella sonnei*.

The result of *in vitro* – antibiotic susceptibility testing of *Shigella* isolates using disc diffusion test demonstrated that most of the isolates (92.3%) was significantly ($P > 0.05$) resistant to ampicillin followed by nalidixic acid, chloramphenicol, and cotrimozaxole in decreasing order. However, ciprofloxacin remained effective against all the *Shigella* isolates tested. However, the relationship between *Shigella* species and antibiotics tested was not statistically significant ($P > 0.05$). In addition, 37 isolates were found resistant to 1 of 3 first-line anti-*Shigella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol).

6. RECOMMENDATION

Mass campaign, public enlightenment should be given more attention on the effective personnel hygiene, food hygiene and environmental sanitation. There is also need for reduced and appropriate consumption of antibiotics both for human and animals. Government should review existing antibiotic use policy to check abuse/misuse of antibiotics and ensure their correct prescription; this would reduce the rate of emergence of *Shigella* resistant in particular and other bacteria in general. Research on development of new antibiotics and vaccines for treatment and prevention of *Shigella* and other bacterial infections should be given more attention.

To reduce this antibiotic resistance, public health reference laboratory with a tool to produce standardized antibiotic susceptibility test results of antibiotic susceptibility tests are important for clinical treatment plans; adequate information must be provided to the health care providers. In addition, susceptibility testing help on providing guidance and monitor of treatment, narrower the spectrum of its antibiotic (the more proffered is it's use when one knows specifically the organism being treated), degree of susceptibility of organism can assist in determining the length of therapy (but not the only factor) and choice of cheaper antibiotic agents with less side effects.

CONSENT

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

"All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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COMPETING INTERESTS

The authors declared that they have no competing interests exist.

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