Prevalence and antibiotic susceptibility of urinary tract infections in febrile children below ten years attending mulago hospital, Uganda

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Ronald Ssemambo
Makerere University COVAB

Isaac Odongo
Makerere University, COVAB

Joseph Kungu  kungu@live.com
Makerere University College of Veterinary Medicine Animal Resources and Biosecurity

Corresponding Author
ORCiD: 0000-0002-2225-463X

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Pediatrics

KEYWORDS
Urinary tract infections, antimicrobial susceptibility, children, Mulago hospital.
Abstract

Background: Urinary tract infections (UTIs) are one of the most common infections in the pediatrics population. This study was performed to determine the prevalence of urinary tract infections amongst febrile children below 10 years attending Mulago National Referral Hospital, and the susceptibility patterns of the isolated uropathogens to common antibiotics. Methods: A cross-sectional study of febrile pediatric patients below 10 years from various ward of Mulago National Referral Hospital was conducted between January and May 2019. Biodata and midstream urine samples were collected from 160 children. The urine samples were cultured onto Blood Agar and Cystine Lactose Electrolyte Deficient (CLED) simultaneously. Growth was considered significant when a pure isolate had $\geq 10^5$ CFU/mL. Susceptibility to 8 antibiotics was set using the modified Kirby-Bauer disc diffusion technique. Results: Out of the 160 urine samples analyzed, 29(18.1%) had significant bacterial growth. The frequency of UTIs was significantly higher in girls 20(69.0%) than boys 9(31.0%). Escherichia coli was the most predominant microorganism (41.4%), followed by Klebsiella pneumoniae (20.7%) and Staphylococcus aureus at (13.8%). Overall susceptibility tests exhibited a very high Antibiotic resistance of uropathogens to ampicillin (96.6%), cotrimoxazole (82.8%) and nalidixic-acid. Nitrofurantoin and imipenem showed the lowest resistances of 34.5% and 31.0% respectively. A total of 24(82.8%) isolates were multidrug resistant. Conclusion: Bacteriuria is a highly prevalent condition amongst febrile children attending Mulago hospital, with Enterobacteriaceae being the most predominant uropathogens. Uropathogens were highly sensitive to nitrofurantoin and imipenem but with significant resistance to ampicillin and cotrimoxazole. This information can be useful in decision making during management of UTIs among children.
Background

Globally, about 150 million people develop a urinary tract infection each year. They occur frequently in developing countries among the low socio-economic populations (1,2). Urinary tract infections (UTIs) affect children of both sexes and all age groups, usually with non specific symptoms (3). Recurrent infections are frequent in girls than in boys, with 50% of the girls having high likelihood to develop another infection (4).

Bacteria are the primary, organisms causing urinary tract infections in children with Enterobacteriaceae being predominant pathogens (5). Particularly, Escherichia coli accounts for about 75% of these infections followed by Klebsiella, Proteus and Enterobacter. However, Pseudomonas aeruginosa, Staphylococci and Enterococci also play an important role depending on the underlying conditions (6). Urinary Tract Infections are treated empirically with antibiotics which when used extensively has resulted in development of drug resistant organisms (7). Numerous reports from miscellaneous hospitals around the globe indicate an increasing incidence of urinary tract infections caused by multi-drug resistant (MDR) organisms (8,9).

Emergence of resistant strains is becoming a serious health burden, it limits the number of available antibiotics with potential to successfully treat these infections hence increasing the costs of treatment (10). Knowledge of local and national antimicrobial resistance patterns is essential and is of utmost importance for translation of evidence-based recommendations to empiric antibiotic treatment of infections (11). The aim of this study was to determine the prevalence and antibiotic susceptibility patterns of urinary tract infections in febrile children below 10 years attending Mulago National Referral Hospital Kampala Uganda.

Methods
Study design

This was a laboratory based cross-sectional study whereby biodata and midstream urine samples were collected from febrile children attending Mulago hospital from February to May, 2019.

Study area

Mulago National Referral Hospital was founded in 1913 and is located on Mulago hill in the northern part of Kampala, Uganda’s Capital city. The coordinates of the hospital are 0°20’16.0”N, 32°34’32.0”E (latitude: 0337786, longitude: 32.575550). It is the largest public hospital in the country with an estimated 1,500 beds. It is the National Regional Hospital for the entire country and a teaching hospital for Makerere College of Health Sciences. It also serves as a general hospital for Kampala metropolitan area.

Sample size determination

The sample size was determined using a formula by Thrusfield as follows (12);

\[ n = \frac{z^2p(1-p)}{i^2} \]

Where; n was the calculated sample size, z the desired level of confidence (1.96), i the standard sampling error (10%), p the estimated prevalence from previous studies 20.3% (13). Although a minimum sample size required was 62, up to 160 febrile children from Mulago hospital who met the inclusion criteria were recruited in the study to increases precision.

Collection of samples
A mid-stream urine sample was collected from each child enrolled into the study. A sterile wide mouthed plastic urine sample container was used. The sample container was labeled with the sample number, sex, patient code, age and time of collection. Care takers of the children were informed on how to collect the sample without contamination. The collected urine sample was then transported to the laboratory and processed within one hour.

Febrile children from two to ten years, presenting with or without any other clinical signs of urinary tract infections were enrolled into the study. Children who were critically ill and needed special care and a sample could not be collected and those who were currently on antibiotic therapies were not enrolled in the study.

**Laboratory examination of the samples**

The samples were subjected to the routine macroscopy, biochemistry, microscopy, culture and sensitivity according to the standard practice.

**Biochemistry**

Biochemistry of the urine was carried out using a dipstick so as to assess the proteins, nitrites, glucose and white blood cells. A dipstick was dipped into the urine so as to wet it after which it was removed and results read within 60 seconds.

**Microscopic examination of the sample**

The samples were examined using a microscope for pus cells, epithelial cells and cellular casts. A wet preparation was made by aseptically transferring a drop of urine onto a slide and covered with a coverslip. The preparation was then be examined using the 10X and 40X objective (14).

**Culturing the specimen**
The urine samples were inoculated onto Cystine Lactose Electrolyte Deficient (CLED) and Blood agar culture media using a 1 µl wire loop. Culture plates were incubated at 37°C for 24 hours. Colonies of the isolated organism were counted, a positive culture was defined as a urine sample containing ≥ 10^5 CFUs/mL of a uropathogen (14).

The colonies of the cultured organism were categorized morphologically so as to identify the organism. Morphology analyses included; size, shape, elevation, margin and surface.

A Gram stain was performed on the cultured organisms so as to determine their Gram reaction. Using an applicator stick, one colony was picked and smeared onto a clean glass slide and then air dried, fixed smear was covered with crystal violet stain for 60 seconds and then rapidly washed off with clean water. All the water was tipped off and then the smear was covered with Lugol’s iodine for 60 seconds, the iodine was then washed off with clean water. The smear was decolorized with acetone–alcohol and then washed off immediately with clean water. The smear was then covered dilute carbol-fuscin stain for 1 minutes. The stain was washed off with clean water, the back of the slide was then wiped clean and placed on a draining rack to air dry. The smear were examined microscopically, first with the 40X objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells (14).

**Biochemical tests**

Biochemical tests were performed so as to identify the genus and species of the causative agents, the process involved the use conventional biochemical tests.

Biochemical tests included;
**Indole**

Indole test was be used to distinguish *E. coli* from *Klebsiella* spp. Colonies of the microorganism were inoculated in a bijou bottle containing about 3 mLs of sterile peptone water, they were incubated at 37 °C for 18 hrs. Thereafter 0.5mls of Kovac’s reagent were added to the bijou bottle and shaken gently. A positive test was indicated by a red ring while a negative test was indicated by absence red ring.

**Catalase test**

This test was used to distinguish between *Staphylococcus* spp. and *Streptococcus* spp. 4 drops of 3% hydrogen peroxide were added to the test tube and a colony of the organism was placed in the tube using an applicator stick. The tube was then observed for immediate bubble formation. If the isolate contained catalase enzyme, bubbles of oxygen were produced. *Staphylococcus aureus* was used as a positive control and *Streptococcus pneumoniae* was used as a negative control.

**Oxidase test**

Paper oxidase test was performed on all Gram negative bacteria. It was used to differentiate between *Pseudomonadaceae* and *Enterobacteriaceae*. A filter paper was placed in a clean Petri dish and three drops of freshly prepared oxidase reagent were added to it. Using an applicator stick, a colony of the test organism was picked from the Petri dish and was smeared on the wet filter paper. Changes in the color of the filter paper were observed in 5 seconds. A positive test was shown by formation of a purple color while the negative test left the reagent colorless. *Pseudomonas aeruginosa* was used as a positive control while *E coli* were used as a negative control.
**Urease test**
This test was used to identify *Proteus spp.* which produce urease enzyme. Urea agar slant was streaked on the surface with a colony from the culture plate and then incubated at 37 for 18 hours. Positive test was indicated by bright pink color while negative test was indicated by no color change.

**Coagulase test**
This test was performed to differentiate *Staphylococcus aureus* from other *Staphylococcus spp.*

**Slide method:** Two drops of normal saline were put onto a slide. Using an applicator stick, the test organism was emulsified in the normal saline. A drop of plasma was then placed on the slide mixed well and then rocked gently for 10 seconds. Clamping on the slide indicated a positive test. If the slide coagulase test was negative, a tube coagulase test was done for confirmation.

**Tube method:** This test was performed on slide coagulase negative organisms or when the slide results were not conclusive. Freshly cultured organisms were used. 0.2ml of plasma were pipetted into a test tube and then 0.8ml of test broth culture was added to the test tube. The contents were mixed gently and then incubated at 37 °C for an hour, if no clotting occurred, the tube was examined again after 3 hours and if the test was still negative, the tube was left at room temperature overnight and examined again. Clotting of the tube contents indicated *Staphylococcus aureus* while no clotting of the tube contents meant a negative test.

Other biochemical tests included; Triple sugar Iron (TSI), Sugars (lactose, glucose, mannitol, and sucrose).

**Antibiotic susceptibility testing**
Antibiotic susceptibility was determined by the modified Kirby-Bauer disc diffusion
technique. Commonly prescribed antibiotics for urinary tract infections at Mulago Hospital were tested. A pure isolate and identified colon of bacteria was be picked and inoculated in peptone water to form a bacterial cell suspension. An appropriate volume of peptone water which contains the cells was uniformly inoculated on Mueller Hinton Agar media. The antibiotic drug discs were then placed onto the culture media and then incubated for 18 hours aerobically. The diameter of the Zone of inhibition was measured. The results were interpreted according to the guidelines of The Clinical and Laboratory Standards Institute (14). The antibiotics used included; Amoxicillin-clavulanate, Ampicillin, Ceftazidime, Ciprofloxacin, Cotrimoxazole, Imipenem, Nalidixic-acid and Nitrofurantoin. For statistical analysis, bacteria with intermediate susceptibility were considered as resistant (15). Multiple drug resistance (MDR) was defined as an isolate resistant to 3 or more unrelated antibiotics tested. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as a control strains.

Data analysis

The collected data were entered in Microsoft Excel, cleaned and then exported to Statistical Package for Social Scientists (SPSS) software, Version 20.0. The Chi-square test was used to test for associations between potential factors with prevalence of UTIs.

Results

Demographic characteristics of the study participants

The study involved 160 febrile children below 10 years with an average age of 5.5 ±2.8 years and a mode of 4 years. Up to 83 (51.9%) were female while 77 (48.1%) were male. A total of 137 (85.6%) were in-patients while 23(14.4) were from the out-patients.

Prevalence of bacteriuria among febrile children
Of the 160 mid-stream urine samples collected and tested, 29 samples exhibited significant growth on either CLED or and Blood agar thus making a prevalence of bacteriuria among febrile children 18.1%. Of the 29 samples which had significant bacterial growth, 20 (68.96%) were from girls and 9 (31.0%) were from boys (P-value 0.042). The age group of children ≤3 years had the highest number of samples with significant bacterial growth 12(41.37%) and the prevalence within this group was 27.27%. The age group of children above 3 to 6 years had the lowest prevalence of 15.55%. Of the 29 samples which had significant bacterial growth, 3(10.34%) where from the inpatients while 26(89.66%) where from the outpatients. Of the 29 samples which had significant bacterial growth, 8 (27.59%) had nitrites and it was statistically associated (P-value 0.007).

**Antibiotic susceptibility patterns of isolated uropathogens**

A total of 29 bacterial isolates were isolated from 160 urine samples collected. The predominant organism isolated was *Escherichia coli* with a percentage of 41.4%, followed by *Klebsiella pneumoniae* at 20.7%, *Staphylococcus aureus* at 13.8%, *Pseudomonas aeruginosa* at 10.3%, *Citrobacter freundii* at 6.9%, *proteus mirabilis* at 3.4% and *Enterobacter spp.* at 3.4%.

Eight antibiotics from various classes were tested. Antibiotic resistance of uropathogens to ampicillin, cotrimoxazole, nalidixic acid, ceftazidime, amoxicillin-clavulanate, nitrofurantoin and imipenem were 96.6%, 82.8%, 75.9%, 69.0%, 51.7%, 48.3%, 34.5% and 31.0% respectively. Of the 29 bacterial isolates, 24(82.8%) were multidrug resistant.

**Discussion**

The findings of this study revealed that the prevalence of bacteriuria among febrile
children below 10 years attending Mulago National Referral Hospital was 18.1%. This is higher than the prevalence reported in a study carried out in Nigeria in a similar age group (16) . This study provides evidence of bacteria being key in causation of UTIs among children in Uganda. This could be due to the poor sanitation levels in their dwelling environment. The study also noted that girls were more affected than boys, a finding which agrees previous studies that showed substantially higher prevalence of UTIs in girls (3,16). This difference is attributed to both the greater proximity of the female urethral orifice to the colonic bacterial reservoir and to the shorter female urethra. Furthermore, a vagina is a well-established reservoir for uropathogenic bacteria in females (3,17).

The most predominant uropathogens isolated were *Escherichia coli* (41.4%) followed by *Klebsiella pneumoniae* (20.7%). These findings concur with those in similar studies carried out in Europe and Asia. The predominance of *Enterobacteriaceae* could be due to their omnipresence in the environment (18,19). Furthermore, *Escherichia coli* is the most predominant normal aerobic intestinal flora with various strains of specific virulence factors that interact with the host to overcome host defenses in the urinary tract and to stimulate an infection. This could also be due to the poor toilet manners amongst children. *Escherichia coli* ascend to the urinary tract thus becoming pathogenic (19).

Bacteria in this study showed a very high resistance to ampicillin (96.6%), cotrimoxazole (82.8%), and nalidixic-acid (75.9%). The lowest resistance was to imipenem and nitrofurantoin. In this study, higher resistance rates to all antibiotics tested with the exception of imipenem and nitrofurantoin may be expounded by the high and uncontrolled usage of these antibiotics, especially third-generation cephalosporins, during the past few years in the country (20,21). Unfortunately, these antibiotics have been widely prescribed not only for urinary tract infections but also for other infections in Uganda (22). Multidrug resistance was exhibited by 24 out of 29 (82.8%) of the isolated bacteria, with
majority of the isolates being *Escherichia coli*. This is in agreement with similar findings reported in Ghana (23). This substantially high multidrug resistance can be greatly attributed to the, easy access to antibiotics without a prescription from a clinician where people misuse and abuse the antibiotics. These high resistance rates negatively impact on the patient outcome. The successful initial empirical treatment is based on susceptibility and resistance patterns obtained from local data, yet these patterns are dynamic. Periodic monitoring of resistance to antibiotic agents is essential to establish standard treatment guidelines for UTIs in children (9,19,24).

**Conclusions**

*Enterobacteriaceae* continue to be the most predominant pathogens causing urinary tract infections in febrile children. They exhibited significant resistance to ampicillin and cotrimoxazole. Therefore, local antibiotic resistance patterns are of utmost importance in choosing empiric antibiotics for pediatric urinary tract infections. More prevalence studies should be done in different regions of the country encompassing the predisposing factors of urinary tract infections in children.

**Declarations**

*Ethical approval and consent to participate*

Research ethical approval was sought and granted from Mulago Hospital Research and Ethics Committee (reference number MHREC 1571). Formal consents were obtained from parents or guardians of the participants before enrollment in the study.

*Consent to publish*

Not applicable in this study.

*Availability of data and materials*

All necessary data has been included in this manuscript submitted.
Competing interests
Authors declare that there are no competing interests.

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Authors’ contributions
RS: Conception and design of study, collection, analysis and interpretation of data; drafting and critical review of manuscript, gave final approval for submission of manuscript.
IO: Collection, critical review of manuscript, gave final approval for submission of manuscript.
JMK: Conception and design of study, critical review of manuscript, gave final approval for submission of manuscript.
All authors have read and approved the manuscript.

List of abbreviations
CLED: Cystine Lactose Electrolyte Deficient, DNA: Deoxyribose nucleic acid, E. coli: Escherichia coli, MDR: Multi-drug Resistant, SPSS: Statistical Package for Social Scientists, TSI: Triple sugar Iron, UTI: Urinary tract infections.

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Tables
Table 1: Demographic characteristics of the study participants
| Variable | Frequency | Proportion (%) |
|----------|-----------|----------------|
| Age group |           |                |
| 0 to 3   | 44        | 27.5           |
| Above 3 to 6 | 52    | 32.5           |
| Between 6 and 10 | 64  | 40             |
| Sex      |           |                |
| Male     | 77        | 48.1           |
| Female   | 83        | 85.6           |
| Ward     |           |                |
| Inpatient | 137     | 85.6           |
| Outpatient | 23     | 14.4           |

Table 2: Cross tabulations of potential factors with prevalence of UTIs
| Variable       | UTI infection | $\chi^2$ |
|----------------|---------------|----------|
|                | Positive | Negative |         |
| **Sex**        |          |          |         |
| Male           | 9        | 68       | 4.144   |
| Female         | 20       | 63       |         |
| **Age group**  |          |          |         |
| 0 to 3         | 12       | 32       | 3.513   |
| Above 3 to 6   | 7        | 45       |         |
| Between 6 and 10 | 10    | 54       |         |
| **Ward**       |          |          |         |
| Out-patient    | 3        | 20       | 0.467   |
| In-patient     | 26       | 111      |         |
| **Nitrites**   |          |          |         |
| Present        | 8        | 12       | 7.37    |
| Absent         | 21       | 119      |         |
| **Proteins**   |          |          |         |
| Present        | 9        | 34       | 0.312   |
| Absent         | 20       | 97       |         |

Table 3: Antibiotic susceptibility patterns of isolated uropathogens
| Antibiotic agent          | Disc content (µg/mL) | Escherichia coli isolates n= 12/29 (41.4%) | Klebsiella pneumoniae isolates n=6/29 (20.7%) | Other uropathogen isolates n=11/29 (37.9%) |
|--------------------------|----------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------|
| Amoxicillin-clavulanate  | 20/10                | 5 (41.7%)                                   | 7 (58.3%)                                    | 4 (66.7%)                               |
| Ampicillin               | 10                   | 0 (0%)                                       | 12 (100%)                                    | 0 (0%)                                  |
| Ceftazidime              | 30/10                | 8 (66.7%)                                    | 4 (33.3%)                                    | 1 (16.7%)                               |
| Ciprofloxacin            | 5                    | 4 (33.3%)                                    | 8 (66.7%)                                    | 5 (83.3%)                               |
| Cotrimoxazole            | 20                   | 2 (16.7%)                                    | 10 (83.3%)                                   | 2 (33.3%)                               |
| Imipenem                 | 10                   | 7 (58.3%)                                    | 5 (41.7%)                                    | 5 (83.3%)                               |
| Nalidixic-Acid           | 30                   | 3 (25%)                                      | 9 (75%)                                      | 4 (66.7%)                               |
| Nitrofurantoin           | 300                  | 11 (91.7%)                                   | 1 (8.3%)                                     | 5 (83.3%)                               |

Susceptibility[^1] n (%)