International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Studies on Biochemical Characterization of Salmonella and Mixed Infection Cases with Schistosomiasis in Kwande LGA, Benue State, Nigeria

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Abstract

Bacterial *Salmonella* infections and helminthic schistosomiasis are classified under Neglected Tropical Diseases (NTD). Biochemical characterization was carried out on *Salmonella* serovars in Kwande LGA, Benue State, Nigeria. The aim was to determine the prevalence of the infection and mixed cases of schistosomiasis. The best biochemical test for the detection of *Salmonella* was also targeted. A total of 180 subjects were randomly investigated from five different locations (Iange, Sati, Kyogyen, Ikov Sati and Adikpo). Two specimens (stool and urine) were collected from each subject. Preparation and culture of specimens followed standard practices. *Salmonella* cases were diagnosed on stool samples using six biochemical tests. *Schistosoma haeamatobium* and *S.mansoni* were diagnosed using urine and faecal specimens respectively. Average prevalence of *Salmonella* infection in Kwande LGA was 13.9% (8.3% female, 5.6% male) consisting of 44% *S. typhi* and 56% of other serovars. Infection was associated with location (χ^2 = 145.79, p=0.000). Ikov Sati had the highest cases (65%) followed by Adikpo (15%). No cases was recorded at Kyogyen. Infection was most common among farmers (8.3% prevalent), thus associated with occupation type (χ^2 =52.63, p=0.000). Among the five biochemical tests, catalase test was the best in the identification of *Salmonella* serovars followed by hydrogen sulphide and citrate.

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Among the 180 subjects studied, six mixed infection cases of Salmonella-Schistosomiasis were recorded with a prevalence of 3% unassociated age, sex and location. However, farmers and school children recorded 2 cases each while okada riders and civil servants had a single case each. Based on organism type, S.typhi-S.mansoni-S.haematobium mixed infection had only one case. Co-infection of S.typhi-S.mansoni also had a single case. Salmonella-S.haematobium co-infection cases were four (2.2% prevalent). From all indications, *Salmonella* cases in the study area were high. Farming practices are implicated. This may be due to unhygienic practices in the handling, washing, and consumption of fresh food crops. Both farmers and school children are more vulnerable to contact Salmonella-Shistosoma co-infections than other occupational status, probably due to water pollution factors. The information given in this report is crucial in the diagnosis and prevention of *Salmonella* infections known to be a major killer in rural areas most especially when combined with shistosomiasis. Urgent control measures should be put in place at the Ikov Sati community recording an alarming rate of *Salmonella* infection among farmers.

Keywords: Biochemical characterization; Co-infection; Control; *Salmonella*; Schistosomiasis; Prevalence.

1. Introduction

Bacterial *Salmonella* infections and helminthic schistosomiasis are classified under Neglected Tropical Diseases (NTD). Schistosomiasis and typhoidal salmonellosis have similar geographical distribution. They are restricted to areas with poor sanitation and warm temperature in the tropical and sub-tropical regions [8]. The causative organisms share similar habitat/microhabitats. They are water borne and enteric. Any species of *Schistosoma* can interact symbiotically with any strain of *Sal. enterica*. It is also possible that a particular *Schistosoma* species interacts with more than one strains of *Sal. enterica* in a host [9]. Both diseases affect rural dwellers especially children and farmers having frequent contact with water bodies [6]. In most studies, *Schistosoma-Salmonella* co-infection cases are much higher in children and young adults than older ages. In other studies, the infections are distributed regardless of age and sex status [8]. Both infections can affect the liver and kidney function and combined action can cause devastating physiological effect. However, the studies on the prevalence of mixed infections of schistosome-*Salmonella* cases are grossly insufficient across African communities [8]. In developing countries where both *Salmonella* and schistosoma are endemic, co-infections may be common and a synergistic interaction may complicate the course of infection and make diagnosis and therapy difficult [8]. High prevalence of mixed infections of the two organisms has been reported in some parts of Nigeria [8,9]. In most cases, two species of *Schistosoma* (*S. haematobium* or *S. mansoni*) are known to interact with any typhoidal *Sal. enterica* strains (Typhi or Paratyphi). Prolonged salmonellosis in schistosome-infected patients is due to difficulty in both diagnosis and treatment because the bacteria evade drug [3]. The worm also provides a multiplication focus for the bacteria in the portal mesenteric system with a persisting bacteremia. The life-threatening effects of the chronic mixed infections have been reported. Many people living in schistosome endemic regions such as Nigeria are asymptomatic carriers of schistosomiasis spreading the communicable infections across communities [6; 8; 11]. The diseases may be accompanied with inactive or active cases. Moreover, both infections are highly prevalent in areas with poor sanitation and contaminated water bodies and they both end as enteric infections [8]. The WHO has expressed the

need for detailed epidemiological data on the prevalence of single and mixed infections of these organisms in all parts of Africa for adequate control measures. Benue State is a North Central State in Nigeria dominated by rural dwellers, farmers, children and adults. One of the largest rivers in Africa, the River Benue, runs across the State where economic activities take place. According to [13], these are targeted areas of schistosomiasis control programme because the life cycle of *Schistosoma* species depend on fresh water bodies. Polluted water bodies are also habitats for *Salmonella* species. Factors that favour the presence of the two infections are present in Benue State where the popular River Benue is situated. The two diseases are highly burdensome and recalcitrant to treat with high mortality rates among infants and adults, most especially where there are cases of co-infections. The aim of this work was to study the biochemical characterization of *Salmonella* with a view to determining its overall prevalence and mixed infection with schistosomiasis in Kwande LGA of Benue State Nigeria.

2. Materials and methods

Study Area and Design

Kwande is a Local Government Area (LGA) of Benue State, Nigeria. The headquarter is Adikpo Town. The LGA has an area of 2,891km2 and a population of 248,697. It is bordered by Vandeikya LGA, Ushongo LGA and Katsina-Ala LGA, Cross River Stat and Takum in Taraba State. The LGA has very big rivers which usually take care of agricultural and industrial needs (www.benuestate.gov.ng). Ethical clearance was obtained from Benue State Ministry of Health and Human Resources**.** Studies were carried out in Zone A Senatorial District across 5 locations: Iange, Sati, Korgyen, Ikov Sati and Adikpo. A total of 180 human subjects were studied. They were: LGEA Primary School Children Iange Adikpo (60); Rural farmers (60) and patients attending General Hospital Adikpo (60). Each subject donated two samples (urine and stool), therefore total sample collected was 360 (180 urine and 180 stool samples).

Detection of *Schistosoma* species

Schistosomiasis was diagnosed through the detection of parasite eggs in stool for *S. mansoni* or urine specimens for *S. haematobium* [5] following WHO standard guideline. Stool samples were examined for eggs of *S. mansoni* identified through their oval shape with lateral spine. Urine filtration and Kato-Katz methods were employed **[7].** Urine samples were examined for *S. haematobium* eggs identified through their oval shape bearing terminal spine. All slides were viewed on the light microscope (Binocular Olympus CH) using appropriate low power objective lenses. Degree of infection was recorded for positive cases using a subjective qualitative assessment. A single plus (+), double plus (++) and triple plus (+++) signs indicated light, intermediate and heavy infections respectively.

Biochemical Characterization of *Salmonella*

Stool sample (1g size) was transferred into a sterile Bijou bottle containing 5mls of selenite F. Broth. Stool samples were transported to the Advanced Biology Research Laboratory Federal University of Agriculture Makurdi for

isolation, biochemical characterisation and detection of *Salmonella* cases. Stool samples were inoculated on sterile XLD (Xylose Lysine Deoxycholate) Agar using the streak plate method [4] and incubated at 37^{o}C for 18-24hours. Colony which shows the characteristic red to pink colour on XLD with or without black centre (Hydrogen sulphide production) were sub-cultured to obtain pure culture. The pure cultures were sub-cultured on sterile nutrient agar plates for 18-24hours from where biochemical test were carried out following standard biochemical tests [4].

Citrate Test

This test was used to study the ability of *Salmonella* to utilize citrate present in Simmons medium, as a sole source of carbon for growth. A colony was inoculated on Simmon's citrate agar which also contained bromothymol blue as indicator. Positive test was indicated by the appearance of growth with blue colour.

Urease Test

This test detected the ability of *Salmonella* to produce urease enzyme. The organism was inoculated on the slant of urea agar and incubated at 37^{o}C. Observations were made after four hours and after overnight incubation. Development of purple-pink colour indicated production of urease.

Indole Test

This was carried out using the Kovac's reagent and peptone water. The organism was inoculated with peptone water broth and incubated at 37^{0}C for 48 hour. 0.5 ml of Kovac's reagent was added as the mixture was shaken gently. A pink/red colour in the alcohol layer (top of the culture) indicated a positive reaction.

Hydrogen Sulfide (H_2S)

The test organism was inoculated into labeled tube by means of stab inoculation in SIM medium. Inoculation was done at 37°C for 24 hours. Formation of black precipitate on the medium indicated positive results

Oxidase Test

With a sterile glass rod, 2-4 drops of oxidase reagent were placed on a piece of Whatman No. 2 filter paper. A test colony was picked with a sterile loop and smeared over a small area impregnated filter paper. A deep purple colour appearing in 20 seconds indicated a positive result. A deep purple colour in 10-60 seconds indicated a weakly positive result.

Catalase Test

Catalase degrades hydrogen peroxide (H_2O_2) and releases oxygen detected as effervescence. A drop of 3% H_2O_2 was placed on test colonies on nutrient agar. Observation was made for effervescence. Prompt effervescence indicated

catalase production.

3. Data Analysis

Statistical analysis was done using Minitab (16.0) software. Data were described and presented in tables and charts. Inferential statistics were carried out using the Chi square at 0.05 level of significance ($P \leq 0.05$).

4. Results and discusion

The prevalence of *Salmonella* infections in Kwande LGA was 13.9%. Ikov Sati had the highest cases of (65%) followed by Adikpo (15%). Sati and Iange recorded prevalences of 10.0% and 5.0% respectively whereas Kyogen was free from *Salmonella* infection (Table 1). Based on sex status, there were 10 males and 15 females with prevalence of 5.6% and 8.3% respectively. S,typhi was 6.1% prevalent while other serovars recorded 7.8% as shown in table 2. The distribution of prevalence the two sex types and between causative serovar was homogenous ($P>0.05$). *Salmonella* infection was most prevalent among farmers (8.33%) while other occupation types had less than 2% prevalence (Table 3). Thus, infection was associated with occupation ($\chi^2 = 52.63$, $P<0.05$). The most sensitive of all the biochemical tests in the detection of *Salmonella* was the catalase test (100%) followed by hydrogen sulphide (60%) and citrate tests (48%) as given in table 4. From all indications, *Salmonella* cases in the study area were high. Farming practices are implicated. This could be due to the compromised level of hygiene by farmers in the handling and consumption of fresh food crops such as fruits and vegetables. Similar views were previously suggested in other studies [8; 6]. *Salmonella* prevalence of 13.9% reported in this work was higher than the 9.3% reported among some students of College of Education in SouthWest Nigeria. It was also higher than the 12.4% reported among patients attending Salama Clinics in Northern Nigeria [2]. On the other hand, higher prevalence was reported in other places such as 31.5% in Lagos State (Ogah and his colleagues 2016) and 62.7% in Karu Narawa State [1]. Among the 180 subjects studied, six (6) co-infection cases of Salmonella-Shistosomiasis were recorded with a total prevalence of 3% as given in table 5. Co-infection was not associated with age, sex and location ($P>0.05$). However, farmers and school children had two cases each (33.3%) while okada riders and civil servants had a lone case each (16.7%). There were more males (4) than females (2) with co-infections (66.7% and 33.3% respectively in proportion) and three locations were affected with equal distribution of two cases each (Adikpo, Sati and Ikov Sati). Based on causative organisms, *Salmonella* typhi-*Schistosoma masoni*-*S.haematobium* mixed infection had only one case (0.56% prevalence). Co-infection of *Salmonella* typhi-*Schistosoma masoni* also had a single case (0.56% prevalence). *Salmonella* typhi-*Schistosoma haematobium* co-infections were four with a total prevalence of 2.2% as shown in figure 1. *Schistosoma mansoni* occurred in two mild cases (+) while *Schistosoma haematobium* cases were either moderate (++) or heavy infections (+++) but not mild (Table 6). In this work, both farmers and school children are more vulnerable to co-infection of schistosomiasis and *Salmonella* than in other occupations probably due to differences in frequency of contact with polluted water bodies. This aligns with the view of [10]. The health implications of the chronic mixed infection cases have been reported [3; 13]. Many people living in schistosome endemic regions are asymptomatic carriers who are likely to spread the infection across

communities [11]. Thus, the occurrence of six co-infections out of 25 *Salmonella* cases was high in the study area and should be taken seriously.

Table 1: Prevalence of Salmonella infections in Kwande LGA.

Location	Number of samples	Number of Infected Subjects	Prevalence (%)
Zone A			
Iange	20	1	5.0
Sati	20	2	10.0
Korgyen	20	0	0.0
Ikov Sati	20	13	65.0
Adikpo	60	9	15.0
Total	180	25	13.9

$\chi^2 = 145.79$, P= 0.00 (P<0.05)

Table 2: Prevalence of Salmonella infections in Kwande LGA based on sex and serotype.

Sex	Prevalence	*Salmonella* type/Number	Prevalence (%) /Proportion
Male	10 (5.6%)	S.typhi (11)	6.1 (44%)
Female	15 (8.3%)	Other serovars (14)	7.8 (56%)
Total	25 (13.9%)	25	13.9 (100%)

P>0.05

Table 3: Prevalence of *Salmonella* infections in Kwande LGA based on occupation.

Occupation	No of infected subject	Prevalence %
Civil servants	2	1.11
Marketers	3	1.67
Okada rider	2	1.11
Student	3	1.67
Farmers	15	8.33

(χ^2 =52.63, P=0.000, P<0.05)

Table 4: Comparative sensitivity of biochemical tests in Salmonella detection.

Biochemical tests	Sensitivity/25	Degree of sensitivity
Catalase	25	100%
Citrate	12	48%
Urease	0	0%
Indole	0	0%
H_2S	15	60%
Oxidase	1	4%

Table 5: Demographic information on subjects with co-infection of salmonella-schistosomiasis.

Location	Occupation	Age (years)	Sex
Adikpo	Civil servant	28	Female
Adikpo	Okada riders	60	Male
Sati	Student	5	Male
Sati	Student	11	Female
Ikov Sati	Farmers	35	Male
Ikov Sati	Farmers	15	Male

Co-infection prevalence= 3%

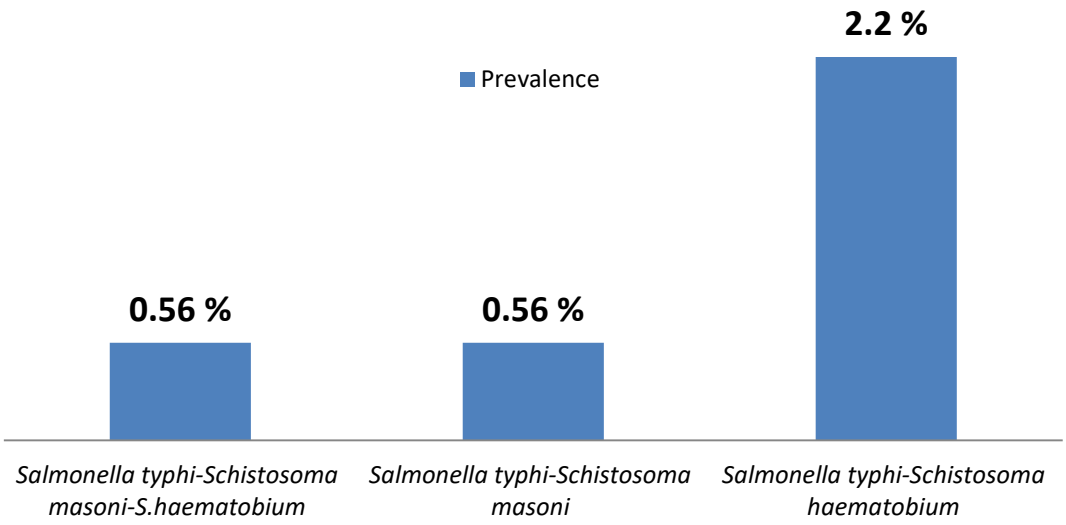

Figure 1: Mixed infection cases of Salmonella-Schistosomiasis and their prevalence in the study area.

Table 6: Degree of infection of schistomiasis in mixed infection with Salmonella.

Degree of infection	*Schistosoma mansoni*	*Schistosoma haematobium*
+ (mild)	2	0
++ (moderate)	0	2
+++ (heavy)	0	2
Total	2	4

5. Conclusion

Prevalence of *Salmonella* infection in Kwande LGA of Benue State was 13.9% while co-infection with schistosomiasis was 3.3%. Cases of *Salmonella typhi-Schistosoma haematobium* were the highest of mixed

infections (2.2% prevalence). *Salmonella* infection was associated with localities and occupation within the study area but mixed infection can occur irrespective of demographic status. Urgent control measures should be put in place at the Ikov Sati community recording an alarming rate of *Salmonella* infection among farmers.This report has given pieces of vital information that are indispensable in the control of the infections studied in this work.

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