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Occurrence of Significant Bacteriuria Among Schistosomiasis Positive Individuals in Ekiti State, Nigeria`

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Abstract
Schistosomiasis, also known as bilharzia, is a disease caused by blood flukes of the genus Schistosoma. The most impacted countries are those in Sub-Saharan Africa, which accounts for nearly 90% of the world's estimated 236.6 million cases. The study was aimed at investigating the occurrence of significant bacteriuria among schistosomiasis positive individuals in Ekiti State, Nigeria.Two hundred and forty-fourindividuals living in riparian areas of Ekiti statewere enlisted for the study. Urine samples were collected from the participants and examined microscopically for presence of Schistosoma eggs, followed by urinalysis and bacteriological investigations. Schistosoma eggs were detected in 35 (14.34%) of the 244 urine samples, while significant bacteriuria was recorded in 16 (6.56%) of the samples.Outof the 16 significant-bacteriuria positive samples only 3(18.75%) were observed to have Schistosoma eggs; while significant-bacteriuria was recorded in 3(8.57%) of the 35 schistosomiasis positive individuals. The bacteriuria was found to be significantly associated with proteinuria (χ^2=25.055; p<0.001) and leukocyturia (χ^2=16.011; p<0.001). The bacteria isolated were Pseudomonas aeruginosa, Vibrio mirabilis, Escherichia coli, Corynebacterium xerosis, Bacillus cereus, Kurthia gibsoni, Staphylococcus aureus and Staphylococcus saprophyticus, among others. The Gram-negative bacterial isolates were highly susceptible to Gentamycin (100%), Tetracycline (85.7%) and Chloramphenicol (78.6%); while the Gram-positive bacterial isolates gave high susceptibility to Gentamycin (84.8%) and Ciprofloxacin (76.1%). Most of the bacteria isolated in this study have been reported to be associated urinary tract infection, except Kurthia gibsoni which has been found to spread from animal to a human by zoophilic sexual intercourse. A case of Schistosomiasis with positive proteinuria and leukocyturia should be taken as UTI and treated accordingly.
Keywords: *Bacteriuria, Kurthia gibsoni, leukocyturia, proteinuria and schistosomiasis.*

INTRODUCTION

Schistosomiasis is an acute and chronic parasitic disease caused by trematode worms of the genus *Schistosoma*. It was estimated that at least 236.6 million people required preventive treatment in 2019. Schistosomiasis transmission has been reported from 78 countries,however, with preventive chemotherapy for schistosomiasis, where people and communities are targeted for large-scale treatment, there remains 51 endemic countries with moderate-to-high transmission that require chemotherapy. The most impacted countries are those in Sub-Saharan Africa, which accounts for nearly 90% of all cases worldwide (WHO, 2022). The disease causes economic and health inconveniences for patients and communities where it is endemic, and Nigeria is the most schistosomiasis-endemic African nation. Nigeria has an overall prevalence of 9.5% with about 24 million persons at risk of schistosomiasis (Anyanti *et al*., 2021). In 2000, WHO estimated the annual death rate at 200,000 globally. This should have decreased considerably due to the impact of a scale-up in large-scale preventive chemotherapy campaigns over the past decade (WHO, 2022).

There are three main types of schistosomiasis, caused by closely related organisms: (1) Japonica, or Eastern, schistosomiasis is caused by *Schistosoma japonicum*, found in Japan, southern China, the Philippines, Thailand, and Indonesia; (2) Manson's, or intestinal, schistosomiasis is caused by *S. mansoni*, found in Africa, the Middle East, the Caribbean, and northern South America; (3) Vesical, or urinary, schistosomiasis is caused by *S. haematobium*, found throughout Africa and the Middle East. Molluscan snails serve as intermediate hosts for S. *haematobium* and *S. mansonias* they develop into infectious larvae in freshwater snail, where they can infect people who come in contact with infected water bodies (Couto *et al*., 2014).

According to Hsiao *et al.* (2016), some bacterial strains and schistosomes have a symbiotic interaction that makes dual infection extremely challenging to identify and cure. *Salmonella* co-infection with either *S. haematobium* or *S. mansoni* has been directly linked by studies conducted in Nigeria, Sudan, and the Democratic Republic of the Congo (Igwe and Agbo, 2018; Mbuyi-Kalonji *et al*., 2020).

The term "urinary tract infection" (UTI) refers to any infection, typically of bacterial origin, that affects any component of the urinary system and is relatively frequent in the human population (Motse *et al*., 2019). Urethritis, cystitis, pyelonephritis and vaginitis were four type of urinary tract infection that affects urethra, bladder, kidneys and vagina respectively (Fosso *et al*., 2017). Today, UTIs cause more than 150 million cases of disease annually throughout the world and it has been a major public health concern (Motseet al., 2019). Uropathogenic *Escherichia coli* (UPEC) reported to cause 80%–90% of UTIs, while *Staphylococcus saprophyticus* causes 5%–10% of UTIs (Abraham and Miao, 2015; Ejrnaes *et al*., 2011). These infections can involve a wide variety of organisms, most notably *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii, Streptococcus,* and *Enterococcus faecalis*, though they are rarely caused by viruses or fungi (Mann *et al*., 2017; Saka and Okunuga, 2017). Women are more prone to than men across all age UTIs are more common in women than men across irrelevant of age, half of female has reported cases of UTI during the life time (Agarwal *et al*., 2020).

The study was aimed at assessing the occurrence of significant bacteriuria among schistosomiasis positive individuals in Ekiti state, Nigeria.

MATERIALS AND METHOD

Sample Collection Processing

Ethical approval was obtained from Ekiti State Ministry of Health, Ado-Ekiti (MOH/EKHREC/EA/P/29), for schistosomiasis investigation in some riparian areas of the state. Two hundred and forty-four individuals living in riparian areas of Ekiti state were enlisted for the study. Fresh urine samples were collected from individuals for urinalysis, microscopical and bacteriological investigation.

Microscopic examination urine for *Schistosoma* eggs

Microscopic examination of urine samples for presence of *Schistosoma* eggs was carried out as described by Cheesbrough (1998). About 10mL of well mix urine was transferred into a conical tube centrifuged at 3,000 revolution per minutes for 5 minutes to sediment the eggs. The supernatant was discarded. Part of the sediment was transferred to a glass slide and covered with a coverslip and examined microscopically for the presence of *Schistosoma haematobium* eggs/ova.

Urinalysis

Biochemical components of the urine were assessed using Combi 9 urinary strip. The quality of the urine samples wasdetermined using the following bio-markers:pH, protein, glucose, leukocyte, nitrite, occult blood, bilirubin, urinobilinogen and ketone.

Isolation, Characterization and Identification of Bacteria

Plate count and Cystein Lactose Electrolyte Deficient (CLED) media were used for primary isolation and determination of bacterial load of the urine samples, by pour plate technique. The plates were incubated at 37°C for 18-24 as well as 48 hours, followed by estimation of microbial loads with the aid of colony counter and values expressed in CFU/mL. A bacterial load of 10^4 CFU/mL was considered significant bacteriuria (Wilson & Gaido, 2004).

Colonies were sub-cultured on sterile nutrient agar plates to obtain pure cultures. This was followed by morphological and biochemical investigations as described by Barrow and Feltham (1993). Biochemical tests carried out on the bacterial isolates were:catalase, oxidase, citrate, blood haemolysis, mannitol salt, MacConkey lactose fermentation, methyl red and Voges – Proskauer, triple sugar iron agar, starch hydrolysis, urease, motility and indole production. Identification of bacteria were based on their phenotypic characteristics using Bergey's identification chart. Isolates that could not be identified phenotypically were subjected to molecular identification.

Molecular characterization of bacterial isolates

Extraction of Deoxyribonucleic acid (DNA) from bacterial cultures was carried out with commercial DNA extraction kit "Quick-DNA" Miniprep plus kit (Zymo Research) with adherence to manufacturer's protocol.

Universal primers targeting the bacterial 16S-rRNA-gene was used for polymerase chain reaction (PCR). Universal 16S rRNA bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R (5'-GGTTACCTTGTTACGACTT-3') were used for polymerase chain reaction (PCR), to amplify 10 ng of genomic DNA extracted from each bacterial isolate (Srinivasan *et al*., 2015). PCR amplification was carried out using Vetrithymal cycler (AppliedBioscience). Electrophoresis was carried out on PCR products using 1% agarose andresolved bands were viewed in gel documentation system (EZ Imager™). The gel was further processed and Sanger sequences were generated at the Bioscience Laboratory, Institute of Tropical Agriculture, Ibadan, Nigeria. This was followed by BLAST of the sequences using NCBI for identification of organisms.

Antibiotic Susceptibility test

All the isolated organisms were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. This was carried out by

making an even spread of the pure isolates on prepared Muller-Hinton agar using sterile swab sticks and aseptic placement of the antibiotics' discs using sterile forceps. The plates were incubated aerobically at 37^0C for 18-24 hours after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2018). Antibiotics used are: Ceftazidime (30g), Cefuroxime (30g), Gentamicin (10g), Cefixime (5g), Ofloxacin (5g), Augmentin (30g), Nitrofurantoin (300g), Ciprofloxacin (5g) for Gram negative isolates; while Gentamycin GEN (10 µg), Ampicillin (30 µg), Cefuroxime (30 µg), Ciprofloxacin CIP (10 µg), Streptomycin (30 µg), Amoxycillin-Clavulanic (30 µg), Vancomycin (5 µg), Chlopromazine (10 µg) and Erythromycin (10 µg), for Gram positive isolates.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 23. Pearson Chi-Square and Spearman correlation statistics were used to test the association of 'significant bacteriuria' with urinalysis parameters, and a value of $p \leq 0.05$ was considered significant.

Results

Schistosoma eggs were detected in 35(14.34%) of the 244 urine samples analysed. Bacteria were isolated from 90% of the urine of the subjects investigated. Significant bacteriuria ($\geq 10^4$CFU/mL) was recorded in 16(6.56%) of the 244 urine samples investigated. Out of the 16 significant-bacteriuria positive samples only 3(18.75%) were observed to have *Schistosoma* eggs; while significant-bacteriuria was recorded in 3(8.57%) of the 35 schistosomiasis positive individuals. The bacteriuria was found to be significantly associated with proteinuria (χ^2=25.055, p<0.001; r_s=0.138, 0.031) and leukocyturia (χ^2=16.011, p<0.001; r_s=0.157; p=0.038). However, bacteriuria was not found to be significantly associated with glucosuria, ketonuria, nitrituria, haematuria, bilirubinuria and urobilinogenuria, (Table 1).

The isolated bacteria from Schistosoma-positive urine samples are presented in Figure 1. The Gram-negative bacteria isolated were *Pseudomonas aeruginosa, Salmonella enterica* serovar Paratyphi, *Citrobacter freundii, Enterobacter aerogenes, Morganella morgani, Vibrio mirabilis, Escherichia coli* and Neisseria species. The isolated Gram-positive bacteria isolated were *Streptococcus pneumoniae, Corynebacterium xerosis, Bacillus cereus, Kurthia gibsoni, Enterococcus faecalis, Staphylococcus aureus, Streptococcusmitis and Staphylococcussaprophyticus. Staphylococcus aureus* had the highest occurrence of 18.8%, followed by *Corynebacterium xerosis* (12.5%) and *Pseudomonas aeruginosa* (9.4%). (Fig. 1).

Plate 1 shows the electrophoresed products of PCR amplification of DNA from bacteria isolated from *Schistosoma* egg positive urine samples. Those characterized molecularly, among the identified bacteria,were *Kurthia gibsonii, Enterococcus faecalis, Salmonella enterica* subsp. *enterica serovar* Paratyphi B, *Kurthiagibsonii* and *Bacillus cereus* (Table 2).

The Gram-negative bacterial isolates were highly susceptible to Gentamycin (100%), Tetracycline (85.7%) and Chloramphenicol (78.6%); with moderate susceptibility to ciprofloxacin (64.3%), Amoxicillin (50%) and Vancomycin (57.1%); low susceptibility to Meropenem (14.1%), Cefuroxime (14.3%), Ceftriaxone (14.3%) and Cefprozil (7.1%);but resistant to Cefotaxime (Fig. 2). The Gram-positive bacterial isolates, on the other hand, gave high susceptibility to Gentamycin (84.8%) and ciprofloxacin (76.1%); moderate susceptibility to Cotrimoxazole (58.7%), Tetracycline (47.8%) and Erythromycin (41.3%); low susceptibility to Chlorpromazine (15.2%), Cefuroxime (4.3%) and Amoxicillin/Clavulanic Acid (4.3%); with no susceptibility to Ampicillin (Fig. 3). The bacterial isolates were observed to show resistance to multiple antibiotics(3-7drugs).

Table 1: Association of bacteriuria with urinalysis outcomes

Analyte	Pearson Chi-Square	Spearman correlation
pH	χ^2=3.193; p=0.203	r_s=0.085; p=0.170
Protein	χ^2=25.055; p<0.001*	r_s=0.138; p=0.031*
Glucose	χ^2=0.938; p=0.333	r_s=0.062; p=0.335
Leukocytes	χ^2=16.011; p<0.001*	r_s=0.157; p=0.038*
Ketone	χ^2=0.361; p=0.948	r_s=-0.039; p=0.655
Nitrite	χ^2=5.011; p=0.082	r_s=0.128; p=0.046*
Blood	χ^2=0.577; p=0.448	r_s=0.050; p=0.450
Bilirubin	χ^2=0.214; p=0.898	r_s=-0.028; p=0.664
Urobilinogen	χ^2=3.707; p=0.157	r_s=0.066; p=0.308

*Significant

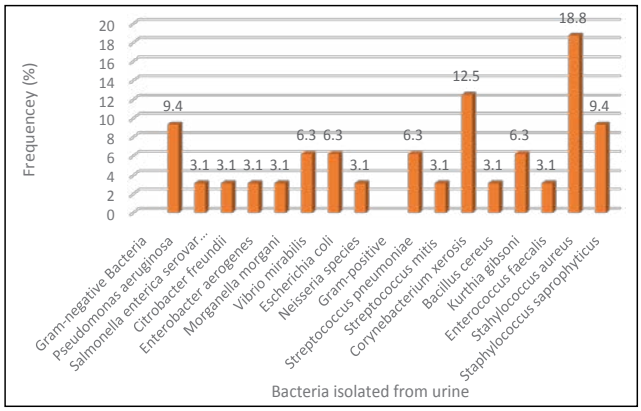

Figure 1: Frequency of bacteria isolation from Schistosoma positive urine samples

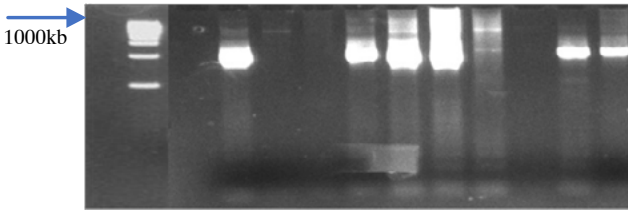

Plate 1: Product of PCR amplification of DNA isolated from bacteria isolated from Schistosoma egg positive urine samples

Table 2: BLAST Hit results of some of the bacterial isolates

S/N	Accession No	Nearest Relatives	Identity (%)
1	MN960342.1	*Kurthia gibsonii* strain TY-06 16S ribosomal RNA gene, partial sequence	92.86%
2	ON778617.1	*Enterococcus faecalis* strain TMPC 20515 16S ribosomal RNA gene, partial sequence	92.33%
3	MH356685.1	*Salmonella enterica* subsp. *enterica* serovar Paratyphi B strain JQ694521.1 16S ribosomal RNA gene, partial sequence	92.96%
4	EF611423.1	*Kurthia gibsonii* strain HC050630C-1 16S ribosomal RNA gene, partial sequence	80.75%
5	MK480518.1	*Bacillus cereus* strain BBS 16S ribosomal RNA gene, partial sequence	82.69%

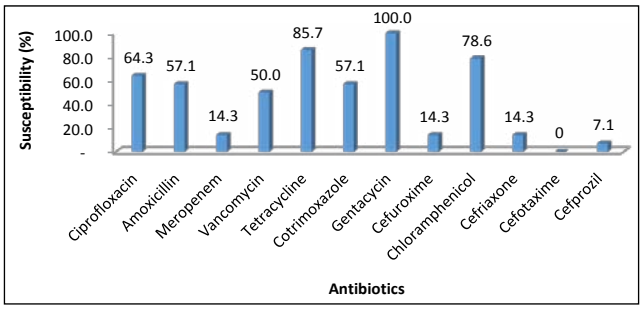

Figure 2: Antibiotic susceptibility pattern of Gram-negative bacterial isolates.

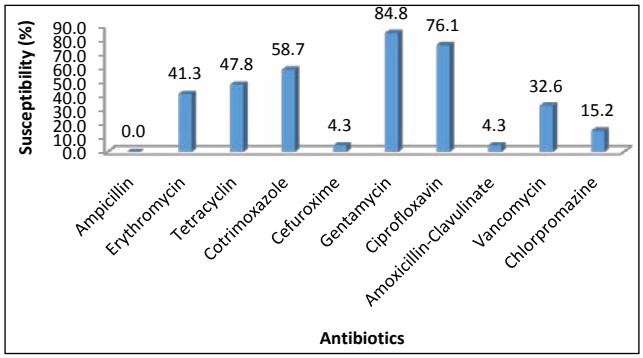

Figure 3: Antibiotic susceptibility pattern of Gram-positive bacterial isolates.

Discussion

The study showed that most of the urine from schistosomiasis positive individuals carried a variety of bacteria. Significant bacteriuria (8.57 %) reported among schistosomiasis positive individuals in this study was lower values reported earlier in other parts of Nigeria (Osai *et al*., 2014; Kone *et al*., 2022).

Most of the bacteria isolated in this study are commonly isolated from urine (Angoti *et al*., 2016; Okiki *et al*., 2015, 2021; Kone *et al*., 2022). Kone *et al*., (2022) had earlier reported isolation of *Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteusvulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *S. saprophyticus* from urine of Schistosomiasis-positive individuals in neighbouring Ondo State, Nigeria. Hsiao et al. (2016) reported that individuals co-infected by both *Schistosoma* and *Salmonella* experienced reduced immunological functioning, exhibited irreversible organ damage due to prolonged schistosomiasis infection, and become latent carriers of *Salmonella enterica* serotypes Typhi and Paratyphi and S. Typhimurium. The sequestration of the bacteria in the parasite was said to lead to ineffective antibiotic treatment because the bacteria cannot be completely killed, and lingering infection may then lead to antimicrobial resistance. These manifestations were likely not just for those dually infected but also for those first infected with schistosomes and, later, *Salmonella*. Isolation of *Salmonella enterica* subsp. *enterica* serovar Paratyphi B from urine of Schistosomiasis positive individuals was reported in present study among Schistosomiasis positive individuals.

However, the isolation of *K. gibsonii* among the studied subjects is great concern. *Kurthia* is a gram-positive, non-spore forming, rod-like bacteria genus from the

Planococcaceae family. *K. gibsonii* can spread from an animal to a human by zoophilic sexual intercourse, and the mucous membranes of the human genital tract can support bacterial survival. Direct contact of genital organs between humans and animals predisposes to mutual transmission of microbes being present either as members of the normal flora or pathogens (Kövesd *et al*., 2016). Kövesd and coworkers (2016) reported isolation of *K. gibsonii* from a male patient with recurring urethritis and balanitis after having repeated unprotected penetrative intercourse with female pigs. It is of note that many of the participants in present study are into animal husbandry.

Majority of the bacterial isolates were resistant to multiple antibiotics. The issue of antibiotic resistance, particularly among members of the Gram-Enterobacteriaceae family, continues to pose a threat to public health (Gupta *et al*., 2016).

Attention should be drawn to the significant association between schistosomiasis on one hand and proteinuria and leukocyturia on the other. It will be recommended that schistosomiasis infected individuals with positive proteinuria and leukocyturia, should be considered as UTI and treated accordingly.

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