UJMR, Volume 2 Number 2 December, 2017 *ISSN: 2616 - 0668*

Received: 30th Nov, 2017 **Accepted:** 4th Jan, 2018

Occurrence of *Salmonella* serovars implicated in Salmonellosis in North-western part of Nigeria

[*]Abdullahi, B.,[1] Abdulkadir, B.[2] and Olayinka, B. O[3].

[1]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

[2]Department of Microbiology, Umaru Musa Yar'adua University, Katsina, Nigeria

[3]Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria

[*]Corresponding Author: albishir13@yahoo.com Tel: +2348054527359

Abstract

Accurate diagnosis of clinical Salmonella enterica and differentiation into its serovars is important from a public health and epidemiological point of view. Standard cultural, biochemical and polymerase chain reaction techniques were employed to isolate and identify *Salmonella* enteric serovars implicated in salmonellosis. This study was carried out between June 2015 and May 2016 in some selected hospitals in North-Western Nigeria. Four hundred and twenty stool samples were collected from patients clinically diagnosed of pyrexia and/or gastroenteritis. Of the 420 samples collected, 27 were positive for Salmonella, given rise to an overall prevalence rate of 6.4%. One hundred and fifty two samples were collected from ≤1-5yrs age-group and 19 (12.5%) were positive for *Salmonella* species. Out of the 199 male patients recruited for this study, 16 (8.0%) had *Salmonella* infection while 11 (5.0%) of221female patients recruited had *Salmonella* infection. Sex of the patients has no significant association with the infection ($p>0.05$). Out of the 27 *Salmonella* isolated, six different serovars were encountered. *S. typhi* and *S. enteritidis* were the most frequently encountered; given rise to 25.9% prevalence rate each. The second most frequently encountered was *S. typhimurium* with 18.5% prevalence rate. The least encountered serovar was S. paratyphi B which had 3.7% prevalence rate. Out of the 27 *Salmonella* isolates, 17 (63%) were isolated from patients presenting with mix symptoms and S.Typhi was the most frequently encountered, given rise to 35.3%. Seven isolates (25.9%) of the 27 Salmonella were isolated from patients presenting with diarrheoa only and *S. enteritidis* (57.1%) was the most frequently encountered. However, frequency of occurrences of serovars has no significant association to the salmonellosis symptoms ($p>0.05$).The prevalence rate in elderly patients (≥60yrs) was also high, for out of the 18 samples collected from them, 5 (27.8%) were positive. The age-group with least prevalence rate was 16-40yrs; all samples collected from them were negative for Salmonella infection. Salmonella infection was significantly associated with the age groups with $p<0.05$.Out of the 16 cases of salmonellosis in male patients, 7 (43.8%) were typhoidal, while 9 (56.3%) were non-typhoidal. Among female patients, 11 salmonellosis cases were recorded, 5 (45.5%) were typhoidal and 6 (54.5%) were non-typhoidal. This result showed that there was reduced in Salmonella infection in part of North Western Nigeria. However, there is need to sustain good sanitary practices in order to curtail increase and reduced children's exposure to the infection.

***Key Words**: PCR; Salmonella Serovars; Salmonellosis; Prevalence.*

INTRODUCTION

Salmonella enteric comprised of six subspecies; they are *S. enteric* subsp. *enterica, S. enteritica* subsp. *salamae, S. enteric* subsp. *arizonae, S. enteric* subsp. diarizonae, *S. enteric* subsp. *indica,* and *S. enteric* subsp. *houtenae* (Porwollik*et al.,* 2003). Of these six subspecies, only *S. enteric* subsp. *enterica* is associated with disease in warm-blooded animals. As of 2002, there are over 2,541 serovars identified within *S. enteric* subsp. *enterica* (CDC, 2010).

Salmonella species cause wide range of human diseases such as enteric fever, gastroenteritis and bacteriamia (Bennasar *et al.*, 2000).

Typhoid salmonellosis is a global problem with an estimated 12-33 million people infected in the world (Kohinur *et al.*, 2010). Non-typhoidal salmonellosis is self-limiting; however, it may lead to systemic symptoms in children, the elderly and immunocompromised (Rayamajhi *et al.*,2008). Bacteremia is reported to occur in 3 to 10 percent of cases of non-typhoidal salmonellosis and in such situations, antimicrobial therapy is lifesaving (White *et al.*, 2001).

Salmonella isolates are serotyped according to the Kaufmann-White scheme using somatic (O), capsular (VI) and flagellar (H) antigens that are present in the cell surface of *Salmonella*. The O factors determine the grouping, while the H factors completely define the serotype identity of a *Salmonella* strain (Popoff, 2001; Fitzgerald *et al*., 2003).

According to Herrera-Leon *et al* (2007), molecular serotyping methods are based on the detection of the same antigens as the Kauffmann-White *Salmonella*. Molecular methods including multiplex PCR offer a consistent and high-throughput approach to typing etiologic agents (Levy *et al*., 2008).

PCR identification of *Salmonella* is important or critical for surveillance, improving prevention and control of food-borne diseases. It has allowed rapid detection, identification of sources, control of outbreaks, and also identification of emerging serotypes and new mechanisms of transmission (Fritzgerald *et al*, 2003).

Therefore, as the salmonellosis becomes so prevalent and life-threatening within the study area couple with no or limited similar studies conducted more especially exploring the use of molecular approach as such the need for the research. However, the objective of this work was to detect and identify *Salmonella enteric* serovars implicated in salmonellosis in part of North-Western Nigeria using monoplex and multiplex PCR techniques.

MATERIAL AND METHODS

Sampling Sites

Six Hospitals were selected for this study in three states of North-Western Nigeria. These Hospitals were Hajia Gambo Sawaba General Hospital, Major Ibrahim Abdullahi B Memorial Hospital, Hasiya Bayero Public Hospital, Muhammadu Abdullahi Wase Specialist Hospital, General Hospital Dutsin-ma and General Hospital Daura. The Hospitals are located in Kaduna, Kano and Katsina States of North-Western Nigeria respectively.

Ethical Consideration, Patient's Consents, Inclusion and Exclusion Criteria:

Approvals for the study were obtained from Kaduna (with Ref NO: MON/ADM/744/VOL.1/286), Kano (with Ref NO: REF/OFF/797/T.I/24) and Katsina (with Ref NO: KHSMB/S.7/VOL.VII) States Ministry of Health respectively (See attached on appendix I-III). Patients' consents were obtained prior to collection of samples. The inclusion criteria were consented outpatients clinically diagnosed by clinicians of having either pyrexia, gastroenteritis or both. The exclusion criteria include non-consented patients and anyone that does not satisfy the inclusion criteria.

Sample Size, Structured Questionnaire and Sample Collection

Seventy stool samples were collected from each of the hospital. This amounted to 420 from the six Hospitals. The samples were collected between January 2015 and December 2015. Structured questionnaires (attached on appendix IV) were administered to the consented patients to obtain information on the socio-demographic data and possible risk factors associated with *Salmonella* infection. Stool specimens were collected from outpatient clinically diagnosed by clinicians having either pyrexia, gastroenteritis or both.Patients were grouped into 5 Age-groups of ≤1-5, 6-15, 16-40, 41-59and ≥60.

Pre- Enrichment and Enrichment of Specimen for Isolation

A loopful of stool sample collected from each patient was directly inoculated into 45ml of buffered peptone water and incubated at 37^{o}C for 16hrs. Aloopful of culture from buffered peptone was inoculated into 9ml of selenite-F broth (Oxiod, Cambridge, UK) contained in test tubes (Akinyemi *et al*., 2007). This was incubated for 18hrs at 37^{0}C for bacteriological culture.

Culture and Biochemical Characterization

A loopful of the culture from selinite-F broth was streaked on xylose lysine deoxycholate (XLD) agar (Oxoid, Cambridge, UK) and incubated overnight at 37^{0}C. The plates of red with black centers colonies or red colonies only on XLD (OIE, 2010) were picked and subculture on XLD inorder to have pure culture for biochemical confirmation. The suspected colonies were then streaked onto Nutrient Agar Slant and incubated overnight at 37^{0}C and later stored in refrigerator. The suspected *Salmonella* isolates were purified again, by streaking on XLD Agar plate from Nutrient Agar Slant and incubated overnight at 37^{0}C. The pure isolated colonies were then used to characterized the organisms biochemically. Series of conventional biochemical testes wereconducted to srceen for *Salmonella* isolatesas recomended by WHO, (2010). Such biochemical tests were Urease production, Lysine Iron Agar (LIA), Triple Sugar Iron (TSI) Agar, Indole, Methyl Red and Voges-Proskauer tests.

DNA Extraction

DNA of the biochemically characterized *Salmonella* species were extracted using Quick-gDNA^{TM} Miniprep (D3024). Two milliliter of an overnight grown *Salmonella* culture in Luria-Bertani Broth was transferred into 2ml micro centrifuge tube and spinned at 5000rpm for 10minutes.

The supernatant was removed and 500µl of genomic lysis buffer was added directly to the cell pellet. The pellet was re-suspended by vortexing for 4-6 seconds and allowed to stand for 5-10minutes at room temperature. The mixture was then transferred to a Spincolumn in a collection tube. This was centrifuged at 10000rpm for one minute. The collection tube was discarded with flow through. The Spin column was transferred to a new collection tube and 200µl of DNA pre-wash buffer was added to it. This was then centrifuged at 10000rpm for 1minute. Five hundred microlitre of g-DNA buffer was added to the spin column and centrifuged at 10000rpm for 1minute. The spin column was then transferred to a microcentrifuge tube and 50µl DNA elution buffer was added to the spin column. This was incubated for 2-5minutes at room temperature and thereafter, centrifuged at top speed for 30 seconds to elude the DNA. The eluted DNA was stored at -20^{0}C for monoplex and multiplex PCR confirmation and typing of *Salmonella* species respectively. All procedure according to manufacturers guidelines.

Monoplex PCRDNA Amplification

A set of primer pair (F-GTGAAATTATCGCCACGTTCGGGCAA and R-TCATCGCACCGTCAAAGGAACC) with 284bp was used to confirm *Salmonella* species (Galan *et al.,* 1992). It is specific for the *invA*gene located on the *Salmonella* pathogenicity island 1 (Galan *et al.,* 1992; Rahn*et al.,* 1992). The primer was commercially available from QIAGEN Operon. The primer was dissolved according to the instruction provided by the manufacturer. The amplification was performed in a final volume of 50µl in micro-amplification tube (PCR tube). The reaction mixture consisted of 5ml of the DNA template, 5ml of PCR buffer (75mM Tris-HCl, pH 9.0, 2mM MgCl2, 50mM KCl, 20mM (NH4)2SO4), 1ml deoxynucleoside triphosphate(dNTPs) (40 mM), 1ml (1U AmpliTaq DNA polymerase), 25 pmol of each primer (*invA*F, *invA* R), and the volume of the reaction mixture was completed to 50ml using DDW. The thermal cycler was adjusted as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of (denaturation at 94°C for 1 min, annealing at 55^{0}C for 1 min and extension at 72°C for 1 min). Final extension was carried out at 72°C for 10 min and the PCR products were stored in the thermal cycler at 4°C until they were collected.PCR products were resolved by electrophoresis in 1.5–2% (w/v) agarose gels and visualized under UV light after ethidium bromide staining (Sambrook *et al*., 1989) using 100bp molecular weight marker.

Multiplex PCR Typing of *Salmonella enteric* Serovars

Set of primer pairs were used. The primers were commercially available from QIAGEN Operon and Bioneer. The primers were dissolved according to the instruction provided by manufacturer. Primers for O-serogrouping were selected based on the *rfb* gene clusters specific for *Salmonella* serogroups A, B, C1, D and E, as well as primers based on *via*B gene to detect Vi-positive strains as reported by Levy *et al*, (2008) and Herrera-Leon *et al*, (2007). Primers used in H typing were also designed by Levy *et al*, (2008). An internal control (primers P1-P2 that amplify *oriC*) was incorporated into the system as described by Levy *et al*, 2008. The multiplex PCR amplification was performed in a final volume of 25µl in micro-amplification tube (PCR tube). Each reaction contained 1X PCR buffer, 25mM $MgCl_2$, 0.25mM of deoxynucleoside triphosphates, 0.4µM (each) of primers prt-F, prt-R, rfbJ-R, vi-F, vi-R, wzxC1-F, wzxC1-R, tyvD-F, tyvD-R, wzxE-F, wzxE-R, 0.2µM of control primers (P1-P2) and 1.75U Promega*Taq*DNA polymerase. Five microliters of a briefly centrifuged, boiled isolate suspension was used as template. PCR was performed in a Mastercycler with a heated lid cover. This prevented evaporation and eliminated the use of mineral oil. The cycling parameters of the PCRs were as follows: denaturation at 95^{0}C for 5minutes, followed by 35 cycles at 95^{0}C for 40 seconds, 50^{0}C for 30 seconds and a final extension at 68^{0}C for 7 minutes. A negative control consisting of double distilled water was included. Multiplex PCR products were separated in 1.5% agarose gel by unidirectional electrophoresis using 0.5X TBE buffer and visualized by staining with ethidium bromide. Fragments (amplicons) sizes were determined by comparison with 100bp DNA ladders. The serovars were then identified.

RESULTS

The conventional biochemically suspected *Salmonella* isolates (48) were reconfirmed using monoplex PCR. Out of the 48biochemically suspected *Salmonella* isolates, 27 were molecularly confirmed to be *Salmonella* species given rise to overall prevalence rate of 6.4% (Table 1).

Out of the 199 samples collected from male patients, 16 (8.0%) had *Salmonella* infection. Two hundred and twenty one samples were collected from female patients and 11 (5.0%) had *Salmonella* infection (Fig. 2). Out of the 27 *Salmonella* isolated, *S. typhi* and *S. enteritidis* were the most frequently encountered, given rise to 25.9% prevalence rate each. The second most frequently encountered was *S. typhimurium* with 18.5% prevalence rate. The

least encountered serovar was *S. paratyphi* B which had 3.7% prevalence rate (Table 2 and 3). Seventeen (63%) isolates were isolated from patients presenting with mix symptoms. Out of this 17 isolates, *S.typhi* was the most frequently encountered, given rise to 35.3%. Seven (25.9%) from the 27 isolates were found to be isolated from patients presenting with diarrhea only and *S. enteritidis* (57.1%) was the most frequently encountered (Table 4).

One hundred and fifty two samples were collected from ≤1-5yrs age-group and 19 (12.5%) were positive for *Salmonella* species. Out of this 19 positive samples, 8 (42.1%) were typhoidal *Salmonellae* and 11 (57.9%) were non-typhoidal *Salmonellae*. The prevalence rate in elderly patients (≥60yrs) was also high, for out of the 18 samples collected from them, 5 (27.8%) were positive. Out of this 5 positive samples, 3 (60%) were typhoidal *Salmonellae* and 2 (40%) were non-typhoidal *Salmonellae* (Table 5).

The overall cases of salmonellosis in male patients were 16. Out of this 16 cases, 7 (43.8%) were typhoidal, while 9 (56.3%) were non-typhoidal. Among female patients, 11 salmonellosis cases were recorded, 5 (45.5%) were typhoidal and 6 (54.5%) were non-typhoidal (Fig. 5).

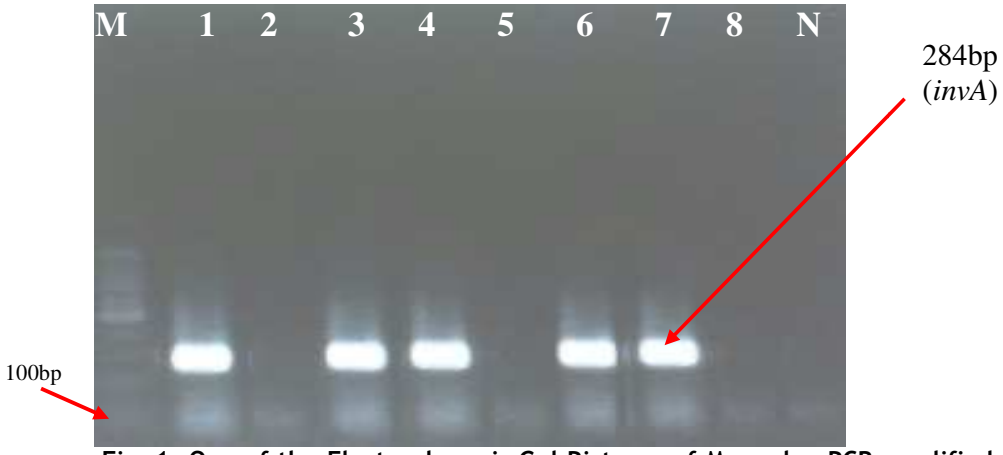
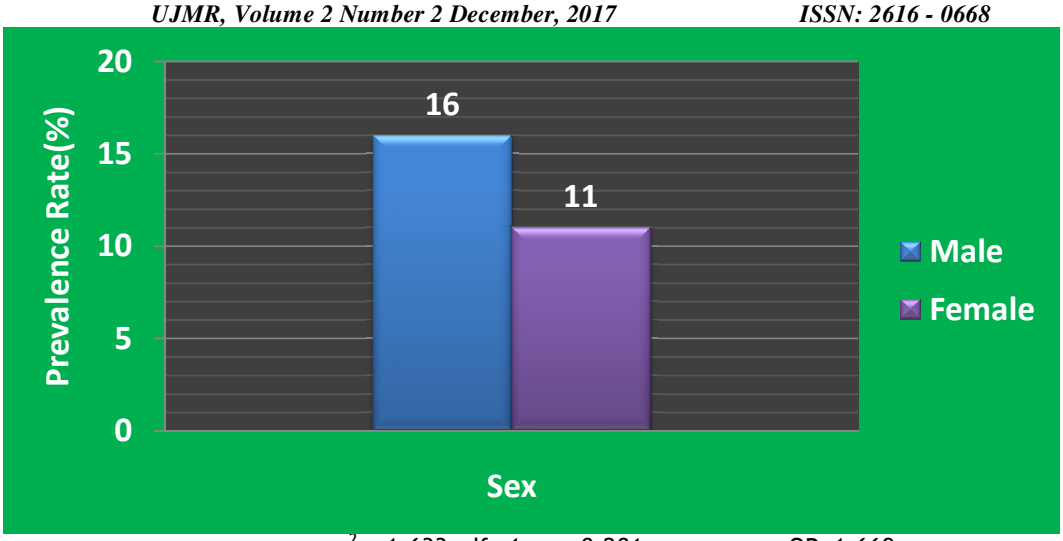


Fig. 1: One of the Electrophoresis Gel Pictures of Monoplex PCR amplified *invA* Genes of *Salmonella* Isolated from Hasiya Bayero Public Hospital (HB), Kano.
Key: Lane M: PCR Ladder Maker (100bp); Lane 1, 3, 4, 6 and 7: Bands (Amplicons) of *invA* Genes; Lane 2, 5 and 8: No *invA* Genes; Lane N: Negative Control.

Table 1: Prevalence Rate of *Salmonella* Infection in the Selected Hospitals in Part of North-Western Nigeria.

Hospitals	No. of Samples	No. Positive (%)
HG	70	6 (8.6)
MA	70	3 (4.3)
HB	70	5 (7.1)
MW	70	5 (7.1)
DT	70	6 (8.6)
DA	70	2 (2.9)
Total	**42027 (6.4)**	

Key: No.: Number, **HG**: Hajiya Gambo Sawaba Hospital Kaduna, **MA**: Mahor Ibrahim Abdullahi Hospital Kaduna, **HB**: Hasiya Bayero Public Hospital Kano, **MW**: Muhammadu Abdullahi Wase Specialist Hospital Kano, **DT**: General Hospital Dutsin-Ma Katsina, and **DA**: General Hospital Daura Katsina.



x^2 = 1.633, df= 1, *p* = 0.201 OR=1.669

Fig. 2: Prevalence of *Salmonella* Infection in Respect of Sex in the Selected Hospitals in Part of North-Western Nigeria

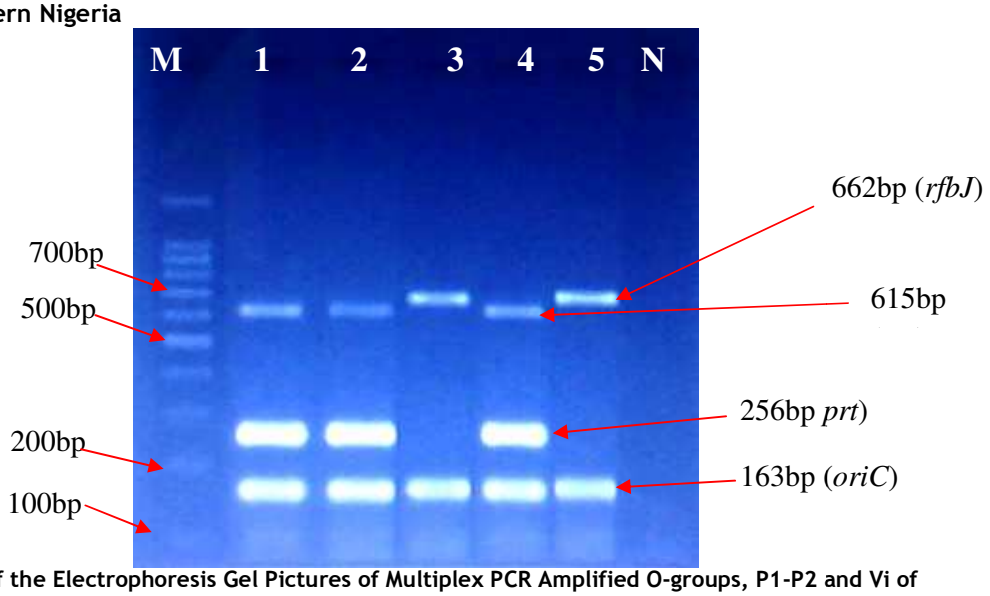


Fig. 3: One of the Electrophoresis Gel Pictures of Multiplex PCR Amplified O-groups, P1-P2 and Vi of *Salmonella* Isolated from Hasiya Bayero Public Hospital (HB), Kano.
Key: Lane M: PCR Ladder Maker (100bp); Lane 1-5: Amplicons of O-Group and Vi genes; Lane N: Negative control.

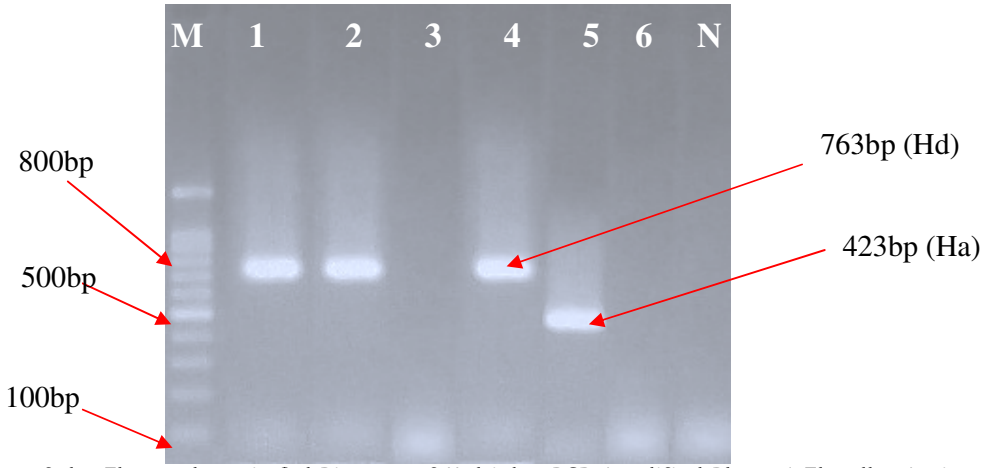


Fig. 4: One of the Electrophoresis Gel Pictures of Multiplex PCR Amplified Phase 1 Flagellar Antigens of *Salmonella* Isolated from Hajia Gambo Sawaba General Hospital (HG), Zaria City, Zaria
Key: Lane M: PCR Ladder Maker (100bp); Lane 1, 2, 4 and 5: Amplicons of Phase 1 Flagellar Genes; Lane 3 and 6:No Phase 1 Flagellar genes; Lane N: Negative control.

Table 2: Results of Multiplex PCR *Salmonella* Typing

ID	P1-P2	H1 Atigens			O-Group Antigens						O Group	Inference
		Ha	Hb	Hd	Prt	rfbJ	wzxC1	Tyv	Vi	wzxE		
HG29	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
HG35	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
HG37	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
HG41	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
HG63	+	+	-	-	+	-	-	-	-	-	A	*S. paratyphi*A
HG70	+	-	-	-	-	+	-	-	-	-	B	*S. typhim*
MA3	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
MA18	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
MA23	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
HB10	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
HB15	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
HB21	+	-	-	-	-	+	-	-	-	-	B	*S. typhim*
HB41	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
HB46	+	-	+	-	-	+	-	-	-	-	B	*S. paratyphi*B
MW8	+	-	-	-	-	+	-	-	-	-	B	*S. typhim*
MW28	+	-	-	-	-	+	-	-	-	-	B	*S. typhim*
MW30	+	-	-	-	+	-	-	+	-	-	D	*S. enteritidis*
MW42	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
MW63	+	-	-	-	-	-	+	-	-	-	C1	*S. infantis*
DT7	+	+	-	-	+	-	-	-	-	-	A	*S.paratyphi*A
DT29	+	+	-	-	+	-	-	-	-	-	A	*S.paratyphi*A
DT53	+	-	-	-	-	+	-	-	-	-	B	*S. typhim*
DT58	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
DT59	+	-	-	-	-	-	+	-	-	-	C1	*S. infantis*
DT67	+	-	-	-	-	-	+	-	-	-	C1	*S. infantis*
DA46	+	-	-	+	+	-	-	+	+	-	D	*S. typhi*
DA68	+	+	-	-	+	-	-	-	-	-	A	*S.paratyphi*A

Key: ID = Isolate Number, **HG**: Hajiya GamboSawaba Hospital Kaduna, **MA**: Mahor Ibrahim Abdullahi Hospital Kaduna, **HB**: HasiyaBayero Public Hospital Kano, **MW**: Muhammadu AbdullahiWase Specialist Hospital Kano, **DT**: General Hospital Dutsin-Ma Katsina, and **DA**: General Hospital Daura Katsina, *S. typhim* = *S. typhimurium*

Table 3: Distribution of Clinical *Salmonella enteric* Serovars in the Selected Hospitals in part of North-Western Nigeria

Hospitals	N	*S. typhi*	*S.paratyphi* A	*S. paratyphi* B	*S. enteritidis*	*S. typhimurium*	*S. infantis*
HG	6	3(50)	1(16.7)	0(0)	1(16.7)	1(16.7)	0(0)
MA	3	1(33.3)	0(0)	0(0)	2(66.7)	0(0)	0(0)
HB	5	0(0)	0(0)	1(20)	3(60)	1(20)	0(0)
MW	5	1(20)	0(0)	0(0)	1(20)	2(40)	1(20)
DT	6	1(16.7)	2(33.3)	0(0)	0(0)	1(16.7)	2(33.3)
DA	2	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)
Total	**27**	**7(25.9)**	**4(14.8)**	**1(3.7)**	**7(25.9)**	**5(18.5)**	**3(11.1)**

$x^2 = 26.314$, df= 25, $p = 0.391$

Key: N: Number of Isolates; Figures in parenthesis indicates percentages.**HG**: Hajiya Gambo Sawaba Hospital Kaduna, **MA**: Mahor Ibrahim Abdullahi Hospital Kaduna, **HB**: Hasiya Bayero Public Hospital Kano, **MW**: Muhammadu Abdullahi Wase Specialist Hospital Kano, **DT**: General Hospital Dutsin-Ma Katsina, and **DA**: General Hospital Daura Katsina

Table 4: Distribution of *Salmonella* Serovars in Respect to Salmonellosis Symptoms. (Isolates=27)

Symptoms	No. Isolates	*S.* Typhi	*S.* PA	*S.* PB	*S.* En	*S.* Tm	*S.* In
Fever	2(7.4)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)
Constipation	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Diarrhea	7(25.9)	0(0)	0(0)	0(0)	4(57.1)	2(28.6)	1(14.3)
Vomiting	1(3.7)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)
Mix-Symptoms	17(62.9)	6(35.3)	2(11.8)	1(5.9)	3(17.6)	3(17.6)	2(11.8)

$x^2 = 21.908$, df= 15, $p = 0.110$

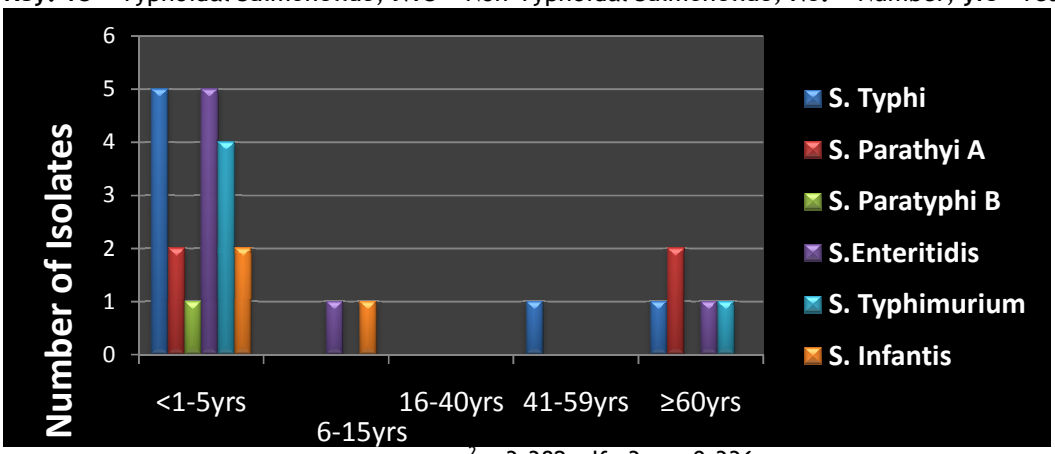
Key: No.: Number; PA: Paratyphi A; PB: Paratyphi B; En: Enteritidis; Tm: Typhimurium; In: Infantis; Number in parenthesis indicates percentages

Table 5: Prevalence of Typhoidal and Non-Typhoidal *Salmonellae* Infection in Respect to Age-Group Patients in Selected Hospitals in Part of North-Western Nigeria.

Age-Groups (yrs)	No. of Sample	No. Negative (%)	No. Positive (%)	*Salmonellae* Infection	
				TS(%)	NTS(%)
<1-5	152	133 (87.5)	19 (12.5)	8 (42.1)	11 (57.9)
6-15	109	107 (98.2)	2 (1.8)	0 (0)	2 (100)
16-40	106	106 (100)	0 (0)	0 (0)	0 (0)
41-59	35	34 (97.1)	1 (2.9)	1 (100)	0 (0)
≥60	18	13 (72.2)	5 (27.8)	3 (60)	2 (40)
Total	**420**	**393 (93.6)**	**27 (6.4)**	**12 (44.4)**	**15 (55.6)**

$x^2 = 34.802$, df= 4, $p = 0.000$

Key: TS = Typhoidal *Salmonellae*; **NTS** = Non-Typhoidal *Salmonellae;* **No.** = Number; **yrs=** Years



$x^2 = 3.382$, df= 3, p =0.336

Fig. 5: Distribution of *Salmonella* Serovars among Age-Group Patients attending Selected Hospitals in Part of North-Western Nigeria.

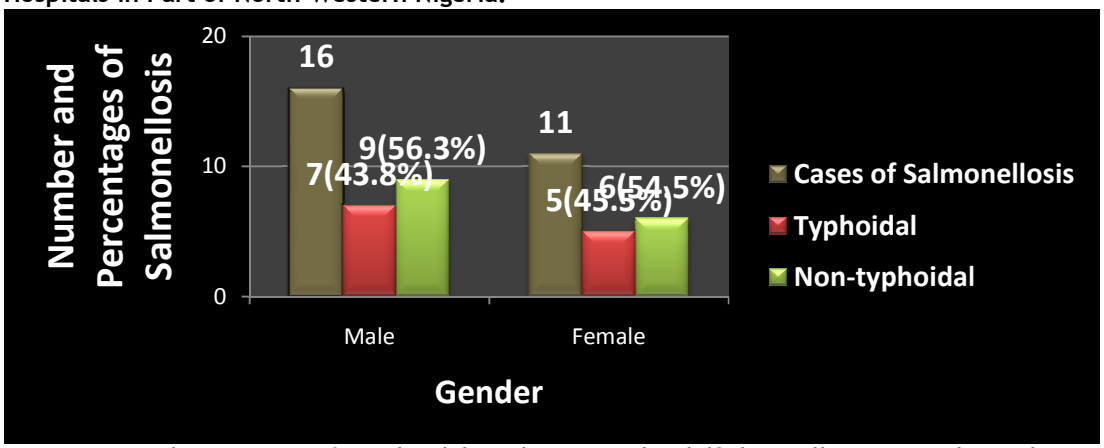


Fig. 6: Prevalence rate of Typhoidal and Non-TyphoidalSalmonellosis in Male and Female Patients attending Selected Hospitals in Part of North-Western Nigeria

DISCUSSION

The occurrence rate was lower in our study compared to other reports elsewhere in Nigeria; in Zaria, Gbonjubola *et al.*(2009) reported 14.1% cases, in Katsina State, Abdullahi *et al*. (2014) reported 15% cases and in Lagos, Muibat *et al.*(2015) reported 35% cases. The decrease in the occurrence (in this study) may be due to better awareness, more effective diagnosis and treatment. More so, out of the 70 samples collected from each of the Hospital, Hajia Gambo Sawaba

General Hospital Kaduna and General Hospital Dutsin-ma Katsina satate had 8.6% prevalence rate each, Hasiya Bayero Public Hospital and Muhammad Abdullahi Wase Specialist Hospital all from Kano state had 7.1% each and General Hospital, Daura from Katsina state had the least prevalence rate of 2.9%. However, there was no any significant association between the sampled Hospitals and the infection (p>0.05).

The frequency of the infection is higher in male (8.0%) than in female (5.0%) (Fig.2 and5).This finding was in agreement with the finding of Zailani *et al*. (2004), who reported higher frequency in male than in female. Also, Isa *et al*. (2013), reported higher prevalence rate in male (43.3%) than female (39.5%) in Biu, Borno, State, Nigeria. Although, there was no significant association between the infection and sex (p>0.05), the difference could be as a result of the fact that outdoor activities are more pronounced in males than females in North-Western Nigeria, there by exposing the males to high risk of infection. It may also be due to the fact that male are more exposed to infection because they tend to eat more road side and fast foods. Most reported cases of salmonellosis are due to contaminated food and water (Threlfall and Ward, 2001 and Wright *et al*, 2005).

In this studysix different serovars were encountered. *S*. typhi and *S*. enteritidis were the most frequently encountered serovars, given rise to 25.9% prevalence rate each.The second most frequently encountered was *S. typhimurium* with 18.5% prevalence rate (Table 3). This finding conformed to the reports ofAkinyemi*et al,* (2007) and Abdullahi *et al* (2014). In most parts of the world, surveys have reported *S. enteritidis* and *S. typhimurium* as the major serovars found in non-typhoidal salmonellosis (Antoine *et al.,* 2008; Saba*et al.,* 2013). In Ghana and most other African countries, they are also the most frequently isolated from diarrheal diseases (Bonkoungou *et al.,* 2013), which is confirmed by this study.

On the other hand, there was no significant association between distribution and frequency of occurrence of the serovars in the sampled hospitals with $p>0.05$. However, *S. typhi* was more encountered in HG than any other hospital and it was not isolated in HB hospital. Out of the 5 *Salmonella* isolates isolated in HB, 4 were non-typhoidal salmonellae. Non-typhoidal salmonellae are usually implicated in non-typhoidal salmonellosis which is self-limiting without the use of antibiotics except in children, elderly and immunocompromised patients (Rayamajhi, *et al.,* 2008). HB is a pediatric hospital and most patients encountered were children, this accounted for more isolation of non-typhoidal salmonellae than typhoidal salmonellae in the hospital.

Comparison of distribution of serovars in respect of salmonellosis symptoms revealed no significant difference in distribution with $P>0.05$(Tables 4). However, 17(62.9%) of the serovars were isolated from patients presenting with mixed-symptoms (fever, constipation, vomiting and diarrhea), while 7 (25.9%) were isolated from patients presenting with diarrhea only. Most people with salmonellosis develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infectionas reported by previous research (Santos *et al.,* 2001).

This study has shown that non-typhoidal salmonellosis was more prevalence than typhoidal salmonellosis with prevalence rate of 55.6% and 44.4% respectively (Table 5). This is in conformity with a study in Lagos-Nigeria by Akinyemi*et al*. (2012), who recorded 64.5% and 35.5% prevalence rate for non-typhoidal and typhoidal salmonellosis respectively but it is in contrast with Abdullahi *et al*. (2014), who recorded more prevalence rate of typhoidal (56.5%) than non-typhoidal salmonellosis (45.5%) in Katsina-Nigeria. The possible reasons for increase in non-typhoidal salmonellosis in part of North-Western Nigeria may be due to changes in cultural habits of eating which increase the patronage of fast foods.

Furthermore, in this study, there was significant association between salmonellosis and age groups with $p<0.05$. Out of the 27 salmonellosis cases recorded, 19 (70.4%) were from age group <1-5yrs and of this value, 11 (57.9%) were non-typhoidal. The age group ≥60yrs had 5 (18.5%) cases while 16-40yrs had no cases recorded against them (Table 5). This outcome may be due to the fact that children and elderly people have weak immunity and are more likely to go down with salmonellosis than the middle age groups. It may also be that the middle age groups usually get over the infection and this may not necessitate them to seek medical attention. Also in this study, it was discovered that most middle age groups usually indulged in self-medication before seeking medical attention and this might have get rid of the infection before samples were

collected for laboratory diagnosis. More so, age group <1-5yrs patients were more encountered than any other age group. It was also reported previously that the highest rate of *Salmonella* infection is found in infants (130 isolates/100,000). One quarter to one third of pediatric typhoid patients are younger than 5 years, of which 6-21% is younger than 2 years (Zaki and Karande, 2011).

All the six different serovars encountered in this study were isolated from age group <1-5yrs with *S. typhi* and *S. enteritidis* been the most frequently encountered. Four of the 6different serovars including *typhi* and *enteritidis* were isolated from age group ≥60yrs with *S. paratyphi* A most frequently encountered. These findings support the report of Saba *et al*. (2013) and Paragrigorakis *et al*. (2007), they reported that *S. enteritidis* and *S. typhi* are the most encountered serovars in non-typhoidal and typhoidal salmonellosis respectively.

CONCLUSION

This study showed that children and elderly patients were the most susceptible to *Salmonella* infection. There was significant association between salmonellosis and age groups with $p<0.05$. In this study, non-typhoidal salmonellosis was more occurrence than typhoidal salmonellosis. We therefore, conclude that, there isneed to sustaingood sanitary practices in order to curtail increasein the infection. Provision of safe drinking and potable water as well as healthcare facilities by authorities at various levels is needed, in order to check mate any possible outbreak of the infection.

Acknowledgment

We acknowledge the Ministry of Health Kaduna, Kano and Katsina States, Nigeria, for the ethical approval given to us to collect samples for this research from some selected hospitals. Also, we acknowledge the support and technical assistance received from the management and staff of the selected hospitals in the course of this research. We also want to thanks TETFund for sponsoring the research.

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MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way,
P.M/;B 2014
Kaduna.
Kaduna State, Nigeria.

MOH/ADM/744/VOL.I/286

2nd June, 2015

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

RE: CHARACTERIZATION OF MULTIDRUG - RESISTANT CLINICAL SALMONIELLA ENTERICA SEROVARS IN PART OF NORTH - WESTERN NIGERIA

Name of Principal Investigator	-	Abdullahi Bashir
Address of Principal Investigator	-	Faculty of Science, Dept. of Microbiology, Ahmadu Bello University, Zaria Kaduna State.
Date of Receipt of Application	-	10th April, 2015
Date of Ethical Approval	-	28th April, 2015

This is to inform you that the research described in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee.

If there is delay in starting the research, inform the HREC so that the dates of approval can be adjusted accordingly.

However, researcher is kindly requested to submit a copy of his/her findings to the state Ministry of Health, please.

DR. B. M. JATAU
Chairman
Research Ethics Committee

REGISTRY
DESPATCHED
SIGN
DATE 2/6/15

KATSINA STATE HOSPITAL SERVICES MANAGEMENT BOARD

P.M.B. 2139, SHAISKAWA, KATSINA
:065-434557

Our Ref: KHSMB/S.7/VOL.VII Your Ref: Date: 02/07/2015

The Medical Director
General Hospital
Dutsin-Ma

INTRODUCTION LETTER IN RESPCET OF BASHIR ABDULLAHI AND KHADIJA ALIYU

The above named students are student of the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria. They are conducting research work title MOLACULAR CHARECTERIZATION OF MULTIDRUG RESISTANCE CLINICAL SALMONELLA ENTRICA SEROVARS in Katsina State.

In view of the above you are required to give the students maximum cooperation with regard to their research work, please.

Sani Yahaya
For:- General Manager

Dr. Salisu Abdu Banye (FWACP) Chairman, Hajiya Mansura I. Bakori Member, Hon. Musa Nuhu Gafia - Member
Alh. Lawal Hussaini Dutsin-ma - Member, Dr. Kabir Garba Dara (FWACS)-General Manager Member,
Lawal A. Bindawa - Board Secretary

HOME OF HERITAGE AND HOSPITALITY

Questionnaire! **Questionnaire!!** **Questionnaire!!!**

RESEARCH TITLE: "*Molecular Characterization of Multidrug-Resistant Clinical Salmonella enterica Serovars in Part of North-Western Nigeria*".

Patient number..... Hospital.....

Sex..... Age.....

Tick appropriately

	Yes	No		<1wk	>2wk
1. Fever	☐	☐	If yes,for how long?	☐	☐
2. Constipation	☐	☐	If yes,for how long?	☐	☐
3. Diarrhoea	☐	☐	If yes,for how long?	☐	☐
4. Vomiting	☐	☐	If yes,for how long?	☐	☐

5. Sources of drinking water

Tap ☐ Pond ☐ Well ☐ Packaged ☐ Borehole ☐

6. Eating of raw egg

Yes ☐ No ☐

7. Eating Unwashed Fruits/Vegetables

Ye ☐ No ☐

8. Self Medication before Coming to Hospital Yes ☐ No ☐

9. If 8 above is Yes, for how long? ☐ days and what Antibiotic/s did you used?

Ampicillin ☐

Cefotaxime ☐ Septrin ☐ Ciprofloxacin ☐ Chloramphenicol ☐ Other ☐

Clinical Diagnosed.....

Signature/Date.....

B. Abdullahi
Investigator