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## Full Length Research Paper

# Salmonella-Bacteraemia and Diversity of Bacterial Uropathogens in Concomitant Urinary Schistosomiasis among Children in Jaba, Kaduna State, Nigeria

Henry Gabriel Bishop\*, Helen Ileigo Inabo, Elijah Ekah Ella

Ahmadu Bello University, Faculty of Life Sciences, Department of Microbiology, Samaru, 81001 Zaria, Kaduna State, Nigeria,

\*Corresponding Author: Tel.: +234(0)8176357626; [gabrielhenrybishop@gmail.com](mailto:gabrielhenrybishop@gmail.com)

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**Abstract.** *Salmonella*-bacteraemia and urinary schistosomiasis affect health and development of Nigerian children. This study was aimed at determining the prevalence of *Salmonella*-bacteraemia and diversity of bacterial uropathogens in concomitant urinary schistosomiasis in Jaba LGA, Kaduna State, Nigeria. Awareness on the diseases was created in pre-selected primary schools from 10 villages in Jaba. 505 pupils participated; 10ml urine and 2ml blood samples were collected from each pupil. Blood samples were pre-enriched in Selenite-F broth and Brilliant-Green-Bile broth. Urine sediments and pre-enriched blood were cultured on SSA and XLD agar. Bacterial isolates were biochemically characterised. Centrifuged urine sediments were microscopically examined for *Schistosoma haematobium* egg(s) and count/10ml urine was recorded. Results/data obtained were statistically analysed at  $P=0.05$ . No *Salmonella* species was found in all examined samples, hence a prevalence of 0.0%. Equally, no bacterial growth was seen on blood cultures. Seven diverse Gram-negative bacteria were isolated from the urine samples. *Citrobacter* spp (6.7%) and *Klebsiella* spp (3.4%) were most prevalent bacteria; others were *Acinetobacter* spp (2.0%) and *E. coli* (1.6%). The least occurring were *Pseudomonas aeruginosa* (0.4%), *Providencia* spp (0.2%) and *Serratia marcescens* (0.2%). *Citrobacter* spp (8.1%), *E. coli* (1.6%) and *Klebsiella* spp (1.6%) were found in co-infection with *Schistosoma haematobium*. Overall concomitant urinary schistosomiasis was 12.3%. *Citrobacter* spp, *E. coli* and *Klebsiella* spp were more prevalent in females. *Acinetobacter* spp was prevalent in males. Drinking water sources were statistically associated with bacterial uropathogens. Concerted efforts are required to eradicate schistosomiasis in Nigeria through snails/cercariae control, awareness and potable water supply.

**Keywords:** Bacteraemia, Jaba LGA, Nigeria, pupils, *Salmonella*, urinary schistosomiasis

## 1. INTRODUCTION

Typhoid and paratyphoid fevers affect many Nigerian children; these diseases have been linked to unsafe water, poor sanitation and unawareness of the risks/routes of transmission. It is important therefore, to evaluate the health conditions of children in rural areas and create considerable awareness of certain tropical diseases, but mass treatment is often difficult. Governmental and non-governmental organizations should intensify efforts in the eradication of these diseases.

Salmonellae are motile facultative anaerobes, non-spore-forming, Gram-negative bacilli, measuring 0.7 to 1.5  $\mu\text{m}$  by 2-5  $\mu\text{m}$  in size (Bell and Kyriakide, 2002; Global Salm-Surv, 2003; Molbak et al., 2006; Pegues and Miller, 2009). The genus *Salmonella* belongs to the family Enterobacteriaceae (Meneses, 2010) consisting of two species: *Salmonella bongori* and *Salmonella enterica* which is divided into 6

subspecies (Global Salm-Surv, 2003; Paccagnella, 2005). *Salmonella* are found in both group 2 and 3 of WHO classification of infective microorganisms (Cheesbrough, 2009). They have been identified as one of the leading causes of foodborne illnesses in the world (Cardinale et al., 2005; Henry et al., 2015). And have caused a number of outbreaks in both developed and developing countries (Meneses, 2010) mainly due to inadequate disposal of sewage, flooding and consumption of unsafe water (Mohager et al., 2014).

Typhoid is caused by *Salmonella* Typhi (Threlfall et al., 2010) but paratyphoid can either be caused by *Salmonella* Paratyphi A, *Salmonella* Paratyphi B or *Salmonella* Paratyphi C. Both typhoid and paratyphoid fevers are commonly grouped together as enteric fever (White, 2010) and are common in less-industrialized countries (Mohager et al., 2014). Children tend to have higher bacteraemia than adults (White, 2010) with typhoid fever occurring most frequently among children and young adults of 3-19

years old, especially in the endemic areas (Cheesbrough, 2009). Usage of ineffective antibiotics has contributed to emergence of antibiotic resistance as well as the phenomenon of bacterial persistence (Barnhill et al., 2011).

Schistosomiasis is a chronic debilitating disease that affects the populations of tropical and subtropical countries, especially children who indulge in water-based activities in unsafe or cercarial-infested water bodies (Noble and Glem, 1982; Ibrionke et al., 2012; Kanwai et al., 2011; Elele and Ewurum, 2013; WHO, 2016). *Schistosoma haematobium* (*S. haematobium*) is the only cause of urinary schistosomiasis among all the schistosomes (Jamjoom, 2006; Ibrionke et al., 2012). The disease can be diagnosed by microscopic detection of egg(s) of *Schistosoma haematobium* in urine, which remains the gold standard (Ibrionke et al., 2012; Barsoum, 2013). Humans become infected via dermo-invasion of intact skin by cercariae which are attracted to the warmth of body and skin lipids (Sakanari and Mckerrow, 2010).

Co-infections of *S. haematobium* and *Salmonella* species can occur, but in the endemic areas the salmonellae are notorious cause of resistant secondary bacterial cystitis (Barsoum, 2013). This occurs because there is an established symbiotic association between schistosomes and certain *Salmonella* species (Muniz-Junqueira et al., 2009; Barnhill et al., 2011; Barsoum, 2013). Enteroinvasive *Salmonella* enter systemic circulation and attach to integuments of adult *Schistosoma* spp (LoVerde et al., 1980; Melhem and LoVerde, 1984) or their intestinal tracts (Lambertucci et al., 1998; Mohager et al., 2014). As such, bactericidal concentrations of antibiotics become largely above the achievable therapeutic levels of the drugs in co-infected individuals. Chloramphenicol-sensitive *Salmonella* Typhi in co-infection with *Schistosoma* sp had been found to be resistant to chloramphenicol (Barnhill et al., 2011), and hence schistosomiasis can increase the cost and duration of treatment of salmonellosis. Therefore, this study aimed to determine the prevalence of *Salmonella*-bacteraemia and diversity of bacterial uropathogens in concomitant urinary schistosomiasis, as well as some socio-demographic factors associated with these infections among pupils in Jaba LGA, Kaduna State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1. Study area and population

The study was conducted in the Local Government Area (LGA) of Jaba, Kaduna State, Nigeria. The area is located in the Northern hemisphere on Latitude 9° 19' 47"N to Latitude 9° 36' 35"N, and in the East on

Longitude 7° 56' 24"E to Longitude 8° 12' 36"E (Maphill, 2013). The area is occupied by the Ham People, who are notable for the rich Nok culture. Archeologists discovered the Nok Terra cotta from Jaba, which was carbon-dated to 2000-2500 years ago. "Kwain" (or Kwoi which means "Community of the United") is the political capital of the LGA (Nokculture, n.d). People of the area are predominantly farmers; majorly, ginger, 'hungry rice' (*Digitaria exilis*), cocoyam, guinea corn, millet and maize are cultivated. The study population was made up of primary school pupils randomly drawn from 10 selected villages in the LGA, namely, Ankun, Bitaro Central Area, Chori, Dura, Gora, Kwoi, Nok, Sambang and Yadi-Pyok. The pupils voluntarily participated in this study after awareness talks were given in pre-selected primary schools within the LGA.

### 2.2. Collection of urine and blood samples

The pupils who consented to participate in the study were briefed and guided on how to collect 10ml of their urine into provided sterile (wide-mouth) sampling bottles with screw caps between 10am - 1pm (Cheesbrough, 2009). From each pupil that submitted a urine sample, 2ml of venous blood sample was collected into EDTA K-3 bottle using new sterile syringe and needle.

### 2.3 Administration of structured questionnaires

A structured questionnaire was administered to each pupil that submitted urine and blood samples. The questionnaire captured some socio-demographic and risk factors associated with urinary schistosomiasis and typhoid fever. Assistance was sought from respective class teachers and head teachers in interpretation from English language into easily understood dialect of the study area.

### 2.4. Packaging of samples

The urine samples were screened away from sunlight by enclosing them in dark polythene bags (to prevent hatching of *S. haematobium* eggs if present). Both sample types from each volunteered pupil were simultaneously labelled and placed into separate ice containers. The samples were analysed at Bacteriology/Parasitology Laboratory in the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

## 2.5. Determination of packed cell volume of blood samples

Packed cell volume (PCV) of each blood sample was determined by the microhaematocrit centrifuge technique (HCT). Two plain capillary tubes were filled with a blood sample to three-fourth their heights and carefully sealed by means of Bunsen flame to a (2 mm) red demarcation on each tube. The tubes were spun at relative centrifugation force (RCF) of 12,000-15,000  $\times g$  for 5 minutes in the microhaematocrit centrifuge, then the PCV were read on the Haematocrit Reader. An average of the two values was recorded; anaemic PCV was  $<34\%$ , normal PCV was  $\geq 34 \leq 45\%$ , high PCV was  $\geq 46\%$  (Cheesbrough, 2009).

## 2.6. Cultural and biochemical identification of *Salmonella* spp and other bacterial uropathogens

For pre-enrichment, 1ml from each blood sample was transferred into 5ml Selenite-F broth (Akinyemi et al., 2004; Mirmomeni et al., 2009), and the remaining blood sample was transferred into 5ml Brilliant-Green-Bile broth and incubated at  $37^{\circ}\text{C}$  for 24 hrs. After the pre-enrichment period, each of the broth was sub-cultured onto prepared sterile plates of *Salmonella-Shigella* Agar (SSA) and Xylose- Lysine-Deoxycholate (XLD) Agar and incubated at  $37^{\circ}\text{C}$  for 24 hrs. Where growth was not detected, the plates were re-incubated for another 48 hrs at the same temperature and periodically observed before discarding as negative culture.

Each 10ml urine sample was gently shaken to stir up any sediment and immediately transferred into labelled plastic centrifuged tubes of 15 ml capacity. Where the sediments remained in the bottle, sterile distilled water was used to rinse them into the centrifuge tube. The samples were centrifuged at 3000 rpm for 3-5minutes (Cheesbrough, 2009). The supernatant was discarded by means of Pasteur pipette. A loopful of the sediment was inoculated on SSA and XLD agar plates and incubated at  $37^{\circ}\text{C}$  for 24 hrs. The sediment was kept for the detection and quantification of *S. haematobium* eggs. Incubation period was increased by another 24 hrs where growths did not occur. Blood and urine cultures were considered negative for *Salmonella* spp only after 48-72 hrs aerobic incubation at  $37^{\circ}\text{C}$ . Other bacterial colonies that grew from the urine cultures were purified on the respective selective media and gram-stained to determine their Gram reaction and morphology. The pure cultures were streaked on

appropriately labelled Nutrient agar (NA) slants and incubated at  $37^{\circ}\text{C}$  for 24 hrs, and stored in the refrigerator for biochemical tests. Periodic sub-culturing of the isolates (after every 2 months) was done to maintain their viability. The following biochemical tests were conducted to identify other Gram-negative bacteria isolated from the urine samples: sugar fermentation, gas and/or hydrogen sulphide ( $\text{H}_2\text{S}$ ) production in Triple Sugar Iron (TSI) agar, indole production, Methyl red (MR), Voges Proskauer (VP), citrate utilisation, urease production, oxidase production, motility, lysine decarboxylation (LDC) and ornithine decarboxylation (ODC) tests (Cheesbrough, 2009).

## 2.7. Detection/quantification of egg(s) of *Schistosoma haematobium* in urine

The sediment obtained in the centrifuge tube (as described in unit 2.6 above) was tapped on the bench and mixed by gentle shaking. A Pasteur pipette was used to transfer all the sediments onto a clean, grease-free glass slide. A drop of Lugol's iodine solution was added and a cover slip was placed over the wet-mount and positioned under the light microscope. The entire wet-mount was screened for egg(s) of *Schistosoma haematobium* using 10x and 40x objectives, while quantitative count was taken. Where the sediments from a sample could not be contained in a single wet-mount, multiple wet-mounts were made from such a sample and the egg counts added together.

## 2.8. Statistical analysis

Data collected of socio-demographic and risk factors of urinary schistosomiasis and bacterial uropathogens, as well as laboratory findings were subjected to Analysis of Variance (ANOVA), Chi Square ( $\chi^2$ ) and Likelihood ratio analyses with the IBM SPSS Statistics Version 21 at  $P = 0.05$ . Final results were summarized in tables, charts and plates.

## 2.9. Benefit of the study to the pupils and school communities

The study benefited the communities by bringing detailed awareness on the risks of using unsafe water bodies and menace of schistosomiasis and salmonellosis. Additionally, each participated pupil was given a test results; and where infection(s) was found, referral to the hospital was made for further medical advice/treatment.

**Table 1:** Prevalence of bacterial uropathogens and parasitic eggs among pupils in Jaba LGA of Kaduna State, Nigeria

Isolated Organism	*Prevalence No. (%)
<i>Acinetobacter</i> spp	10 (2.0)
<i>Citrobacter</i> spp	34(6.7)
<i>E. coli</i>	8(1.6)
<i>Klebsiella</i> spp	17(3.4)
<i>Providencia</i> spp	1(0.2)
<i>Pseudomonas aeruginosa</i>	2(0.4)
<i>Serratia marcescens</i>	1(0.2)
Negative cultures	432 (85.5)
<i>Enterobius vermicularis</i> ovum	1(0.2)
<b>Total</b>	<b>505 (100.0)</b>

\* n = 505

**Table 2:** Prevalence of *Salmonella* species and its co-infections with urinary schistosomiasis in Jaba LGA

Sample Type	No. of Samples Examined	No. Positive (%)	No. Negative (%)	Co-infection No. Positive (%)
Blood	505	0(0.0)	505 (100.0)	0 (0.0)
Urine	505	0(0.0)	505 (100.0)	0(0.0)
<b>Total</b>	<b>1010</b>	<b>0(0.0)</b>	<b>1010(100.0)</b>	<b>0(0.0)</b>

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Results

Out of 505 volunteered pupils who submitted blood and urine samples each, comprising of 251 females (with mean age of 9.87years) and 254 males (with mean age of 9.82 years), Gram-negative bacterial uropathogens were isolated from 73(14.5%) urine samples; the remainder 432(85.5%) were culture-negative (Figure 1).

Seven diverse bacterial uropathogens were biochemically identified. The most occurring bacteria were *Citrobacter* spp (6.7%) and *Klebsiella* spp (3.4%), but *Providencia* spp and *Serratia marcescens* were the least occurring bacteria. One ovum of *Enterobius vermicularis* was identified from a urine sample of a female pupil.

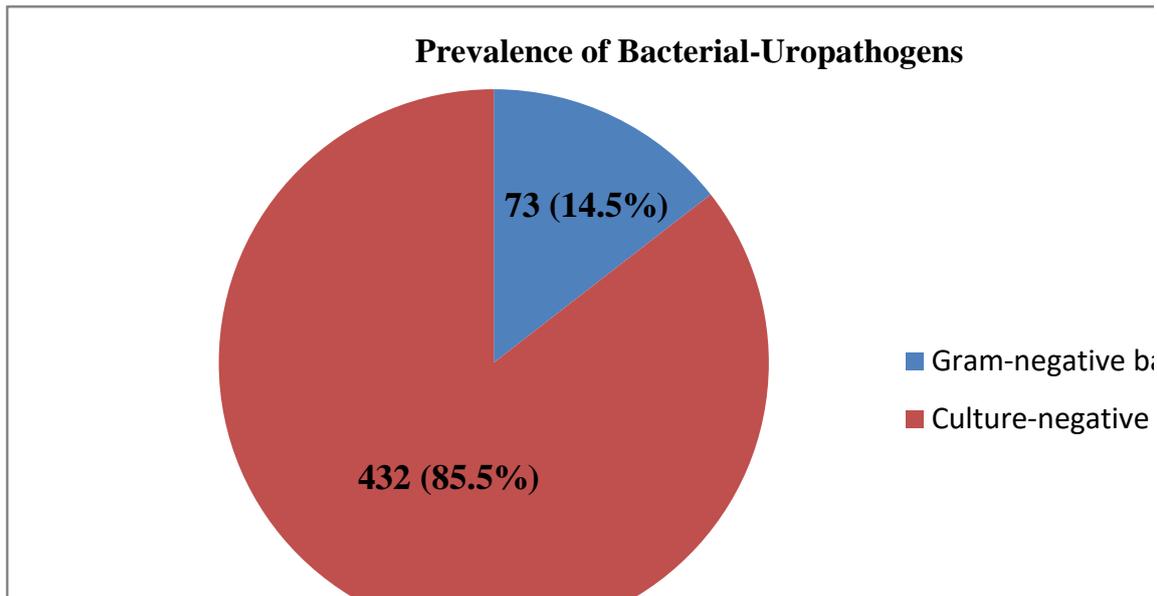
There was no occurrence of *Salmonella* spp from the blood and urine samples of pupils in Jaba LGA and hence an overall prevalence of 0.0%. Similarly, there was no co-infection between *Schistosoma haematobium* and *Salmonella* spp (0.0%) in this study (Table 2), but concomitant urinary schistosomiasis among the pupils was 12.3% (Figure 2). The urinary schistosomiasis was diagnosed by the detection of characteristic eggs of *Schistosoma haematobium* in the urine samples of the pupils. The eggs were golden-

brown in colour with terminal spines as shown in Plate I.

Co-infections of *Schistosoma haematobium* with *Citrobacter* spp (8.1%), *E. coli* (1.6%) and *Klebsiella* spp (1.6%) occurred among the pupils but the relationship was insignificant ( $\chi^2 = 2.874$ ,  $df = 7$ ,  $P = 0.896$ ;  $LR = 4.707$ ,  $df = 7$ ,  $P = 0.696$  (Table 3).

There was absence of *Providencia* spp, *Pseudomonas aeruginosa* and *Serratia marcescens* among the females, but all the isolated uropathogens occurred in at least a case among the males (Table 4). The gender distribution of these uropathogens was not statistically significant ( $\chi^2 = 11.157$ ;  $df = 7$ ;  $p = 0.132$ ). However, higher occurrences of *Citrobacter* spp, *E. coli* and *Klebsiella* spp were observed in the females; *Acinetobacter* spp were more frequently isolated from the males.

The association between uropathogens and swimming by pupils in rivers/streams was statistically insignificant ( $\chi^2 = 13.324$ ;  $df = 7$ ;  $p = 0.065$ ). Among those pupils who claimed to swim in unprotected water bodies, there was a higher occurrence of *Acinetobacter* spp; all the other uropathogens except *Serratia marcescens* were isolated from their urine samples. On the other hand, pupils who claimed not to indulge in swimming had similar uropathogens in their urine, except *Providencia* spp and *Pseudomonas aeruginosa* which were absent as indicated in Table 5.



**Fig. 1:** Prevalence of Gram-negative bacterial uropathogens among pupils in Jaba LGA, Kaduna State, Nigeria

**Table 3:** Co-infection of bacterial uropathogens in concomitant urinary schistosomiasis

Co-infection	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
No infection	443	10(2.3)	29(6.5)	7(1.6)	16(3.6)	1(0.2)	2(0.5)	1(0.2)	377(85.1)
Urinary schistosomiasis	62	0(0.0)	5(8.1)	1(1.6)	1(1.6)	0(0.0)	0(0.0)	0(0.0)	55(88.7)
Total	505	10(2.0)	34(6.7)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	432(85.5)

$$\chi^2 = 2.874, df = 7, P = 0.896; LR = 4.707, df = 7, P = 0.696$$

**Table 4:** Gender distribution of bacterial uropathogens among pupils in Jaba LGA of Kaduna State, Nigeria

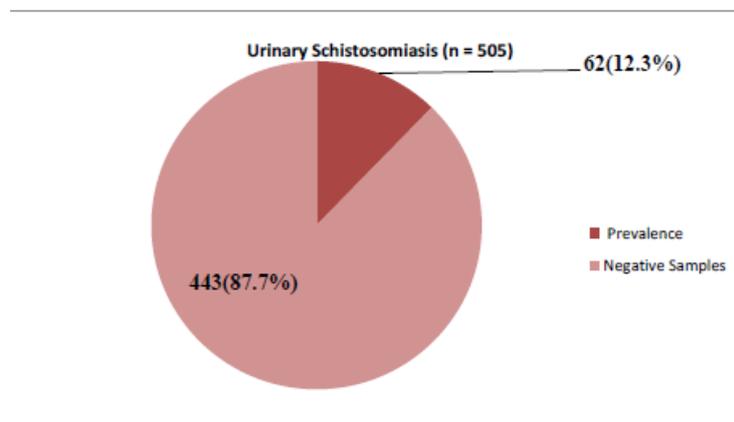
Gender	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
Female	251	4(1.6)	19(7.6)	7(2.8)	11(4.4)	0(0.0)	0(0.0)	0(0.0)	210(83.7)
Male	254	6(2.4)	15(5.9)	1(0.4)	6(2.4)	1(0.4)	2(0.8)	1(0.4)	222(87.4)
Total	505	10(2.0)	34(6.7)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	432(85.5)

$$\chi^2 = 11.157; df = 7; P = 0.132, LR = 13.290; df = 7; P = 0.065$$

**Table 5:** Effect of swimming activity on acquisition of bacterial uropathogens by pupils in Jaba LGA

Swimming in River/Stream	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
No	178	1(0.6)	13(7.3)	5(2.8)	10(5.6)	0(0.0)	0(0.0)	1(0.6)	148(83.1)
Yes	327	9(2.8)	21(6.4)	3(0.9)	7(2.1)	1(0.3)	2(0.6)	0(0.0)	284(86.9)
Total	505	10(2.0)	34(6.7)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	432(85.5)

$\chi^2=13.324$ ;  $df = 7$ ;  $P = 0.065$ ,  $LR = 14.772$ ;  $df = 7$ ;  $P = 0.0390$



**Fig. 2:** Prevalence of urinary schistosomiasis among pupils of Jaba LGA, Kaduna State

**Table 6:** Occurrences of bacterial uropathogens in relation to drinking-water sources of the pupils in Jaba LGA

Drinking Water Source	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
Borehole	277	8(2.9)	15(5.4)	3(1.1)	5(1.8)	1(0.4)	2(0.7)	1(0.4)	242(87.4)
River/Stream	71	2(2.8)	1(1.4)	2(2.8)	3(4.2)	0(0.0)	0(0.0)	0(0.0)	63(88.7)
Tap water	50	0(0.0)	10(20.0)	1(2.0)	4(8.0)	0(0.0)	0(0.0)	0(0.0)	35(70.0)
Well	107	0(0.0)	8(7.5)	2(1.9)	5(4.7)	0(0.0)	0(0.0)	0(0.0)	92(86.0)
Total	505	10(2.0)	34(6.7)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	432(85.5)

$\chi^2=33.398$ ;  $df = 21$ ;  $P=0.042$ ,  $LR = 34.226$ ;  $df = 21$ ;  $P = 0.034$

The source of drinking water significantly determined the occurrences of bacterial uropathogens among the study population ( $\chi^2=33.398$ ;  $df = 21$ ;  $P=0.042$ ). *Citrobacter* spp (20.0%) and *Klebsiella* spp (8.0%) were mostly isolated from the urine samples of

pupils who drink tap water. *E. coli* (2.8%) was frequently isolated from the urine samples of pupils who drink water fetched from rivers/streams. There was absence of *Providencia* spp, *Pseudomonas aeruginosa* and *Serratia marcescens* among the pupils

who drink from rivers/streams, taps and wells. More cases of bacterial uropathogens occurred in those who drink from borehole water (Table 6).

Only 6.3% of the study population complained of having pains during micturition. Among this

population, no uropathogen was isolated from their urine samples. However, among those who claimed to have normal micturition, uropathogens were isolated from their urine samples. The relationship was not statistically significant as  $p = 0.566 > 0.05$  (Table 7).

**Table 7:** Effect of bacterial uropathogens on onset of some signs/symptoms among the pupils in Jaba LGA, Kaduna State, Nigeria

Sign/Symptom	Response Category	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
Painful Micturition*	Absent	473	10(2.1)	34(7.2)	8(1.7)	17(3.6)	1(0.2)	2(0.4)	1(0.2)	400(84.6)
	Present	32	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	32(100.0)
Haematuria#	Absent	480	10(2.1)	34(7.2)	7(1.5)	15(3.1)	1(0.2)	2(0.4)	1(0.2)	410(85.4)
	Present	25	0(0.0)	0(0.0)	1(4.0)	2(8.0)	0(0.0)	0(0.0)	0(0.0)	22(88.0)
Frequency of Micturition^	Normal	496	10(2.1)	34(7.2)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	423(85.3)
	Frequent	9	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	9(100.0)

\* $\chi^2 = 5.773$ ;  $df = 7$ ;  $P = 0.566$ ,  $LR = 10.352$ ;  $df = 7$ ;  $P = 0.170$

# $\chi^2 = 5.165$ ;  $df = 7$ ;  $P = 0.640$ ,  $LR = 6.817$ ;  $df = 7$ ;  $P = 0.448$

^ $\chi^2 = 1.548$ ;  $df = 7$ ;  $P = 0.981$ ,  $LR = 2.838$ ;  $df = 7$ ;  $P = 0.900$

**Table 8:** Effects of level of urinary schistosomiasis on the occurrence of bacterial uropathogens

Intensity of Urinary Schistosomiasis	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
Heavy	10	0(0.0)	4(40.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(60.0)
Light	52	0(0.0)	1(1.9)	1(1.9)	1(1.9)	0(0.0)	0(0.0)	0(0.0)	49(94.2)
None	443	10(2.3)	29(6.5)	7(1.6)	16(3.6)	1(0.2)	2(0.5)	1(0.2)	377(85.1)
Total	505	10(2.0)	34(6.7)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	432(85.5)

$\chi^2 = 22.372$ ;  $df = 14$ ;  $P = 0.071$ ,  $LR = 16.579$ ;  $df = 7$ ;  $P = 0.279$

Among our study population, 5.0 % of them had haematuria with occurrences of 1(4.0%) and 2(8.0%) of *E. coli* and *Klebsiella* spp respectively in their urine samples. Other uropathogens did not occur in haematuria-reported urine samples. But all the seven

uropathogens occurred in the urine samples of those without haematuria. This relationship was statistically insignificant as  $P = 0.640 > 0.05$  (Table 7).

The relationship between the presence of uropathogens and the onset of frequent micturition

among the pupils was statistically insignificant as  $P = 0.981 > 0.05$ . Though only 1.8% (9/505) had high frequency of micturition, no uropathogen was isolated from their urine samples. However, those with normal micturition rather had occurrences of the seven different types of uropathogens (Table 7).

In Table 8, only four cases of *Citrobacter* spp occurred in urine samples of pupils diagnosed with heavy infection of *Schistosoma haematobium* (with count of  $\geq 50$ eggs/10ml urine). One case each of *Citrobacter* spp, *E. coli* and *Klebsiella* spp occurred in urine samples of pupils with light *Schistosoma haematobium* infection (with count of  $< 50$ eggs/10ml urine). More isolation of bacterial uropathogens was

from urine samples of pupils without urinary schistosomiasis but not statistically significant as  $P=0.071 > 0.05$ .

There was only a single case of *E. coli* in urine samples of anaemic pupils. *Providencia* spp did not occur in the urine samples of individuals with high PCV. Also, there was no occurrence of *Serratia marcescens* in urine samples of those with normal PCV. Both urine samples of pupils with high and normal PCVs were found with various bacterial uropathogens. Generally, most of the bacterial uropathogens were isolated from urine samples of pupils with high PCV. Since  $P = 0.325$ , the distribution was statistically insignificant (Table 9).

**Table 9:** Effects of bacterial uropathogens on packed cell volume (PCV) of Pupils in Jaba LGA, Kaduna State

PCV Group	No. of Samples Examined	<i>Acinetobacter</i> spp No. (%)	<i>Citrobacter</i> spp No. (%)	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp No. (%)	<i>Providencia</i> spp No. (%)	<i>P. aeruginosa</i> No. (%)	<i>Serratia marcescens</i> No. (%)	Negative samples No. (%)
Anaemic PCV	41	0(0.0)	0(0.0)	1(2.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	40(97.6)
High PCV	274	8(2.9)	21(7.7)	6(2.2)	8(2.8)	0(0.0)	2(0.7)	1(0.4)	228(83.2)
Normal PCV	190	2(1.1)	13(6.8)	1(0.5)	9(4.7)	1(0.5)	2(0.5)	0(0.0)	164(86.3)
Total	505	10(2.0)	34(6.7)	8(1.6)	17(3.4)	1(0.2)	2(0.4)	1(0.2)	432(85.5)

$\chi^2 = 15.811$ ;  $df = 14$ ;  $P = 0.325$ ,  $LR = 22.436$ ;  $df = 14$ ;  $P = 0.070$

### 3.2. Discussion

The overall prevalence of *Salmonella* species in this study population was zero because both the blood and urine samples examined were culture-negative for *Salmonella* species. Pegues and Miller (2009) and Singh et al. (2011) reported that the isolation of *Salmonella* from urine was rare, even in the endemic areas. *Salmonella* species are not continuously excreted, but may be isolated from urine of 25% of typhoid patients in the third week of infection, especially those with concomitant urinary schistosomiasis (Cheesbrough, 2006). However, it contrasted the report of Singh et al. (2011) who obtained 15.0% prevalence for *Salmonella* spp from urine samples. Explanations for our finding are not far-fetched: firstly, the study population (i.e., volunteered primary school pupils in Jaba LGA) was made up of apparently healthy individuals. Secondly only 18(3.6%) and 9(1.8%) of this population reported the presence of fever and abdominal pain respectively, and *Salmonella* was not isolated from their blood and urine samples. Typhoid fever presents with discomforting symptoms, with capacity to immobilize a sufferer. Hence, infected pupils were likely missed

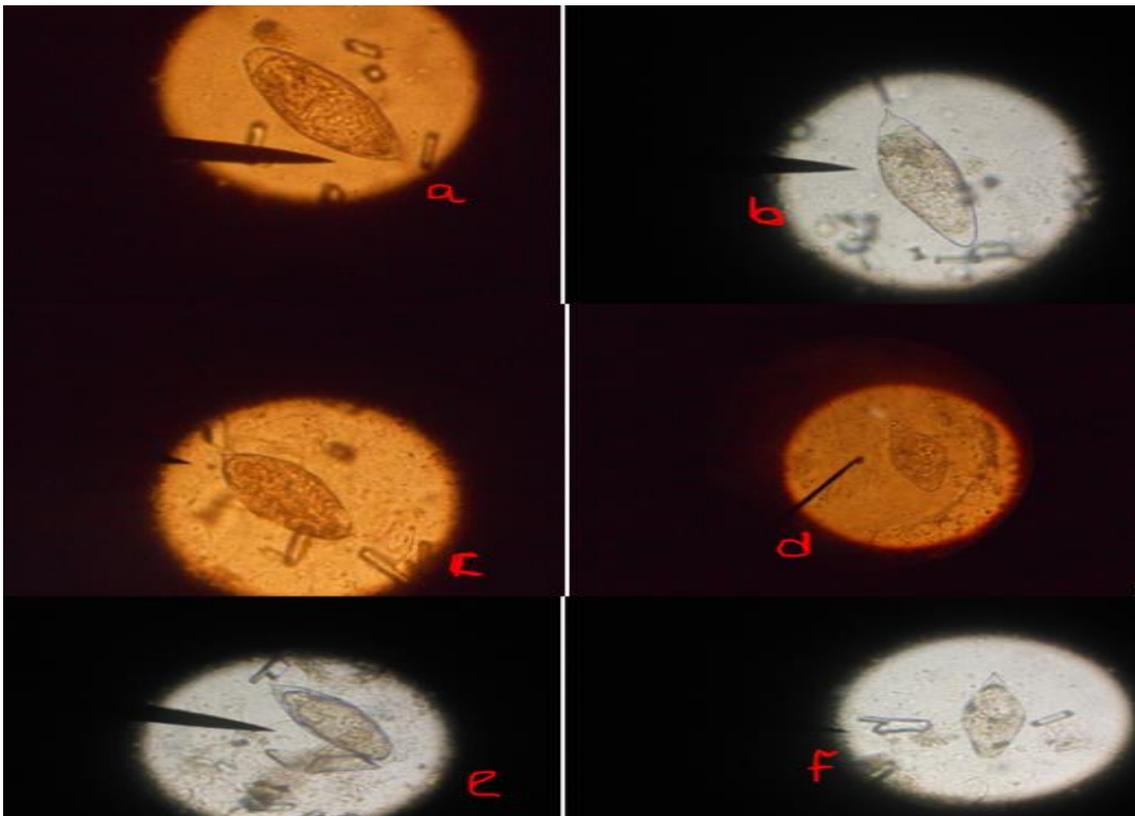
during sampling as they might not have reported to school. However, Abdullahi et al. (2015) showed that among 500 hospitalized patients, 104 *Salmonella* culture-positive samples were obtained. All the hospitalized patients first presented with typical symptoms of *Salmonella* infections: (vomiting and/or diarrhoea, headache, abdominal pain, body pain, dyspnea, weight lost, constipation and anaemia).

The zero prevalence for *Salmonella* spp in blood samples of pupils from Jaba LGA agreed with the research findings of Akinyemi et al. (2004) who also reported 0.0% prevalence. This further indicated the absence of *Salmonella*-bacteraemia among the study population. Transport stress (Mikoleit, 2010), immunological factors affecting the organism within the blood and the inhibitory effect of ethylene-diamine-tetraacetic acid in the EDTA K-3 containers can affect isolation of bacteria from blood. The inhibitory effects of EDTA had been monitored by Root et al. (1988), Stevens et al. (1991), Chudzik et al. (2007), Hinton and Ingram (2010). Hence, a combination of some or all of these factors can risk chances of recovering *Salmonella* spp from blood samples.

There was no co-infection of *Schistosoma haematobium* and *Salmonella* spp in this study. This finding revealed that these co-infections are not always common in urinary schistosomiasis endemic areas, though the work of Melhem and LoVerde (1984) and Barnhill et al. (2011) have shown from *in vitro* studies the synergism of *Schistosoma-Salmonella* interactions. The zero co-infections between *S. haematobium* and *Salmonella* spp in this study also disagreed with the findings of Igwe and

Agbo (2014) for co-occurrence of *Salmonella* spp and *Schistosoma* spp in endemic areas.

An egg of *Enterobius vermicularis* (0.2%) was identified in the course of this study. It was observed in a urine sample of a female pupil. Eggs of this parasite are occasionally found in urine of young females. The female adult worms migrate at night to the perianal and external genitalia to deposit their eggs, which may be carried in urine during micturition (Cheesbrough, 2006). Hence, the female pupil was suffering from enterobiasis.



**Plate 1:** Various appearances of *S. haematobium* ova in urine samples of pupils from Jaba LGA, Kaduna State, Nigeria. (a, c, d in wet mounts stained with Lugol's iodine; b, e, f without Lugol's iodine)

Concomitant bacteruria among the pupils revealed seven diverse bacterial uropathogens of which *Citrobacter* spp and *Klebsiella* spp were the most prevalent. Many studies have implicated *E. coli* as the most occurring bacterial uropathogens. Uneke et al. (2009) reported that *E. coli* (19.0%) and *S. aureus* (31.8%) were the most prevalent uropathogens among children of Ngbo-West and Ezza-North LGAs respectively in Ebonyi, Nigeria. Also, the research findings of Ossai et al. (2014) and Bishop and Shehu (2016) implicated *E. coli* as the most prevalent cause of UTIs among other pathogens. These UTIs result from the invasion and colonization of tissues of parts of the urinary tract, with inflammatory responses; though asymptomatic cases occur but complicated cases are often discomforting (Bishop and Shehu, 2016).

Though painful and frequent micturition are characteristics of symptomatic UTIs, findings from our research revealed the complete absence of the Gram-negative bacterial uropathogens in 32 and 9 urine samples of pupils who experienced painful and frequent micturition respectively. Asymptomatic bacteruria is not accompanied with overt pains or inflammation of the urinary tract. The bacteruria found in larger proportion of the pupils without painful and frequent micturition was an evidence of asymptomatic cases. Symptomatic cases of urinary tract infections (UTIs) are not always due to bacteria; other pathogens can be responsible (Bishop and Shehu, 2016).

The distribution of the isolated uropathogens by gender in this study was not statistically significant. Though *Providencia* spp, *P. aeruginosa* and *Serratia*

*marcescens* were absent in the urine samples of female pupils, they had higher proportions of *Citrobacter* spp, *E. coli* and *Klebsiella* spp than the males. Ossai et al. (2014) also found out that the distribution of uropathogens in concomitant urinary schistosomiasis by gender was not statistically significant, though *E. coli*, *S. aureus* and *Klebsiella* spp were the most prevalent in their study. The location and structure of urethral and vaginal openings of females are by far less effective in preventing bacterial entry (Warren et al., 1999). Also, any habit of unhygienic manner of wiping the anus from back to front can help to transfer faecal-borne pathogens into the vulva and vagina (Arul et al., 2012), and hence may lead to UTIs.

The eggs of *Schistosoma haematobium* were detected as oval in shape, golden-brown in colour and having terminal spines through microscopy. This method of diagnosing urinary schistosomiasis remains the gold-standard (Cheesbrough, 2009). Light infections occurred more than heavy infections among the pupils. The overall prevalence of urinary schistosomiasis (12.3%) in our study was considerably lower compared to other studies. Kanwai et al. (2011) and Omenesa et al. (2015) reported higher urinary schistosomiasis prevalence of 19.5% and 25.11% respectively. Our study area had few fast-running streams/rivers which are unfavourable for the breeding of snails and schistosome cercariae. Also, there was reduced water activities among the pupils compared to other reports.

Even though *Citrobacter* spp occurred most in pupils with heavy infections with *Schistosoma haematobium*, it had no statistical association. Equally, distribution of the bacterial uropathogens had no statistical association with anaemia, though *E. coli* had its highest occurrence in the urine samples of pupils with anaemia. However, the distribution of the bacterial uropathogens had statistical significance based on source of drinking water. Pupils who claimed to drink borehole water had more of the bacterial uropathogens.

Unlike some parasitic infections (malaria and schistosomiasis) that can adversely affect red blood cells of infected individuals (Leder and Weller, 2011), the bacterial uropathogens found in our research had no statistical relationship with the pupils' PCV.

#### 4. CONCLUSION

There was absence of *Salmonella*-bacteraemia and the overall prevalence of *Salmonella* spp among the pupils in Jaba LGA was zero. Concomitant urinary schistosomiasis was 12.3%. Seven diverse bacterial uropathogens were isolated from urine samples of the pupils. Co-infections of *Schistosoma haematobium*

with *Citrobacter* spp (8.1%), *E. coli* (1.6%) and *Klebsiella* spp (1.6%) occurred among the pupils. The females had higher occurrences of *Citrobacter* spp, *Klebsiella* spp and *E. coli*, but *Acinetobacter* spp occurred more in the males. There was absence of *Providencia* spp, *Pseudomonas aeruginosa* and *Serratia marcescens* in the females. Occurrence of bacterial uropathogens had no statistical relationships with swimming in streams/rivers, anaemia, haematuria, painful micturition, frequent micturition, gender of pupils and urinary schistosomiasis. However, the presence of these bacterial uropathogens was statistically associated with the type of drinking water sources: *Citrobacter* spp, and *Klebsiella* spp were most found among pupils who drink tap water, but *Acinetobacter* spp, *Providencia* sp, *Pseudomonas aeruginosa* and *Serratia marcescens* were most isolated from urine samples of pupils who drink borehole water. However, *E. coli* associated with drinking of water from streams/rivers.

Urinary schistosomiasis will continue to pose health challenge on African children unless active roles of government, school authorities, parents and researchers are intensified in its control and prevention.

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Mr. Henry Gabriel **Bishop** is a Nigerian. He obtained his first degree (BSc. Microbiology, FIRST CLASS) from Ahmadu Bello University Zaria (A.B.U.), Nigeria in 2012. He was a recipient of Certificate of Recognition and A.B.U. Student of Excellence Award by the Vice Chancellor, Student Affairs Division, A.B.U. Zaria in May, 2013. He is also a writer (of novels and inspirational books). He is a lecturer at the Department of Microbiology A.B.U Zaria and a member of Nigerian Society for Microbiology (NSM). He has conference papers and journal publications. His area of research is Medical Microbiology.



Helen Ileigo **Inabo** is a Nigerian. She is Professor of Microbiology at Ahmadu Bello University (A.B.U.), Zaria, Nigeria where she obtained her first degree in 1984. She later pursued her Masters degree in Medical Microbiology (Parasitology) in A.B.U. Zaria and graduated in 1991. She obtained her doctorate from A.B.U. Zaria in 1996. She has several (international and internal) journal publications and conference papers. Her area of research is parasitology.



Dr. Elijah Ekah **Ella** is a Nigerian. He is a Senior Lecturer at the Department of Microbiology, Ahmadu Bello University (A.B.U.), Zaria, Nigeria. His area of research is immunology and virology. He has several journal publications and conference papers, and is a member of Nigerian Society for Microbiology (NSM).