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Shigellosis and Socio-Demography of hospitalized Patients in Kano, North-West, Nigeria.

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Abstract:
Aim: *The aim of the study was to determine the prevalent of Shigellosis in relation to socio-demographic characteristics 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.*
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 frequency of shigellosis was highest in other patients (without occupation), patients with informal level of education, using tap water as sources of drinking water, with more than one of all clinical manifestations of Salmonella infections and patients on treatment. However, there was a significant difference between the rate of Salmonella infections and socio-demographic characteristics of patients studied (p<0.05).*
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. The frequency of shigellosis was highest in other patients (without occupation), patients with informal level of education, using tap water as sources of drinking water, with more than one of all clinical manifestations of Salmonella infections and patients on treatment.*
Keywords: *Shigellosis, Shigella, Questionnaires, Blood specimens, Socio-demography, Kano.*

I. Introduction

Socio-demographic characteristic like feeding habit, occupation, literacy, hygiene among others of individual have been reported to be the most predisposing factors that increases the risks of *Shigella* infection in Nigeria and other developing countries [1]. In Kano, it was documented that 80% of those with informal education had not heard of *Shigella* infections in 1987. Up to date those who knew about *Shigella* infections believed it was retribution from God for promiscuity or it is an enemy's intervention/evil spirit [2]. The factors militating against the maintenance of good hygiene; may attributed to the ignorance of the people and lack of awareness to proper hygiene. It is a well-known fact that modern education is a gateway to awareness and sanitation, sometimes, the local customs of the people and poverty affects food habits and preparation [3].

Similarly, Iruka *et al.* [4] reported that, the poverty-resistance cycle within a larger cycle of poverty and disease particularly among unemployed people. Selective pressure for resistance is in almost all cases, a response to actual or supposed infection and resistant *Shigella* are largely spread through the same routes as pathogens. In Kano metropolis, the uses of antibiotics sold in local markets that are counterfeit, sub-standard or expired and improperly stored add to the problems [4].

Water is the most important commodity man consumes and the consumption of water goes steadily up, a number of diseases are related in one way or another to water, some of which are water-borne, water-washed, water based or due to insect vector using water body as breeding site [5]. A reciprocal relationship exists between health, agriculture and the general social level of a nation. The promotion of health has a beneficial effect on the economy of a nation, which in turn will affect the nation's cultural and social advancement. Health promotion is not dysgenic, clear up diseases and people cannot feed themselves [1].

Control of faecal–orally transmitted pathogens is inadequate in many developing countries, in particular, in sub-Saharan Africa. Acquired resistance to antimicrobial drugs has becoming more prevalent among *Vibrio cholerae*, *Shigella enteritidis*, diarrhoeagenic *Escherichia coli*, and other pathogens in this region. The poor, who experience most of the infections caused by these organisms, bear the brunt of extended illness and exacerbated proportion of deaths brought about by resistance [6,7]. Shigellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots. The organisms can usually be detected in 75-90% of patients during the first ten days of infection and in about 30% of patients during the third week in the blood [5,8]. Therefore this study was conducted in Kano metropolis, to determine the relationship between Shigellosis and socio-demographic characteristics of hospitalized subjects in Kano metropolis, Nigeria.

II. 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 [8].

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 [8]. 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 [9].

Bacterial isolates obtained were further sub-cultured by stabbing into nutrient agar slants and stored at 4^{o}C after aerobic incubation 37^{o}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^{o}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 [10], Habtamu *et al*. [11], and OIE [12]. A 24h 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) [13].

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 flexneri*, *Shigella dysenteriae*, *Shigella boydii* or *Shigella sonnei* subgroup was confirmed, no further testing with antisera needed to be conducted [13].

2.9 Statistical analysis of results

Statistical Package for Social Science (SPSS) version 14 was used [14]. 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.

III. 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* inf
ections by level of educational of hospitalized subjects in Kano metropolis, Nigeria.

Out of 28 patients infected, highest rate of *Shigella* infections were recorded from patients with informal education with 11(39.3%) followed in that order by those with primary 8(28.6%) and secondary education 6(21.4%). However, the least infection rate was recorded from patients with tertiary education 3(10.7%). In addition, the difference among these groups was statistically significant ($p < 0.05$) (Table 1).

Table 1: The distribution of *Shigella* infections by level of educational of hospitalized subjects in Kano metropolis, Nigeria.

Level of educational	No. examined	No. and % infected
Informal	117	11(39.3)
Primary	142	8(28.6)
Secondary	157	6(21.4)
Tertiary	84	3(10.7)
Total	**500**	**28(100)**

$X^2 = 20.628$ p value = 0.0001 - Significant

KEY: NO. = Number; % = Percentage of total number of patients infected (28).

The study revealed that, in Table 2 the frequency of infection was highest among other patients (without occupation) with 12(42.9%) followed in that order by business 8(28.6%) and civil servants 6(21.4%). However, the least frequency of infections was recorded from farmers 2(7.1%) and the association among these groups was statistically significant ($p < 0.05$).

Table 2: The distribution of *Shigella* infections by occupation of hospitalized subjects in Kano metropolis, Nigeria.

Occupations	No. examined	No. and % infected
Business	158	8(28.6)
Civil servants	101	6(21.4)
Farmers	51	2(7.1)
Others	190	12(42.9)
Total	**500**	**28(100)**

$X^2 = 10.681$ p value = 0.0136 - Significant

KEY: NO. = Number; % = Percentage of total number of patients infected (28).

When the 500 patients and assed in relation to sources of drinking water use, 13(6.6%) were using tap water, 5(10.2%) were using borehole water, 8(2.4%) were using packaged water and 2(1.6%) were using well water. Out the 28 infected patients, the frequency of infection was highest among patients using tap water with 13(6.6%) followed in that pattern by those using borehole 8(2.4%), packaged water 5(10.2%). However, the least frequency of infection was recorded from those using well water as sources of drinking 2(1.6%). In addition, the difference among these groups was also statistically significant ($p< 0.05$) (Table 3).

Table 3: The distribution of *Shigella* infections by sources of drinking water of Hospitalized subjects in Kano metropolis, Nigeria.

Sources of drinking water	No. examined	No. and % infected
Borehole	120	8(2.4)
Packaged water	102	5(10.2)
Tap water	51	13(6.6)
Well water	89	2(1.6)
Total	**500**	**28(100)**

$X^2 = 13.827$ p value = 0.0032 - Significant

KEY: NO. = Number; % = Percentage of total number of patients infected (28).

Of 28 patients with *Shigella* infections recorded in Table 4, highest number of patients were presented with more than one of all clinical manifestation of *Shigella* infections with 10(35.7%), followed in that pattern by those presented with fever 5(17.9%), headache 3(10.7%), vomiting 7(25.0%) and diarrhoea 2(7.1%). In addition, the least frequency of infection was recorded from patients presented with abdominal pain 1(3.6%) and the difference among these groups was also statistically significant ($p< 0.05$).

Table 4: The distribution of *Shigella* infections by clinical manifestations of Hospitalized subjects in Kano metropolis, Nigeria.

Clinical manifestations	No. examined	No. and % infected
Abdominal pain	33	1(3.6)
Diarrhoea	63	2(7.1)
Fever	103	5(17.9)
Headaches	34	3(10.7)
PWMCM	242	10(35.7)
Vomiting	25	7(25.0)
Total	**500**	**28(100)**

$X^2 = 21.325$ p value = 0.0007 - Significant

KEY: NO. = Number; P.W.M.C.M. = Patients with more than one of all clinical manifestation;
% = Percentage of total number of patients infected (28).

Of 500 patients used in the study, 320(64.0%) were patients on treatment and 180(36.0%) were patients not on treatment. However, out of 28 patients recorded with *Shigella* infections, there was significant different ($P< 0.05$) between the patients on treatment with 11(39.3%) and those who were not on treatment with 17(60.7%) prevalence rate (Table 5).

Table 5: The distribution of *Shigella* infections by treatment status of hospitalized subjects in Kano metropolis, Nigeria.

Treatment status	No. examined	No. and % infected
Patients on treatment	320	11(39.3)
Patients not on treatment	180	17(60.7)
Total	**500**	**28(100)**

Fisher exact test p value = 0.0009 – Very Significant
KEY: NO. = Number; % = Percentage of total number of patients infected (28)

Out of 28 presumptive *Shigella* isolates obtained in this study, Although, the relationship between *Shigella* species and sex of patients was not statistically significant (P > 0.05), the species of *Shigella flexneri* (predominant) with 14(50.0%) followed by *Shigella dysenterie* 5(17.9%), *Shigella boydii* 4(14.3%) and *Shigella sonnei* was the least prevalent serovar with 2 (7.2%). Although not statistically associated, males were more infected than females patients with 18 (64.3%) and 10 (35.7%) respectively (Table 6).

Table 6: The recovery and distribution of *Shigella* species according to sex of Hospitalized subjects in Kano metropolis.

Sex	*Shigella* species/%				Total/%
	Shigella flexneri	*Shigella dysenterie*	*Shigella boydii*	*Shigella sonnei*	
Male	9(32.1)	4(14.3)	3(10.7)	1(3.6)	**18(64.3)**
Female	5(17.9)	1(3.6)	1(3.6)	1(3.6)	**10(35.7)**
Total/%	**14(50.0)**	**5(17.9)**	**4(14.3)**	**2(7.2)**	**28(100)**

KEY: No. = Number; % = Percentage of total number of *Shigella* isolated (28).

IV. Discussion

The result obtained in the present study showed that socio-demographic characterizations of individual have an impact in the acquisition and spread of resistant *Shigella* in the community. As it was found out that the frequency of infection was significantly (p< 0.05) highest in other patients (without occupation), patients with in formal level of education, using tap water and with more than one of all clinical manifestations of *Shigella* infections and patients on treatment. Although, *Shigella* species and sex of patients was not statistically significant (P > 0.05), the species of *Shigella flexneri* (predominant) and *Shigella sonnei* was the least prevalent *Shigella* species isolated. However, males were more infected than females'patients.

This agrees with the work of Iwalokun *et al.* [3]; Ngwu and Agbo, [15]; Ngozi and Onyenekwe [16] and Oboegbulam *et al.* [17].Patients with informal education were more infected than those with tertiary level of education. This is probably being due to knowledge about *Shigella* infections was indeed low, and filled with several misconceptions, especially among those with informal education. Most of their wives are illiterate, with no good knowledge on the use of toilet coupled with improper disposal wastes. In addition, most of their houses are too congested and there is no provision of refuse collection points, no good drainages [2].On the other hand, patients with tertiary education had an opportunity to acquire knowledge on what *Shigella* infection is all about through new papers, journals and internet this could be a reasons for low recovery of *Shigella* infection among them [3, 18].

High record of infection among other patients (without occupations) was due to the fact that, lack of occupation is among the risks factors associated with *Shigella* infection. This is indeed obvious, due to the fact that such category of people prepare to use orthodox medicines that are readily available, cheaper and sometime free up charge for the treatment of *Shigella* infection than modern medicines. However, persons earning low daily wages often procure drugs 1 dose at a time in hospitals and standard pharmacies. This leads to the inappropriate use of drugs and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of antibiotics results in an incomplete course being purchased, sufficient only to alleviate the symptoms [15].

However, majority of patients without occupations cannot afford their personal houses but are renting in houses with many households were toiled, bathroom, kitchen, laundry and compound were shared this would probably increase the level of *Shigella* infection among them [15].In addition, malnourished or otherwise immune-compromised patients are more likely to have inadequate economic resources due to unemployment and they become the target of resistant *Shigella* when it's prevalent [19].

This study revealed that *Shigella* infection had highest frequency among patients using tap water as their sources of drinking water, and patient using well water were the least infected. This could be attributed to the fact that in the study area, tap water is the main source of drinking water most of the pipelines that provided water were too old, rusty and are usually leaking. As such there is high tendency of contaminants to be transported either by wind, rain, humans, animals and other sources to pipelines through leakages. However, the

bacteriological analyses of water that can confirm whether a water supply has been faecally contaminated have not been practices. In piped water distribution systems, a sanitary inspection will often not detect problems occurring during distribution, e.g. pipes buried underground might be damaged, allowing in pollution [6].

However, in Kano metropolis investigation of the water supply showed that tap water is not supplied frequently. To ensure water was available, the residents collected the water in open containers and residents usually make a provision of large water storage tanks without regular washing and chlorination [20].

Most of the patients presented during study were patients with more than one of all clinical manifestations of *Shigella* infection. The presentation of patients during study with more than one of all clinical manifestations of *Shigella* infection could be due to so many factors particularly drugs abuse, because of therapeutic intervention in suspected cases of *Shigella* infection due to attitude of self- medication. This prevents early reporting of patients to the hospitals at the onset of disease symptoms, except when complications had occurred, as observed in this study, where more untreated cases of *Shigella* infections by self-medication were brought to the hospitals [17, 21].

The rate of *Shigella* infection was significantly high ($p < 0.05$) among patients who were not on treatment with 56(53.8%) than those who were on treatment with 48(46.2%) prevalent rates. Low recovery of *Shigella* from patients on treatment could possibly be due to bad attitude of patients in the study area on attended their nearby chemist before coming to the hospital (self-medication) and coupled with lack of withdrawal of drugs for at least three days prior to blood culture. These can in turn affect the recovery of *Shigella* in their blood specimens [22].

The highest incidence in males patients recorded is in consonance with the works of Mashi [23] and Abdullahi [24], probably due to the fact that most of males in the study area were more prone to contaminations than their female counter part, males are usually eat and drink outdoor and they do not recognize the state of the food or drink they eat and nature of the environment in which they are prepared. In addition, the highest prevalence of *Shigella* infection in males could also connected with water exposure by these individuals in their community. Similarly, sharing of public toilet in school, marked, bus stop by male would probably increase the level of infection among the males [24]. On the other hand, females are less likely to be seeing eating, drinking and defaecating outdoor possibly because of culture and religious inclination. It was observed that males were responsible for 98% of activities involving contamination and water exposure [23].

In this study, four (4) species of *Shigella* were, encountered in the present study. Among the *Shigella* species isolated, *Shigella flexneri* was predominant followed by *Shigella dysenteriae*, *Shigella boydii* and *Shigella sonnei*. This finding is in consonance with studies by Akinyemi *et al.* [25] and Asma *et al.* [26]. Contrary to the work conducted in Kano metropolis (the study area) by Abdullahi [24] who revealed that *Shigella dysenterie* was the most predominant specie. The highest susceptibility to *Shigella flexneri* in the study area could probably because *Shigella* infections occurs by ingesting organisms in contaminated food or water from contaminated hands. In the area where this research was conducted, residents experiences serious problem of inadequacy of water supply that result to high consumption of unhygienic water. However, sewages contaminated water used for irrigation contained high loads of *Shigella flexneri*. On the hand, *Shigella sonnei* was the least because it prefer an area with good hygiene (developed countries) [27].

V. Conclusion And Recommendation

In the present study, it was observed that there was a significant difference between the rate of *Shigella* infections and socio-demographic characteristics of patients studied ($p > 0.05$). The frequency of *Shigella* infection was higher in other patients (without occupation), patients with in formal level of education, using tap water as sources of drinking water, with more than one of all clinical manifestations of *Shigella* infections and patients on treatment. *Shigella flexneri* remains the prominent causative agent of Shigellosis in the population under study and *Shigella sonnei* was the least. The males were found to be more susceptible to Shigellosis than their females' counterparts.

Systemic immunizations with good potent vaccines, observation of personnel hygiene and environmental sanitation, cleaning hand with soap/detergent immediately after defecation as well as primary health care education could surely help in controlling and/or preventing Shigellosis. Measures should also be taken encourage residents on self-employments, patronized hospital immediately and be sent early to the laboratory for effective diagnosis and susceptibility test of organisms to ensure quick, specific and effective treatment of *Shigella* infection [7].

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