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Schistosoma Haematobium and Klebsiella Pneumoniae Co-Infections, Antibiotic Susceptibility and Multiple Antibiotic Resistance Index in School Children in Zaria, Nigeria

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Abstract:

Urinary schistosomiasis in concomitant bacteriuria is a problem in many African countries. Antibiotic susceptibility and multiple antibiotic resistances (MAR) index are important in drug selection. A total of 170 school children in Zaria were involved in the study. Urine sample (10mls) was collected from each participant and a questionnaire was administered. The samples were cultured on MacConkey agar plates. Pure cultures were subjected to Gram staining, biochemical characterization and antibiotic susceptibility testing. For detection of *Schistosoma haematobium*, the urine samples were centrifuged at 3000 revolutions per minute for 5 minutes. The sediments were examined as wet mounts with drops of Lugol's iodine using 10x and 40x objectives of the light microscope. Results were subjected to Chi Square and Odd Ratio analyses on IBM SPSS version 21. Overall prevalence of urinary schistosomiasis was 22(12.9%), while *Klebsiella pneumoniae* was 16(9.4%). Co-infections of the two organisms were 4(2.4%). Male children were significantly more infected with *Schistosoma haematobium* (19.6%, p=0.003, OR =5.684) than the females 3(4.1%). Also the male children had more infections with *Klebsiella pneumoniae* than the females, and all co-infections were found among the males. Children in secondary schools and those between 22-24 years of age had more infections with *Schistosoma haematobium* and *Klebsiella pneumoniae* than those in primary schools and of age 5-6 years. Identified risk factors for urinary schistosomiasis in this study were swimming in river/streams, washing in dams, use of stream as main source of water and fishing. Those with co-infections were more at risk of developing abdominal pain (P=0.004, OR=13.6). Isolates of *Klebsiella pneumoniae* were susceptible to: Oflaxacin (87.5%), Gentamicin (68.75%), Ciprofloxacin (62.5%), Streptomycin (62.5%), and Septrin (56.25%); and resistant to Nalidixic acid 8(50%), Ampicillin (43.75%) and Cephalexin (37.5%). The four isolates of *Klebsiella pneumoniae* in concomitant urinary schistosomiasis had very high MAR indices of 0.7, 0.9, 1.0 and 1.0 respectively.

Keywords:

Schistosoma haematobium, Klebsiella pneumoniae, Urine, Co-infections, Antibiotic Susceptibility, Children, Zaria, Nigeria

1. Introduction

Schistosoma haematobium (*S. haematobium*) causes urinary schistosomiasis. There are over 240 million cases worldwide. Its occurrence in tropical and subtropical countries, especially in poor communities, is mainly due to poor water supply and inadequate sanitation [1]. Children are most prone to urinary schistosomiasis due to their indulgence in indiscriminate water-contact activities [2]. Invasion of the urinary tract by pathogenic organisms can lead to urinary tract infections (UTIs) [3]. UTIs are common bacterial infections that affect all ages and sexes, but women are most prone to UTIs [2]. As an important opportunistic pathogen and a common cause of nosocomial infection, *Klebsiella pneumonia* can occur in any body site, but the urinary and respiratory tract are most affected [4]. Recurrent infection and antibiotic resistance by *Klebsiella pneumoniae* pose threats to public health [5]. *Klebsiella pneumoniae* (*K. pneumoniae*) is second to *Escherichia coli* in causation of uncomplicated UTIs; however some Gram-positive bacteria and some fungi can cause UTIs [5]. Parasite-bacterial co-infections can exacerbate the condition of a patient due to complex interactions that occur between them, which can result in antibiotic resistance [6]. The association between *Salmonella* and *Schistosoma* had been studied during co-infection: the bacterium becomes resistant to many antibiotics [6,7].

2. Materials and Methods

2.1. Study Area

Zaria was the study area, located on latitude: 11 °06' 40.61" N and longitude: 7 °43' 21.72" E. It is a major city in northern part of Kaduna State, Nigeria [8].

2.2. Study Population

A total of 170 school children in both primary and secondary schools in Zaria were involved in the study. The children voluntarily participated in the study following an enlightenment talk in their schools. Consents were obtained from the managements of selected schools, as well as from parents and their children.

2.3. Collection of Urine Samples and Administration of Questionnaires

Urine sample (10mls) was collected from each participant [7,9]. The children were guided on how to collect 10ml each of their urine samples into sterile wide-mouth, screw-capped sampling bottles. The urine samples were shielded from light by enclosing them in dark cold containers. Structured questionnaire encompassing socio-demographic, risk factors, and signs/symptoms of urinary schistosomiasis and UTIs was administered on each participant [9]. The samples were conveyed for analysis at the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria.

2.4. Isolation and Biochemical Characterization of Klebsiella pneumoniae

Each urine sample was mixed by gentle shaking before opening the cap. Aseptic inoculation was made on MacConkey agar followed by aerobic incubation at 37^{o}C for

24h [7]. Pure cultures with characteristic mucoid appearances were Gram-stained. The pure isolates were maintained on Nutrient Agar slants at 4℃ for further laboratory investigations. Biochemical confirmation of *Klebsiella pneumoniae* included: citrate utilization, motility test, indole test, sugar fermentation in Triple Sugar Iron (TSI) agar slants, methyl red, Voges-Proskauer, oxidase, urease, motility, lysine decarboxylation, and ornithine decarboxylation tests [7].

2.5. Antibiotic Susceptibility Tests

Standardized inocula were made to 0.5 McFarland and susceptibility was determined by Kirby-Bauer method on sterile Muller-Hinton agar. The following antibiotics were used: Ampicillin (30µg), Augmentin (30µg), Nalidixic acid (30µg), Ofloxacin (10µg), Ciprofloxacin (10µg), Gentamicin (10µg), Streptomycin (30µg), Cephalexin (10µg), Septrin (30µg), and Pefloxacin (10µg). The zones of bacterial growth inhibition were measured to the nearest millimeter and interpreted by the standard of Clinical Laboratory Standard Institute.

3. Results and Discussion

3.1. Results

Out of 170 urine samples examined, overall prevalence of *Schistosoma haematobium* infections was 22(12.9%). The eggs of the parasite were yellow-brown and oval in shape with terminal spines. The prevalence of *Klebsiella pneumoniae* infection was 16(9.4%). The bacterium was a Gram-negative short rod, fermented slant and butt of TSI medium with all acid and gas production, and without hydrogen sulphide (H_2S) production. It was negative to methyl red, indole, oxidase, motility, and ornithine decarboxylation tests; but it utilized citrate and was positive to Voges-Proskauer, urease and lysine decarboxylation tests. There were co-infections of 4(2.4%) between the two pathogens as shown in Table 1.

Table 1. *Prevalence and co-infections S. haematobium and K. pneumoniae in children in Zaria.*

Infection (n=170)	Positive Number (%)	Negative Number (%)
Schistosoma haematobium	22(12.9%)	148(87.1%)
Klebsiella pneumoniae	16(9.4%)	154(90.6%)
Co-infection	4(2.4%)	166(97.6%)

Occurrence of *Schistosoma haematobium* had statistical significance with age (P=0.000), gender (P=0.003) and school level (P=0.016) of the children. There was no any infection in school children of 4-6years of age, compared to those of 19-21years and 22-24years of age that had most of the infections. The male children were 5.7 times more at risk of *Schistosoma haematobium* infections than the female children. Those in the secondary school were 3.2 times more at risk of *Schistosoma haematobium* infection (Table 2).

Those within the age group of 22-24 years had the highest cases of *Klebsiella pneumoniae* infections (33.3%) and co-infections (33.3%) but there was no any statistical significance. The male children had higher cases of *Klebsiella pneumoniae* infection (12.4%) and all the co- infections (4.1%) were found in the males. Those in the secondary schools had more *Klebsiella pneumoniae* infections as well as co-infections (Table 2).

Table 2. *Distribution of S. haematobium and K. pneumoniae infections in school children in Zaria.*

Factors	Category (years)	Number examined	*S. haematobium*[a] Number positive (%)	*K. pneumoniae*[b] Number positive (%)	Co-infection[c] Number positive (%)
Age group	4-6	4	0(0)	0(0)	0(0)
	7-9	26	2(7.7)	5(19.2)	1(3.8)
	10-12	48	2(4.2)	1(2.1)	0(0)
	13-15	34	6(17.6)	3(8.8)	1(2.9)
	16-18	47	6(12.8)	6(12.8)	1(2.1)
	19-21	7	3(42.9)	0(0)	0(0)
	22-24	3	3(100)	1(33.3)	1(33.3)
Gender	Male	97	19(19.6)	12(12.4)	4(4.1)
	Female	73	3(4.1)	4(5.5)	0(0)
School level	Primary	87	6(6.9)	7(8.0)	1(1.1)
	Secondary	83	16(19.3)	9(10.8)	3(3.6)

Age: ${}^{a}\chi^2=31.071$, *df=6, P=0.000;* ${}^{b}\chi^2=9.858$, *df=6, P=0.131;* ${}^{c}\chi^2=14.292$, *df=6, P=0.270*
Gender: ${}^{a}\chi^2=8.857$, *df=1, P=0.003, OR=5.684;* ${}^{b}\chi^2=2.320$, *df=1, P=0.128, OR=2.435;* ${}^{c}\chi^2=3.083$, *df=1, P=0.079, OR=1.043*
School level: ${}^{a}\chi^2=5.779$, *df=1, p=0.016, OR=3.224;* ${}^{b}\chi^2=0.390$, *df=1, p=0.532, OR=1.390;* ${}^{c}\chi^2=1.123$, *df=1, p=0.289, OR=3.225*

The risk factors (in Table 3) that statistically associated with *Schistosoma haematobium* infection were swimming, laundry activities at the dam/stream, fishing, and use of stream as source of water ($P \leqslant 0.05$). Children that claimed to be aware of urinary schistosomiasis and UTIs were rather more significantly infected with *Schistosoma haematobium* 7(36.8%) than those that were even unaware. However, children that were unaware had more cases of *K. pneumoniae* infections 13(7.6%).

Table 3. *Risk factors of S. haematobium and K. pneumoniae co-infections in children in Zaria.*

Risk factors	Category	Number Examined	*S. haematobium*[a] Number positive (%)	*K. pneumoniae*[b] Number positive (%)	Co-infection[c] Number positive (%)
Swimming	No	130	13(10.0)	13(7.6)	3(2.3)
	Yes	40	9(22.5)	3(7.5)	1(2.5)
Awareness	Unaware	151	15(9.9)	13(7.6)	1(0.6)
	Aware	19	7(36.8)	3(1.8)	3(1.8)
Place of laundry	Dam	3	3(100)	0(0.00)	0(0.0)
	Home	160	16(10)	15(9.4)	3(1.9)
	Stream	7	3(42.9)	1(14.3)	1(14.3)
Source of water	Borehole	66	6(9.1)	7(10.6)	2(3.0)
	Dam	11	2(18.2)	0(0)	0(0)
	Stream	12	5(41.7)	1(8.3)	1(8.3)
	Tap	36	2(5.6)	4(11.1)	1(2.8)
	Well	45	7(15.6)	4(8.9)	0(0)
Fishing	No	140	10(7.1)	12(8.6)	2(1.4)
	Yes	30	12(40.0)	4(13.3)	2(6.7)

Swimming: $^{a}\chi^2$*=4.24, P=0.039, OR=2.613;* $^{b}\chi^2$*=0.224, P=0.636, OR=0.730;* $^{c}\chi^2$*=0.005, P= 0.944, OR=1.085*

Awareness: $^{a}\chi^2$*=10.846, P=0.001, OR=5.289;* $^{b}\chi^2$*=1.020, P=0.312, OR=1.990;* $^{c}\chi^2$*=16.809, df=1, P=0.000, OR=28.125*

Laundry: $^{a}\chi^2$*=26.971, P=0.000;* $^{b}\chi^2$*=0.507, P=0.776;* $^{c}\chi^2$*=4.570, df=2, P=0.102*

Water source: $^{a}\chi^2$*=11.941 , P=0.018;* $^{b}\chi^2$*=1.406, P=0.843;c*χ*2=2.377, P=0.479*

Fishing: $^{a}\chi^2$*=23.674, P=0.000, OR=8.667;* $^{b}\chi^2$*=0.657, P=0.418, OR=1.641;* $^{c}\chi^2$*=2.950, P=0.860, OR=4.929*

From Table 4, children that presented with visible haematuria were more infected with *Schistosoma haematobium* (45.5%, P= P=0.000, OR=9.4), *Klebsiella pneumoniae* (13.6%, P=0.467, OR=1.6) and also had most of the co-infections (9.1%, P=0.025, OR =7.3). Those with co-infections were more at risk of developing abdominal pain (P=0.004, OR=13.6).

Table 4. *Signs/symptoms of S. haematobium and K. pneumoniae co-infections in school children.*

Signs/ Symptoms	Category	Number Examined	*S. haematobium*[a]	*K. pneumoniae*[b]	Co-infection[c]
Visible haematuria	No	148	12(8.1)	13(8.8)	2(1.4)
	Yes	22	10(45.5)	3(13.6)	2(9.1)
Painful urination	No	128	13(10.2)	12(9.4)	3(2.3)
	Yes	42	9(21.4)	4(9.5)	1(2.4)
Frequent urination	No	128	13(10.2)	11(8.6)	3(2.3)
	Yes	42	9(21.4)	5(11.9)	1(2.4)
Abdominal pain	No	137	14(10.2)	9(6.6)	1(0.7)
	Yes	33	8(24.2)	7(21.2)	3(9.9)

Haematuria: $^{a}\chi^2$*=23.711, df=1, P=0.000, OR=9.444;* $^{b}\chi^2$*=0.529, df=1, P=0.467, OR=1.640;* $^{c}\chi^2$*=4.993, df=1, P=0.025, OR=7.300*

Painful urination: $^{a}\chi^2$*=3.567, df=1, P=0.059, OR=2.413; b*χ*2=0.001, df=1, P=0.977, OR=1.018;* $^{c}\chi^2$*=0.000, df=1, P=0.989, OR=1.016*

Frequent urination $^{a}\chi^2$*=3.567, df=1, P=0.059, OR=2.413;* $^{b}\chi^2$*=0.407, df=1, P=0.524, OR=1.437;* $^{c}\chi^2$*=0.000, df=1, P=0.989, OR=1.016*

Abdominal pain: $^{a}\chi^2$*=4.642, df=1, P=0.31, OR=2.811;* $^{b}\chi^2$*=6.688, df=1, P=0.10, OR=3.829;*

$^{c}\chi^2$*=8.092, df=1, P=0.004, OR=13.600*

The *Klebsiella pneumoniae* isolates were most sensitive to Ofloxacin (87.8%), Gentamicin (68.8%), Ciprofloxacin (62.5%) and Streptomycin (62.5%). In terms of antibiotic resistance, it was mostly observed on Nalidix acid (50.0%), Ampicillin (43.8%), and Augmentin (31.3%) as shown in Table 5.

Table 5. *Antibiotic susceptibility profile of K. pneumoniae isolated from school children in Zaria.*

Antibiotic (Potency)	Number of Isolates	Resistant Number (%)	Intermediate Number (%)	Sensitive Number (%)
Ampicillin (30µg)	16	7(43.75)	1(6.25)	8(50.00)
Augmentin (30µg)	16	5(31.25)	3(18.75)	8(50.00)
Cephalexin (10µg)	16	6(37.50)	3(18.75)	7(43.75)
Ciprofloxacin (10µg)	16	2(12.50)	4(25.00)	10(62.50)
Gentamicin (10µg)	16	3(18.75)	2(12.50)	11(68.75)
Nalidixic acid (30µg)	16	8(50.00)	5(31.25)	3(18.75)
Ofloxacin (10µg)	16	2(12.50)	0(0.00)	14(87.75)
Pefloxacin (10µg)	16	3(18.75)	4(25.00)	9(56.25)
Septrin (30µg)	16	4(25.00)	3(18.75)	9(56.25)
Streptomycin (30µg)	16	4(25.00)	2(12.50)	10(62.50)

Multiple antibiotic resistance (MAR) indices of the four isolates of *Klebsiella pneumoniae* in concomitant urinary schistosomiasis among the pupils were 0.7, 0.9, 1.0 and 1.0 respectively (Table 6).

Table 6. *MAR Index of K. pneumoniae in concomitant urinary schistosomiasis in school children.*

Number of Antibiotic tested against (a=10)	Isolate Number	Number of antibiotic resistant to (b)	MAR index (a/b)
Ampicillin (PN), Augmentin (AU), Cephalexin (CEP), Ciprofloxacin (CPX), Gentamicin (CN), Nalidixic acid (NA), Ofloxacin (OFX), Pefloxacin PEF Septrin(STX), Streptomycin (S),	76	STX, S, PN, CEF, OFX, NA, PEF (b=7)	7/10 = 0.7
	118	AU, CPX, STX, S, PN, CEP, OFX, NA, PEF (b=9)	9/10 = 0.9
	127	CN, AU, CPX, STX, S, PN, CEP, OFX, NA, PEF (b=10)	10/10 = 1
	163	CN, AU, CPX, STX, S, PN, CEP, OFX, NA, PEF (b=10)	10/10 =1

3.2. Discussion

Urinary schistosomiasis is diagnosable by microscopic detection of terminal spine eggs of *Schistosoma haematobium* in urine sediment [7,9,10]. It is a peculiar problem in Nigeria [10]. A prevalence of 12.3% was previously reported among school children [9]. *Klebsiella pneumoniae* has been implicated in UTI with prevalence reports of 3.4% [11], 6.0% [3] and 17% [12]. It has been reported that *Klebsiella pneumoniae* is second to *Escherichia coli* in terms of causation of urinary tract infections [12,13,14]. It is also one of the six bacteria known to possess multidrug resistance (MDR). These bacteria are commonly called by the acronym 'ESKAPE' including *Enterococcus facium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter species* respectively [15,16]. Urinary schistosomiasis and concomitant urinary tract infection, especially by *Klebsiella pneumoniae*, will present more difficulty in diagnosis and treatment. The two infections must be treated simultaneously which is dependent on accurate diagnosis. It has been found by other researchers that association between schistosomes and *Salmonella* exists: where the concomitant *Salmonella* resist antibiotic therapy [6]. The significantly high prevalence of urinary schistosomiasis among the study subjects of 22-24 years and 19-21years of age could have been due to their involvement in predisposing activities like swimming in cercarial-infested water bodies, fishing and irrigation farming. The secondary students were far much older than the primary school pupils; hence they are capable of indulging more in high risk water-contact activities. They also stand a higher risk of urinary tract infection by *Klebsiella pneumoniae*.

Comparatively, the male subjects had more cases of urinary schistosomiasis than the female because the males indulge more in indiscriminate water-contact activities. Hence, they are about 6 times more at risk of the infection. High risk of urinary schistosomiasis among male subjects had been reported by other researchers [17,18]. Underlying urinary schistosomiasis must have predisposed them to UTI caused by *Klebsiella pneumoniae*.

Swimming in unsafe water bodies, or washing in dams, fetching of water from streams and fishing were identified as important risk factors for the transmission of

urinary schistosomiasis. Such risk factors had been severally reported [9,10,17,18]. Awareness about the disease is not enough to stop the transmission of the infection, as most of the children that claimed to be aware of urinary schistosomiasis were rather the most infected. Lack of proper preventive strategies, inadequate safe water supply and poor monitoring of children could have endangered them to these infections. Important sign of urinary schistosomiasis included visible haematuria. Also, from this study, the co-infection of *Schistosoma haematobium* and *Klebsiella pneumoniae* associated with onset of haematuria and abdominal pain.

The isolates of *Klebsiella pneumoniae* were most resistant to Ampicillin and Nalidix acid. Another study by Bishop and Shehu [3] showed that *Klebsiella pneumoniae* were most resistant to Gentamicin, Streptomycin and Ampicillin. From this study however, Ofloxacin demonstrated the best activity against the isolates. It has been reported that Ofloxacin is potent against *Klebsiella* species, while Ampicillin had no activity against them [13]. Successfully treatment of UTIs has been hampered by rise of multidrug resistance by some clinical isolates.

All the four isolates of *Klebsiella pneumoniae* in concomitant urinary schistosomiasis were highly resistant to the antibiotics tested: the first was resistant to 7 antibiotics; the second was resistant to 9 antibiotics, but the last two completely resisted the actions of all the tested antibiotics. This study shows that *Klebsiella pneumoniae* as an agent of UTI, occurring in concomitant urinary schistosomiasis, poses great threat to successful treatment because they have high MAR indices of 0.7 to 1.0. In such a dilemma, the way out is to find newer drugs for treatment during *Schistosoma haematobium-Klebsiella pneumoniae* co-infections. This very high MAR index of *Klebsiella pneumoniae* in concomitant urinary schistosomiasis demonstrates possible parasite-bacterial interactions that promote their ability to resist many antibiotics. The consequence of this will be the onset antibiotic therapy failures and prolongation of treatment course.

4. Conclusions

The study revealed the prevalence of 12.9%, 9.4% and 2.4% for *Schistosoma haematobium*, *Klebsiella pneumoniae* and their co-infections respectively in school children in Zaria, Nigeria. Those at higher risk of urinary schistosomiasis included the male subjects, children in secondary school and of the age of 21-24 years. Important risk factors for the transmission of urinary schistosomiasis included swimming in unsafe water bodies, fetching water from streams, washing in dams and fishing. Visible haematuria is a sign of urinary schistosomiasis while abdominal pain was associated with the co-infections. Ofloxacin activity proved excellent and should be recommended for the treatment of *Klebsiella pneumoniae* during UTI. However, in concomitant urinary schistosomiasis they have very high MAR index, even Ofloxacin failed—as such newer drugs will be needed for successful treatment. There is need to raise considerable awareness on the danger of using unsafe water sources for recreational and domestic activities in Nigerian rural areas. There is also need for regular surveillance on antibiotic resistance for effective control and to guide the choice of antimicrobial agents for treatment. We therefore speculate the possibility of a unique interaction between *Klebsiella pneumoniae* and *Schistosoma haematobium* for the observed high MAR indices. Therefore, during screening for antibiotics to be administered to patients with UTI caused by *Klebsiella pneumoniae*, it should be done in view of any co-infection with *Schistosoma haematobium*.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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