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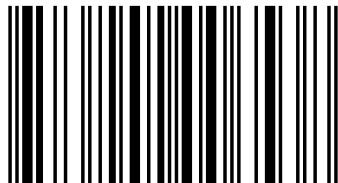
In this book we discussed the urinary tract infections caused by trematodes, certainly *Schistosoma haematobium* which is transmitted through penetration of skin by forked tail cercaria which present in polluted water and that caused by bacterial infections. We concentrate our efforts to know the prevalence of bacterial and *Schistosoma haematobium* co-infection among school children in villages southern to Shendi, River Nile state, Sudan. We try to give clear pictures about the prevalence of urinary tract infections caused due to association of bacterial strains and *Schistosoma haematobium* between children selected in this study and also we identify the antimicrobial agents to that's pathogens. We hope to introduce real science to all Microbiology and parasitology students in the world.



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978-620-2-00653-8



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Cover image: www.ingimage.com

Publisher:

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17 Meldrum Street, Beau Bassin 71504, Mauritius

Printed at: see last page

ISBN: 978-620-2-00653-8

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Dedication:

To our Mothers & Fathers

To all medical students around the world

Abstract

Bacterial infections are often recurrent and important complications of the inactive stage of urinary schistosomiasis. The research was carried out to determine the frequency of bacterial urinary tract infection among schistosomiasis school children. In this study 120 urine samples were collected from school pupils in some villages of rural southern Shendi town, their average age between 5-16 years, and examined for the presence of *S. haematobium* eggs using centrifugation technique and for bacteriuria by routine bacteriological methods. A total of 120 school children was included in the study, 84 was suffering of schistosomiasis and 36 uninfected. Out of 84 who had schistosomiasis 37 (44.05%) showed significant bacteriuria, 47 (55.95%) had insignificant bacteriuria (p.value =0.001). The bacterial isolated including: *Klebsiella* species, *Escherichia coli*, *E. faecalis*, *Salmonella* species, *Proteus* species, and *Pseudomonas* species. *Escherichia coli* occurred more frequently 17 (45.95%) than the rest of the bacterial species isolated. The antimicrobial susceptibility test of isolates revealed varying patterns of susceptibilities by all isolates. This study clearly suggests that bacteriuria is a potent complication in the management of urinary schistosomiasis.

Introduction

Urinary tract infections (UTI) represent one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group ⁽¹⁾. Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population ⁽²⁾. 150million individuals have been reported to be affected by UTIs annually worldwide ⁽³⁾. Urinary tract infections occur as a result of the microbial colonization of urine and the invasion of any structure of the urinary tract by microbial organisms such as bacteria, viruses, yeasts and parasites ⁽⁴⁾. UTIs Of both bacterial and parasitic origins have been associated with high incidence of squamous cell carcinoma of the bladder and the cervix ⁽⁵⁾.

Escherichia coli and *Schistosoma* spp. are the most widely reported UTI causing bacteria and parasite respectively ⁽⁶⁾.

The resulting disease conditions from UTI include cystitis and pyelonephritis which is known to be non-age discriminatory as it affects both older persons and infants. Moreover, pyuria as evidenced by the inflammation of the genitourinary tract is common in subjects with asymptomatic bacteriuria ⁽⁷⁾. Asymptomatic UTI in particular has been associated with an increased risk of developing pyelonephritis, maternal and infant morbidity, pre-term labour and Low birth weight ⁽⁸⁾.

Asymptomatic UTIs occur when urinary tract pathogens enter into the bladder without causing apparent symptoms. Typically the pathogens are usually eliminated by host defense factors when they persist only for a short time in the human host, however when such pathogens stay in the urinary system for a long time, symptomatic urinary tract infections result ^(6,9).

The presence of bacteria in urine is known as bacteriuria. Asymptomatic bacteriuria is a urinary tract infection that occurs in both males and females where bacteria are present in urine with the absence of clinical signs or symptoms in the host ⁽¹⁰⁾. Asymptomatic bacteriuria is defined by the presence of at least 10⁵ colony forming units (CFU) of organism per milliliter in cultures of urine specimens in the absence of symptoms of infection referable to the urinary tract ⁽¹¹⁾. Early detection of bacteriuria is expected to aid the detection of correctable abnormalities of the urinary tract and the prevention of renal scarring obstructive atrophy, hypertension and renal insufficiency among other serious health maladies that may develop as a direct result of asymptomatic urinary tract infection. Moreover, treatment is more effective when the culprit organism is correctly identified as indiscriminate treatment with antibiotics is known to result in microbial resistance to antibiotics. In addition to *E. coli*, other bacteria implicated in causing bacteriuria includes *Protues spp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella spp.*, and *Streptococcus spp.* ⁽¹²⁾.

The risk factors identified with high prevalence of UTIs in young adult females include sexual intercourse, spermicide based contraception, and a history of UTIs ⁽¹³⁾.

Schistosomiasis still remains the second most prevalent tropical parasitic disease after malaria, and a leading cause of severe morbidity in many parts of the world ⁽¹⁴⁾. The disease is caused by the parasitic helminth of the genus *Schistosoma* and

transmitted through fresh water snail intermediate hosts. About 200 million people worldwide are estimated to be infected with *S. haematobium* of which 70% live in sub-Saharan Africa⁽¹⁵⁾.

Schistosoma haematobium inhabit the venous plexus that drains the urinary bladder of man. The mature worm, deposit terminal spined eggs which clog the venous plexus thus impeding blood flow. This causes the veins to burst then blood and eggs enter the urinary bladder, giving rise to the characteristic symptom⁽¹⁶⁾. *S. haematobium* infection however could cause haematuria, dysuria, nutritional deficiencies, lesion of the bladder, kidney failure, and an elevated risk of bladder cancer and in children growth retardation^(17, 18). Accordingly the estimates for morbidity and mortality in affected populations are high with school age children usually presenting with the highest prevalence and intensity infection^(19, 20). Studies in Nigeria among school aged children in various parts of the country and in both rural and urban environments have shown that *S. haematobium* is clearly a problem of this age group, Prevalence among school aged children ranges from 20-40 % in typical communities⁽²¹⁾. Urinary schistosomiasis and concomitant bacteriuria was investigated in the Federal Capital Territory (FCT) Abuja. Single urine samples collected from subjects aged 5 years and above examined for the presence of *S. haematobium* eggs using centrifugation technique and for bacteriuria by standard bacteriological methods. A total of 1,150 subjects comprised of 667 males and 483 females were studied from the 6 Area Councils of the FCT. Overall, 360 (31.3%) had the eggs of *S. haematobium* in their urine while 289 (80.3%) of the 360 who had eggs of *S. haematobium* in their urine, had bacterial growth. Prevalence of bacteriuria in urinary schistosomiasis ranged from 74-86% ($P=0.125$). The bacteria isolated included: *klebsiella* species, *Escherichia coli*, *Enterococci* species, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Salmonella* species, *Proteus*

species, and *Pseudomonas* species. *Escherichia coli* occurred more frequently (70%) than the rest of the bacteria species isolated. The antimicrobial susceptibility pattern of isolates revealed varying percentage susceptibilities by all isolates. The study clearly suggests that bacteriuria is a potent complication in the management of urinary schistosomiasis ⁽²²⁾.

Ibadan, the largest city in Southwestern Nigeria, reported a 57.5% prevalence rate of Urinary schistosomiasis with over 75% of infected school pupils exhibiting concomitant bacteriuria. The results show a linear relationship

Between contact and usage of stream water and increase in rate of infection; bacteriuria seems most often to occur concurrently with parasitic urinary tract infections. Including *Schistosoma* spp. (particularly *S.hematobium*) ^(23, 24, 25).

Also a study conducted in Enugu State of the 842 pupils, the prevalence of urinary schistosomiasis was 34.1%. Infection rate was higher (52.8%) among 13-15 years (Prevalence Ratio=2.45, 95% Confidence Interval 1.63-3.69). Significant bacteriuria among pupils with urinary schistosomiasis was 53.7% compared to 3.6% in the uninfected (PR=30.8, 95% CI 18.91- 52.09). The commonest implicated organism was *Escherichia coli* ⁽²⁶⁾.

Urinary tract:

Anatomy:

The urinary system consists of the kidneys, ureters, bladder, and urethra .often, urinary tract infections (UTIs) are characterized as being either upper or lower based primarily on the anatomic location of the infection: the lower urinary tract encompasses the bladder and urethra, and the upper urinary tract encompasses the ureters and kidneys ⁽²⁷⁾.

Commensals:

The bladder and urinary tract are normally sterile. The urethra however may contain a few commensals and also the perineum (wide variety of gram positive and gram negative organisms) which can contaminate urine when it is being collected. With female patients, the urine may become contaminated with organisms from the vagina. Vaginal contamination is often indicated by the presence of epithelial cells and a mixed bacterial flora. Most urine specimens will contain fewer than 10^4 contaminating organisms per ml providing the urine has been collected with care to minimize contamination and the specimen is examined soon after collection before the commensals have had time to multiply significantly ⁽²⁵⁾.

Urinary tract infections:

Types of infection and their clinical manifestations:

UTI encompasses a broad range of clinical entities that differ in terms of clinical presentation, degree of tissue invasion, epidemiologic setting, and requirements for antibiotic therapy. There are five major types of UTIs:

Urethritis, asymptomatic bacteriuria, cystitis, the urethral syndrome and pyelonephritis ⁽²⁷⁾.

Urethritis:

Urethritis is infection of the urethra, it is a common infection associated with dysuria (difficult urination) and frequency .Chlmydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis are common causes of urethritis ⁽²⁷⁾.

Asymptomatic bacteriuria:

Asymptomatic bacteriuria or asymptomatic UTI is the isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs of urinary infection. Asymptomatic bacteriuria is common but its prevalence varies widely with age, gender, and the presence of genitourinary abnormalities or underlying diseases ⁽²⁷⁾.

Cystitis:

Cystitis is infection of the bladder, typically patient with cystitis complain of dysuria, frequency, and urgency (compelling need to urinate) .These symptoms are due not only inflammation of the bladder but also to multiplication of bacteria in the urine and urethra .Often, there is tenderness and pain over the area of the bladder. In some individuals, the urine is grossly bloody .The patient may note urine cloudiness and a bad odor. Because cystitis is a localized infection, fever and other signs of a systemic illness are usually not present ⁽²⁷⁾.

Acute urethral syndrome:

Patients with this syndrome are primarily young, sexually active women, who experience dysuria, frequency, and urgency but yield fewer organisms than 10⁵

colony forming units of bacteria per milliliter (CFU / ml) urine on culture. Almost 50% of all women who seek medical attention for complaints of symptoms of acute cystitis fall into this group ⁽²⁷⁾.

Pyelonephritis:

Pyelonephritis refers to inflammation of the kidney parenchyma, calices and pelvis, and is usually caused by bacterial infection. The typical clinical presentation of an upper urinary tract infection includes fever and flank (lower back) pain and frequently , lower tract symptoms (frequency , urgency ,and dysuria) ⁽²⁷⁾ .

Some times UTIs are classified as:

Uncomplicated UTI:

Uncomplicated infections occur primarily in otherwise healthy females and occasionally in male infants and adolescent and adult males. Most uncomplicated infections respond readily to antibiotic agents to which the etiologic agent is susceptible ⁽²⁷⁾.

Complicated UTI:

Complicated infections occur in both sexes. In general, individuals who develop complicated infections often have certain risk factors. In general, complicated infections are more difficult to treat and have greater morbidity (e.g., kidney damage, bacteremia) and mortality compared with uncomplicated infections ⁽²⁷⁾.

The clinical presentation of UTIs may vary, ranging from asymptomatic infection to pyelonephritis. Some UTI symptoms overlap considerably in patients with lower UTIs and in those with upper UTIs ⁽²⁷⁾.

Clinical features and Complications:

Acute infections of the lower urinary tract are characterized by a rapid onset of:

- Dysuria (burning pain on passing urine)
- Urgency (the urgent need to pass urine)
- And frequency of micturition.

Upper UTIs causes a fever and lower urinary tract symptoms ⁽²⁸⁾.

Route of infection:

Bacteria can invade and cause a UTI via two major routes:

Ascending pathways:

Although the ascending route is the most common route of infection in females, ascent in association with instrumentation (e.g., urinary catheterization , cystoscopy) is the most common cause of hospital acquired UTIs in both sexes ⁽²⁷⁾. For UTIs to occur by the ascending pathway, enteric gram negative bacteria and other microorganisms that originate in the gastrointestinal tract must be able to colonize the vaginal cavity and /or the periurethral area ⁽²⁷⁾. Once these organisms gain access to the bladder, they may multiply and then pass up the ureters to the kidneys. UTIs occur more often in women than men, at least partially because of the short female urethra and its proximity to the anus. Also sexual activity can increase chances of bacterial contamination of the female urethra ⁽²⁷⁾.

In most hospitalized patients, UTI is preceded by urinary catheterization or other manipulation of the urinary tract by bacteria colonize the patient's skin, gastrointestinal tract, and mucous membranes, including the anterior urethra. It is estimated that approximately 10 % to 30% of catheterized patients will develop bacteriuria (presence of bacteria in urine)⁽²⁷⁾.

hematogenous pathways :

Hematogenous spread, or blood borne route usually occurs as a result of bacteremia. Any systemic infection can lead to seeding of the kidney, but certain organisms, such as staphylococcus aureus or salmonella spp, are particularly invasive. Although most infections involving the kidneys are acquired by ascending route, yeast (usually Candida albicans), mycobacterium tuberculosis, salmonella spp, leptospira spp, or staphylococcus aureus in the urine often indicates pyelonephritis acquired via the hematogenous spread, or the descending route. Hematogenous spread accounts for less than 5% of UTIs ⁽²⁷⁾.

Acquisition and etiology:

Bacteria that cause UTI:

Bacterial infection is usually acquired by the ascending route from the urethra to the bladder. The Gram-negative rod Escherichia coli is the commonest cause of ascending UTI, **Other members of the Enterobacteriaceae are also implicated includes:**

.Proteus mirabilis is associated with urinary stones.

.Citrobacter, Klebsiella, Enterobacter, and Pseudomonas aeruginosa (hospital-acquired UTI).

Among the Gram-positive species; Staphylococcus saprophyticus (especially in young sexually active women), Staphylococcus epidermidis and Enterococcus species are more often associated with UTI in hospitalized patients (especially those with AIDS).

In some instances; capnophilic species (organisms that grow better in air enriched with carbon dioxide), including corynebacteria and lactobacilli, have been

implicated as possible causes of UTI. Obligate anaerobes are very rarely involved⁽²⁸⁾.

When there has been haematogenous spread to the urinary tract, other species may be found, e.g. *Salmonella typhi*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* (renal tuberculosis)⁽²⁸⁾.

Association between schistosomiasis and bacterial urinary tract infection:

Urinary tract disease is a specific trait of infection with *Schistosoma haematobium* which affects in a diffuse manner the entire genitourinary tract⁽²⁹⁾. Bacterial infections are often recurrent and important complications of the inactive stage of urinary schistosomiasis which may be instrumental in precipitating renal failure. In schistosomiasis of the urinary bladder, secondary bacterial infections are common and in men can involve the seminal vesicles, spermatic cord, and to a lesser extent, the prostate. In women, infection can involve the cervix and fallopian tubes and can cause infertility⁽³⁰⁾. Opined that it seems possible that agricultural workers and others who are regularly exposed to contaminated water are occasionally simultaneously infected with both the schistosome parasite and pathogenic bacteria⁽³¹⁾.

Urinary tract pathology due to *S. haematobium* infection and subsequent obstructive uropathy has been postulated to increase the risk of secondary bacterial infection. Several epidemiological surveys in Egypt, Gambia, Nigeria and Niger revealed significant differences among rates of bacterial urinary tract infection in patients with schistosomiasis or in communities' endemic for urinary schistosomiasis in comparison with rates in control groups without schistosomiasis or in comparable non endemic areas^(32, 33, 34, and 35). In Egypt, bacteriuria was found in 20 out of 390 (5.1%) school boys in an endemic area with a prevalence rate of 66% which is more than 10 times greater than that observed in the non-endemic control area⁽³³⁾. In an

endemic community in Gambia, prevalence of bacteriuria in males under 25 years of age as 6.6% (10/152) while in a comparable but non endemic area it was absent (0/153) ⁽³⁵⁾. In Nigeria significant bacteriuria was observed in 3.2% of males in an endemic area as compared to 0.5% of those of the control group ⁽³⁴⁾. The rate of urinary tract infections in an endemic village in Niger was five times higher than in a control village ⁽³²⁾. In hospital studies, due to the high frequency of obstructive uropathy, the association between bacteriuria and schistosomiasis is usually significant ⁽³⁶⁾. The predominant organisms isolated from urine in urinary schistosomiasis were *Escherichia coli*, *Staphylococcus albus*, *Streptococcus faecalis*, and *Proteus* species, *Pseudomonas*, *Klebsiella* and *Salmonella* ^(34, 36, and 37). Association of salmonella with schistosoma infection in man has long been known. Scanning electron microscopic observations, revealed that salmonella adhered by its pili to the surface tegument of *S.haematobium*. The association of salmonella and schistosoma may therefore contribute to the persistence of salmonella infection ⁽³⁸⁾. Persistent bacteraemia may accompany salmonella bacteriuria. The schistosoma may play a role as both the source and vehicle of the bacteria. Even without antibiotics, after treatment of schistosomiasis, a reduction of salmonella carriers has been reported ⁽³⁶⁾. Both antibiotics and antischistosomal drugs are recommended in treatment of prolonged *E.coli* bacteraemia associated with *S.haematobium* infection ⁽³⁹⁾.

Chronic bacterial infection in schistosomal obstructive uropathy was considered to be an important etiological factor by several authors ^(40, 41). Urinary bacteria produce nitrosamines, which are well known to be carcinogenic to the bladder, from their precursors in urine. High levels of nitrosamines have been found in the urine of *S.haematobium* infected cases with bladder cancer ⁽⁴²⁾. Several studies have implicated bacteriuria co-infection with urinary schistosomiasis in the aetiology of

bladder cancer and other complications ⁽³¹⁾. Studies have shown that it may take up to 10-20 years after initial coinfection for terminal complications such as renal failure and squamous cell carcinoma of the bladder to develop ^(43,44). The potential association of the urinary schistosomiasis with other infectious diseases (e.g., urinary bacteria) is so far not well understood. Control measures that are instituted by various public health agencies pay little attention to the complexity of schistosomiasis morbidity and its assumed dependency on co-infection with bacteriuria ^(45, 46). When the mucosal barrier is broken down which happens with urinary schistosomiasis, the urinary tract becomes an easy target for invading bacteria. These bacteria accelerate the multi-stage process of bladder carcinogenesis as experimental evidence has shown by the formation of N-nitroso compounds, produced from amine precursors and nitrate in urine during bacterial infections ^(47, 48). Some of the compounds like N-butyl-N-(4-hydroxy butyl) nitrosamine (BHBN) and N-methyl-N-nitro-urea (NMU) are known bladder carcinogens ⁽⁴⁸⁾. Systematic knowledge about bacterial coinfection and schistosomiasis in the 5-15years age group is scanty which is understandable since methods for schistosomiasis surveys are not optimal for detecting bacteriuria ⁽⁴⁹⁾.

Laboratory diagnosis:

Specimen collection:

Prevention of contamination by normal perineal, and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen ⁽²⁷⁾.

Methods of collection:

Mid-stream urine (MSU) sample:

An MSU sample should be collected into a sterile wide-mouthed container after careful cleansing with soap (not antiseptic) and water, and after allowing the first part of the urine stream to be voided, as this helps to wash out contaminants in the lower urethra ⁽²⁸⁾.

Special urine samples are required to detect *Schistosoma haematobium* :

These include:

The last few millilitres of a urine sample collected early afternoon after exercise for detection of *S. haematobium* ⁽²⁸⁾.

Screening procedures:

Many screening methods have been advocated for use in detecting bacteriuria and/or pyuria. These include microscopic methods, colorimetric filtration, bioluminescence, electrical impedance, enzymatic methods, photometric detection of growth, and enzyme immunoassay ⁽²⁷⁾. Commonly used methods are:

Gram stain:

A Gram stain of urine is an easy, inexpensive means to provide immediate information as to the nature of the infecting organism (bacteria or yeast) to guide empiric therapy. After a drop of well mixed urine is allowed to air dry, the smear fixed, stained and examined under oil immersion (100x) for the presence of more than 1 or 5 bacteria per oil immersion field (OIF). Gram stain should not be relied on for detecting polymorphonuclear leukocytes in urine because leukocytes deteriorate quickly in urine that is not fresh or not adequately preserved ⁽²⁷⁾.

Laboratory investigations:

Urine specimens should be examined macroscopically and microscopically and should be cultured by quantitative or semi quantitative methods ⁽²⁶⁾.

Macroscopically examination:

Describe the colour and appearance (cloudy or clear) of the specimen ⁽²⁵⁾.

Microscopic examination of urine allows a rapid preliminary report:

Bacteria may be seen on microscopy when present in the specimen in large numbers. However, they are not necessarily indicative of infection, but may indicate that the specimen has been poorly collected or left at room temperature for a prolonged period of time. The presence of red and white blood cells, although abnormal, is not necessarily indicative of UTI. Hematuria may be present in association with:

- Infection of the urinary tract and elsewhere (e.g. bacterial endocarditis)
- Renal trauma
- Calculi
- Urinary tract carcinomas
- clotting disorders
- Thrombocytopenia.

Occasionally, red blood cells may contaminate urine specimens of menstruating women. White blood cells are present in the urine in very small numbers (e.g. < 10/mL) in health; a count of over 10/mL is considered abnormal, but is not always associated with bacteriuria. Sterile pyuria is an important finding and may reflect:

- Concurrent antibiotic therapy
- Other diseases such as neoplasms or urinary calculi
- Infection with organisms not detected by routine urine culture methods⁽²⁸⁾.

Renal tubular cells, seen in the urine of aspirin-misusers, may be confused with white blood cells. Urinary casts are also indicative of renal tubular damage⁽²⁸⁾.

Culture:

The common urinary pathogens grow well on simple and selective media within 24 h of aerobic incubation at 37 C°. Nutrient agar, blood agar, MacConkey agar and CLED agar are the media most often used and laboratories generally limit the range to only one or two of these. CLED and MacConkey media have the advantages of distinguishing lactose from nonlactose fermenters and of inhibiting proteus from swarming, and as CLED is the less inhibitory to staphylococcus saprophyticus, its use is most strongly recommended.

Blood agar has the advantage of promoting the growth of nutritionally exacting strains, which may additionally require incubation for up to 48 h in air with added 5-10 % CO₂, but these and anaerobic pathogens are relatively uncommon in urinary tract infections and their culture should be attempted only in cases of pyuria from which significant numbers of a commoner pathogen have not been grown on the routine media⁽⁵⁰⁾.

Culture methods:

Standard loop method:

An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and spread it over a plate of agar culture medium. The plate is incubated, the number of colonies counted or estimated, and this number used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.004 ml loopful of urine yields 400 colonies, the count per ml will be 10^5 , or just indicative of significant bacteriuria ⁽⁵⁰⁾.

Filter paper method:

This method of semi-quantitative culture is rapid and very economical in the use of culture medium, but growths are often confluent and, if mixed, require to be plated out to obtain pure subcultures for identifying and sensitivity tests. A standard 6 mm wide strip of absorbent fluffless blotting or filter paper is bent into an L shape with a 12 mm long foot (area 12*6 mm) and sterilized at 160 C° for 1 h. Dip the whole of the angulated end and foot into the mixed uncentrifuged sample of urine, withdraw it and wait a few seconds to allow all the excess fluid to be absorbed into the paper. Then press the foot on to the surface of a marked section of a well dried plate of agar culture medium ensuring that the whole area of the foot makes contact with medium. Remove the strip and discard it into disinfectant. Up to 8 or 10 samples can be tested in duplicate on different areas of a 9 cm plate. Incubate the plate and afterwards count the colonies growing on the impression area. Up to 50 colonies may be countable and heavier growths are noted as being semi-confluent (+) or confluent (++) . Estimate the number of viable bacteria per ml of urine from the count of colonies on the impression area or the pattern of semi-confluent or confluent growth ⁽⁵⁰⁾ .

Dip slide method:

The dip-slide is a small plastic tray carrying a layer of an appropriate agar culture medium. Opposite sides of the tray may carry different media, e.g. CLED agar medium on one side and MacConkey, brain heart infusion or pseudomonas selective

agar on the other .The slide is supplied in a universal type container ,being held on a stalk fastened rigidly to the inside of the screw cap of the container .Such outfits are available commercially ⁽⁵⁰⁾ .

The method of semi-quantitative culture on dip slides or dip spoons is the least laborious for the laboratory and as the medium is seeded with urine immediately it has been passed, obviates the difficulty of having to prevent bacterial multiplication during transport to the laboratory. It is especially convenient for the routine screening of large numbers of patients and for use in clinics and practices remote from the laboratory. Its disadvantages are that it does not provide material for microscopical examination for the cellular content of the urine and that when the bacterial count is high and the growth on the dip slide confluent, it difficult to judge whether the growth is pure or mixed and to obtain a unmixed inoculum for identifying and sensitivity tests ⁽⁵⁰⁾.

Automated and semi-automated systems:

Automated screening systems offers the promise of a large throughput with minimal labor and rapid turnaround time compared with conventional cultures. However, these advantages may be offset by a substantial cost for the instrumentation. Often these costs can be justified only in laboratories that receive many specimens ⁽²⁷⁾. Several automated or semi-automated urine screening systems that are either bacterial growth independent or dependent are commercially available ⁽²⁷⁾.

Identification and sensitivity tests:

If similar colonies are found in numbers suggesting significant bacteriuria, a separate colony or apportion of apparently pure growth should be sub cultured for identification and testing of its sensitivity to antibiotics .The appearance of primary

growth on CLED or MacConkey medium will suggest the kind of organism that is present. How much more precisely it should be identified is a matter for

Consideration. Probably coliform bacilli should be differentiated into *E.coli*, *klebsiella* , *proteus* ,*pseudomonas* and other coliform ; *s.saprophyticus* and *S.aureus* should be distinguished from other staphylococci, and enterococci should be distinguished from other streptococci. Detailed characterization and typing of isolates may be done in epidemiological studies of cross infection and in cases where it is important to distinguish between re infection of a patient with a new strain and relapse of infection with a strain that was formerly present ⁽⁵⁰⁾.

Antibiotic sensitivity tests are best done with an appropriately diluted inoculum of a pure subculture, but if prior microscopy has indicated that infection may be present, primary sensitivity tests may be set up at the same time as the initial culture by flood inoculating the urine on to a suitable medium, drying the surface and applying sensitivity test disks. As antibiotics are concentrated in urine to higher levels than are found in the tissues, high content test discs should be used .If the patient is attending a general practice or outpatient clinic, drugs suitable for oral administration should be tested ⁽⁵⁰⁾ .

Treatment:

Depending on clinical evaluation of the patient and local antimicrobial resistance trends, uncomplicated UTI is typically treated with an oral antibacterial for 3 days. Uncomplicated UTI (cystitis) generally resolves spontaneously within 4 weeks in up to 40% of patients; however, treatment with antibacterial agents reduces symptoms and ensures bacterial eradication .Oral antimicrobial chemotherapy is generally administered twice a day for 3 days, depending upon the drug and clinical evaluation of the patient. The choice of agent should be based on the results of susceptibility

tests. In addition to antibacterial therapy, the patient should be advised to drink large volumes of fluid to help the normal flushing process ⁽²⁸⁾.

Several different classes of antibacterial are available in oral formulations and suitable for treatment of UTI. Children and pregnant women with asymptomatic bacteriuria should be treated with antibacterials and followed up to check for

Eradication of the infection. Instrumentation of the urinary tract should be delayed in patients with significant bacteriuria until appropriate treatment has rendered the urine sterile ⁽²⁸⁾.

Complicated UTI (pyelonephritis) should be treated with a systemic antibacterial agent, should continue until the signs and symptoms subside. It can then be replaced by oral therapy. The usual length of treatment is at least 10 days, but longer treatment may be necessary to sterilize the kidney ⁽²⁸⁾.

Hospital acquired infections or recurrent infections, particularly in catheterized patients, may be caused by antibiotic resistant organisms, and the agent of choice will depend upon the antibacterial susceptibility pattern ⁽²⁸⁾.

Prevention:

Recurrent infections in otherwise healthy women can be prevented by regularly emptying the bladder , This washes bacteria out of the urinary tract .The prophylactic use of antibiotics may also prevent recurrent infections, but in the presence of underlying abnormalities ,there is a tendency to select antibiotic-resistant strains ,which subsequently cause infections that are more difficult to treat. Infection in catheterized patients is very common, but can be reduced by good catheter care procedures. Catheterization should be avoided if possible or kept to a minimum duration ⁽²⁸⁾.

Rationale

Bacterial infections are often recurrent and important complications of the inactive stage of urinary schistosomiasis, the aim of this study to determine the frequency of bacterial urinary tract infections in schistosomiasis school children in rural southern Shendi town in which high prevalence of urinary schistosomiasis and people in these areas focus on treatment of schistosomiasis without detected of bacterial urinary tract infection.

Objectives

General objective:

To determine the Frequency of urinary tract bacterial infection among schistosomiasis school children.

Specific objectives:

- To isolate and identify the organisms that cause UTI in schistosomiasis school children.
- To determine the association between schistosomiasis and urinary tract bacterial infection in school children.
- To determine antimicrobial susceptibility patterns of isolated organisms.

Materials and Methods

Study design:

A cross-sectional study.

Study area:

Study was conducted in rural southern shendi town.

Shendi locality is one of the River Nile state localities. It is bounded by Eddamer locality northern of the River Nile State, Khartoum state to the north, River Nile to the west and Gadarif state to the east. Geographically it lies between line 360east to 310west longitudinal and line 190 north to line 150 south latitudinal in the arid zone of Sudan. The Rural areas of the Shendi locality are composed of about 96 villages, 63 of these are at southern side of the locality. The sources for drinking water in these villages are various. The study is conducted in three endemic villages (Bannt, Wad Nora and Gandato) in which agricultural projects are found.

Study period:

During 2016 from March to July.

Study Population:

Primary School males pupils.

Inclusion Criteria:

Test group:

Males in primary School infected with schistomasis.

Control group:

Males in primary School non infected with schistosomiasis.

Exclusion Criteria:

Males in primary School infected with schistosomiasis and take medications.

Specimen:

About 20ml of midstream urine samples collected in 50ml capacity, sterile, wide-mouthed, screw cap universal containers by subjects themselves, who are previously carefully instructed with illustration aids.

Sample size:

84 urine samples collected from males schistosomiasis pupils, and 36 urine samples from control group.

Tools of Data collection:

Data including age, gender, residence and history of diseases was collected in suitable data sheet.

Data Analysis:

SPSS was used for data analysis to compare between test group and control group (version 16).

Ethical Consideration:

Verbal consent was obtained from the parents and teachers of primary schools. Participation by pupils was voluntary after obtaining assent. Information collected

from participants was maintained with utmost confidentiality as names were not used on any sample but codes.

Methodologies :

Collection of Urine sample :

urine examination microscopically :

10 ml of urine sample was poured aseptically into a centrifuge tube and spun in the centrifuge at 500 - 1000 rpm for 5 minutes. The supernatant was decanted leaving the sediment at the bottom of the tube. A drop of the sediment was pipetted and placed on a microscope slide, then covered with a cover slip. The deposit was examined using x40 objective of the microscope detected of eggs of *S. hematobium* (25, 51 and 52).

-Urine culture :

The urine samples aseptically cultured on cystine lactose electrolyte deficiency (CLED) Medium using sterile wire loop . The culture plates were incubated aerobically at 37C° for 24 hours. Culture plates without visible growth were further incubated for an additional 24 hours before being discarded. Bacterial isolates were identified based on a combination of cultural, morphological and biochemical characteristics (53)

Identification of isolated bacteria:

CLED medium distinguishes between lactose fermenters (yellow colonies) and non lactose fermenters (green) (54). The colonial morphology ; its size and shape, whether it is opaque or translucent, mucoid or dry, and pigmented also was described (54).

Smear preparation and Gram stain was done by procedure describe by Abba M.El-Mishad ⁽⁵⁴⁾.

Biochemical test for gram positive cocci:

Catalase:

A small particle of separated colonies is picked with clean sterile loop and this is inserted into a small clean tube containing 1ml of 3% H₂O₂ solution, the production of gas bubbles indicates a positive reaction ⁽⁵⁰⁾.

Esculin hydrolysis test:

Inoculate the culture on to the slant and incubate at 37 C° for 48 hrs and look for blacking ⁽⁵⁰⁾.

Litmus milk reduction test:

Test organism was inoculated into 0.5 ml of sterile litmus milk medium. Incubate at 35–37 C° for up to 4 hours, examining at half hour intervals for a reduction reaction as shown by a change in colour from mauve to white or pale yellow ⁽²⁵⁾.

Biochemical test for gram negative bacilli:

Oxidase test:

The tested colony was smeared over the filter paper which soaked with a freshly prepared 1% solution of tetramethyl-p-phenylene diamine dihydrochloride, a positive reaction is indicated by an intense deep - purple hue ⁽⁵⁰⁾.

Urease test:

The test organism was Inoculated on slope of the medium and incubate at 37 C° .Examine after 4 hrs and after overnight incubation, urease positive cultures change the colour of the indicator to purple –pink ⁽⁵⁰⁾.

Citrate test:

The test organism was Inoculated on slope of the Simmons , citrate agar and incubate for 96 hrs at 37 C° , positive test is blue colour and streak of growth ⁽⁵⁰⁾.

Motility test:

The test organism was Inoculated on semi-solid media and incubte at 37 C° and examined, motile bacteria diffuse in agar ⁽⁵⁰⁾.

Kiligler iron agar test:

The test organism was Streaked a heavy inoculums over the surface of the slope and stab into the butt and Incubate aerobically at 37 C° for 24 h ⁽⁵⁰⁾.

Indole test:

The organism was inoculated in peptone water and after incubation at 37 for C° 24hrs. ,the kovacs reagent was added presence of red ring indicated a positive results ⁽⁵⁴⁾.

Antimicrobial Susceptibility Testing:

Antibiotic susceptibility testing of the isolated test organisms, namely Escherichia coli, Salmonella typhi, Klebsiella spp , Pseudomonas aeruginosa ,proteus spp and E.facalis was carried out using the following antibiotics: Amikacin (30 Mg), Chloramphenicol (30Mg), Cephalexin (30mcg), Norfloxacin (10 Mg), Levofloxacin (5Mg) , tetracycline (30 Mg) , Ciprofloxacin (5 Mg) , Ofloxacin (5 Mg) , Nitrofurantion (300 Mg) , Ampicillin /sulbactam (20 Mg) , Sparfloxacin (5 Mg) , Co-trimoxazole (25Mg)] .

The test organism was placed and emulsified in 10 ml of the normal saline in a test

Tube using sterile loop then incubated for 2hours. Mc Farland 0.5 was use. Turbidity was compared to Mc Farland standard ⁽⁵⁵⁾.

A sterile swab was dipped in the suspension and remove the excess fluid by pressing and rotating it against the side of the tube above the level of the suspension .The swab was streaked evenly over the surface of the medium in three directions,rotating the plate to insure even distribution , allow 3-5 minutes for surface of the agar to dry . Antimicrobial discs was put on the surface of the inoculated plate suitably spaced (25mm from disc to disc and 15mm from the rim). Plates were incubated at 37 C° for 18-24 hrs , then examined for the presence of zones of inhibition of bacterial growth around antibiotic discs . These are measured by a ruler, read from standard charts as sensitive, moderate or resistant to the different antibiotics ⁽⁵⁴⁾.

Results

Association between UTI and schistosomiasis among test population :

A total of 120 school children was included in the study their age range between 5-16 years, 84 was suffering of schistosomiasis and 36 non infected. out of 84 who had schistosomiasis 37 (44.05%) showed significant growth, 47 (55.95%) had no growth (p.value =0.001) as shown in table (3.1).

Frequency of Bacterial Isolates in the schistosomiasis Urine Samples:

Out of the 37 subjects with significant a symptomatic bacteriuria, Escherichia coli remains the most frequently occurring culprit accounting for 17 (45.95%) of the infections followed by 7 (18.92%) recorded for salmonella spp.; 5 (13.51%) of the subjects had klebsiella spp and pseudomonas spp, 2 (5.41%) had proteus species and 1 (2.70%) had E.facalis as as shown in table (3.2) .

Antibiotic susceptibility pattern (%) of isolated organisms :

The antibiotics used during the course of study were multiple discs for urinary tract isolates which included; Amikacin (30 Mg), Chloramphenicol (30 Mg), Cephalexin (30mcg) , Norfloxacin (10 Mg) , Levofloxacin (5Mg)

Tetracycline (30Mg), Ciprofloxacin (5mcg), Ofloxacin (5mcg), Nitrofurantoin (300Mg), Ampicillin / sulbactam (20 Mg), Sparfloxacin (5 Mg) and Co-trimoxazole (25Mg).

All gram negative isolates showed resistant to amikacin (AK) , cephalosporins (PR) and ampicillin (AS) in different percentages , E.coli and klebsiella resistance to Co-trimoxazole and moderate to high sensitivity to other antibiotics , high resistance rate

appear with E.coli and Klebsiella . E.facalis moderately susceptibility to amikacin (AK) ,cephalexin (PR),tetracycline (TE) and Nirofuranation (FD) , resistance to Co-trimoxazole and highly susceptibility to remaining antibiotics as shown in table (3.3) .

Table (1) : comparison between Bacteriuria and Urinary Schistosomiasis among test population :

Categories	Urinary schistosomiasis Cases	Non urinary schistosomiasis cases	Total	P.value
Presenceof bacteriuria	37(44.1%)	0.0 (0.0%)	37	0.001
Absence of urinary Bacteriuria	47(55.9%)	36(100%)	83	
Total	84(100%)	36(100%)	120	

Table (2) : frequency of Bacterial Isolates in test population samples :

Isolates	Number & percentage%
E. coli	17 (45.95%)
Klebsiella spp.	5 (13.51%)
Pseudomonas spp.	5 (13.51%)
salmonella spp.	7 (18.92%)
E. facialis	1 (2.70%)
Proteus spp	2 (5.41%)
TOTAL	37 (100%)

Table (3): percentages of the patterns of resistant to antibiotics by isolated organisms (%) :

Organism isolated	AK	CH	PR	TE	NX	LE	CP	OF	FD	AS	SC	BA
E. coli n(17)	94	18	88	30	24	30	30	6	35	88	6	100
Klebsiella spp. n(5)	100	20	20	20	0.0	60	40	80	40	100	20	100
Pseudomonas spp. n(5)	20	20	100	40	40	20	0.0	20	80	100	20	40
Salmonella spp. n (5)	100	29	43	43	100	71	14	0.0	14	57	71	0.0
E .fecialis n(1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Proteus spp n(2)	100	0.0	100	50	0.0	0.0	0.0	0.0	0.0	50	0.0	50

Key: [S = Sensitive/susceptible, R = Resistance, I = Intermediate, AK = Amikacin (30_{Mg}) ,CH = Chloramphenicol (30_{Mg}) , PR = Cephalexin (30_{Mg}) ,NX = Norfloxacin (10_{Mg}) , LE = Levofloxacin (5_{mcg}) , TE =tetracycline (30_{Mg}) , CP = Ciprofloxacin (5_{Mg}) , OF = Ofloxacin (5_{Mg}) , FD =Nirofuranation (300_{Mg}) ,AS =Ampicillin /sulbactam (20_{Mg}) , SC =Sparfloxacin (5_{Mg}) , BA = Co-trimoxazole (25_{Mg})] .

Discussion

In this study frequency of bacteria among urinary schistosomiasis children is 44.5 % and this study assessment between urinary schistosomiasis and urinary tract bacterial infection, this finding is agreed with study carried out by Okechukwu et al 2012, who stated that concomitant bacterial urinary tract infection in children, prevalence of significant bacteriuria was 53.7% among those infected with urinary schistosomiasis and 3.6% in those that were not infected with urinary schistosomiasis (p value<0.001) ⁽²⁶⁾.

Also comparable to the findings in 2008 in Ngbo west with a bacteriuria prevalence of 48.3% was reported among people with urinary schistosomiasis by C.J. Uneke et al ⁽⁵⁶⁾. Urinary schistosomiasis and concomitant bacteriuria was investigated in Abuja Prevalence of bacteriuria in urinary schistosomiasis ranged from 74-86% ⁽⁵⁷⁾. And finally Reports from Egypt indicated that some 39 - 66 % of subjects with schistosomiasis were found to have a bacterial infection in the urinary tract ^(58,59).

The reasons why individuals with *S. haematobium* infection appear to be more susceptible to UTI or the mechanism by which this occurs remain obscure. Nevertheless, some studies have noted that the association between schistosomal and bacterial infection could result from a relationship (possibly symbiotic) in which the bacteria either become fixed on the cutaneous surface of the worms in clearly defined place or, colonize the caecum of the parasite as observed by Otteens and Dickerson ^(60,61 and 62). Another previous report had suggested that schistosomiasis appears to enhance the susceptibility of infected persons to bacteria causing. Some earlier studies have evaluated the prevalence of UTI caused by bacteria and the

relationship with urinary schistosomiasis in different epidemiological, clinical and experimental studies and suggested a possibility of a link between the two conditions⁽⁶³⁾ .

The six bacterial species encountered in this study (*E.coli* ,*Klebsiella* spp, *proteus* spp ,*salmonella* spp, *pseudomonas* spp and *E.facalis*) confirm the results of studies of Prevalence of bacteria Isolates among School children in Ezza-North LGA of Ebonyi country and the Prevalence of bacteria Isolates among School children in Ngbo-West LGA of Ebonyi country⁽⁶⁴⁾ .

The isolation of *Esherichia coli* more frequently than the rest isolates this conforms to reports of many researchers as finding of Laughlin et al2013. ,also is agreed to the findings in some other states in Nigeria such as study by Uneke et al in Ebonyi State and Normosi et al in Ogben rural community of Edo state these are a confirmation of my findings⁽⁶⁵⁾ .

The antimicrobial susceptibility test of isolates revealed varying patterns of susceptibilities by all isolates. This study clearly suggests that bacteriuria is a potent complication in the management of urinary schistosomiasis. Therefore the complimentary incorporation of antibacterial therapy appear essential . most of the organisms encountered during the course of this study were resistant to amikacin , cephalexin and impicillin in different percentages . *Klebsiella* spp . and *E.coli* resistance to Co-trimoxazole and in particular showed a high levels of resistances to most antimicrobial agents . Similar results have been reported by R. M. Mordy and etal 2006 and P. Galia,etal2003 found that high level of resistance of antibiotics with *E.coli*^(66, 67) .

Conclusion

From this study it concluded that there was high prevalence of bacteriuria co-infection among school age children with urinary schistosomiasis in Rural Southern Shendi town . This co-infection might portend some danger in later years of life as it increases the risk of bladder cancer. It is pertinent to state that since the potential exist for possible interaction between *S. haematobium* infection and bacteria UTI in urinary schistosomiasis endemic areas .

Recommendations

In the light of the above I recommended that :

- All children infected with urinary schistosomiasis should be screened for bacteriuria and appropriate antibiotics concurrently administered.
- These children should also be followed up to monitor for later complications.
- Access to safe water, improved sanitation, health education, health communication, and appropriate case management. These strategies will improve the health of children in endemic areas.
- Further studies are urgently required using the more sophisticated molecular and immunological tools to clearly elucidate this association.

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