Antibacterial Susceptibility Patterns of Common Bacterial Species associated with Urinary Tract Infections in Patients Attending Kam Medical and Diagnostic Centre, Kampala Uganda.

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Abstract

Background:[a]
Urinary tract infection (UTI) is defined as the presence of microbial pathogens within the urinary tract. It is primarily caused by *Escherichia coli* (*E.coli)*, accounting for 75% of all bacterial UTI cases. Other bacteria such *Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Enterococcus faecalis* have also been reported as causative agents. The study aimed to determine the antibiotic susceptibility patterns of Uropathogenic bacteria in urine samples of patients with suspected UTI in Kam Medical and the diagnostic Centre.

Methodology:
This was a cross-sectional study where 120 urinary samples from Kam Medical and Diagnostic Centre in 2019. The urine specimens were cultured on CLED (Cysteine Lactose Electrolyte – Deficient) and blood agar media. Kirby-Bauer's standard disk diffusion method was applied to test the susceptibility of the drug for Mueller-Hinton culture agar plates.

Results:
All 120 patients suspected of UTI had bacterial pathogen causing UTI. Among the urinary pathogens, *Escherichia coli* was the most common in 85/120 (70.8%) of the patients followed by *S.aureus* 13/120 (10.8 %), *Klebsiella spp* 4/120 (9.2%), *Enterococcus spp* with 4/120 3.3 %), *Pseudomonas aeruginosa* with 4/120 (3.3%) and *Proteus* with 3/120 (2.5%). According to the results of the antibiogram, the highest resistance was observed for Nalidixic acid (64.2%), Ampicillin (61.7%), and Cotrimoxazole (54.2 %). The highest susceptibility (antibiotic sensitivity) was observed with imipenem (97.5%), Nitrofurantoin (49.2 %), Ciprofloxacin (45.8%), and Clotrimazole (44.2 %)

Conclusion and recommendations:
The bacterial pathogens associated with UTIs in this study were *E.coli* species, *Staphylococcus aureus*, *Klebsiella, Enterococcus species, Pseudomonas* species, and *Proteus* species. *E.coli* was the most common isolate followed by *S.aureus*, *Klebsiella spp*, *Pseudomonas spp,* and *Enterococcus spp*, and lastly *Proteus* spp. The highest levels of bacterial resistance were recorded against first-generation antibiotic drugs The bacterial isolates in this study were highly susceptible to broad-spectrum, second/ third generation antibiotics drugs.

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1 Background

Urinary tract infection (UTI) is defined as the presence of microbial pathogens within the urinary tract Muthulakshmi *et al.,* (2017). It is primarily caused by *Escherichia coli* (*E.coli*), accounting for 75% of all bacterial UTI cases. Other bacteria such as *Klebsiella pneumonia*, *Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Enterococcus faecalis* have also been reported as causative agents (Antwi *et al.,* 2008; Boye *et al.,* 2012).

UTI can be classified based on the site of infection; Infection of the bladder is known as cystitis, and infection of the kidneys is called pyelonephritis (Flores-Mireles *et al.,* 2015). It can also be classified clinically as either complicated or uncomplicated depending on the extent of infection (Bennett *et al.,* 2014). UTIs are highly intricate and are difficult to treat. Complicated urinary tract infections can lead to structural abnormalities that lower the ability of the urinary tract to flush out the urine hence bacteria are provided with better scope for growth (Sabih *et al.*, 2021).

Globally, UTIs are a major public health burden leading to increased morbidity and the associated high healthcare cost (Flores-Mireles *et al.,* 2015). According to World Health Organization (WHO), UTIs are responsible for 8.3 million hospital visits and more than 1 million hospitalizations leading to an overall annual cost of over $1 billion (WHO, 2013). Worldwide, it is evident that urinary tract infections are more common among young girls because women have a shorter urethra as compared to men (Odoki *et al.,* 2019) show that an estimated 50% of women report having had a UTI at some point in their lives. In Uganda, the prevalence of UTI was reported to be 13.3% by Odoki *et al.,* (2019). In Tanzania, a study conducted on patients in the pediatric ward of Muhimbili Hospital and Mulago National Referral Hospital in Uganda between five to ten years reported prevalence of UTI to be 16.8%and 14.6% respectively.

Antibiotics play an important role in minimizing morbidity and mortality associated with UTIs. The most common antibiotics used for the treatment of UTI are cefuroxime; amoxicillin/clavulanic acid, trimethoprim/sulphamethoxazole, and fluoroquinolones. However, in Ugandan clinical practice, there is the unorthodox treatment of patients suspected of UTI, typically patients have blindly prescribed antibiotics without valid microbial culture and sensitivity laboratory results. This affects the efficacy of the treatments due to the rise of antimicrobial-resistant bacteria which leads to treatment failure, hence increased morbidity and ultimately high cost of treatment (Uganda Clinical Guidelines, 2016). One way to avert this problem is detailed knowledge of the prevalence of bacteria causing UTI and the antibiotic susceptibility pattern. Thus, this study will investigate the prevalence and antibiotic susceptibility pattern of Uropathogenic bacteria isolated from urine samples of patients with suspected UTI, in Kam Medical and Diagnostic center Kampala, Uganda.

The rise of antimicrobial-resistant bacteria (AMR) is a global public health concern because it leads to treatment failure, hence increased morbidity and mortality. It also leads to higher treatment costs, therefore, placing a greater burden on the economy. This rise is partly driven by the blind indiscriminate use of antibiotics. In Uganda suspected UTI patients have blindly prescribed antibiotics without valid microbial culture and sensitivity laboratory results and this contributes to treatment failure. Secondly, most patients with suspected UTI, out of ignorance visit local drug shops often run by under-trained medical personnel who sell antibiotics drugs for treatment without any prescriptions, further leading to treatment failure and antibiotic resistance.

Despite this, in sub-Saharan Africa, for example, Uganda, where bacterial infections are common and there is a paucity of information on the common causes of UTIs and antibiotic susceptibility patterns of the causative species.

Given the above limitations, this study will elucidate the bacterial pathogens commonly associated with UTIs at KAM Medical and Diagnostic Centre and the antimicrobial susceptibility patterns to aid successful prognosis.

Materials And Methods

Study area

The study was carried out in the Microbiology (bacteriology) department of the Kam Medical and Diagnostic Centre. Kam medical is a private medical center with four departments in the Kawempe division Kampala, Uganda. It lies between Mulago National Referral hospital and Kubiri roundabout Kalerwe road. The Kam Medical and diagnostic Centre is a newly constructed medical center and

started in 2015 in the Kawempe division. It caters to people living in and around Kampala capital city.

Study design

This was a cross-sectional study where120 patients presenting with signs and symptoms for UTI attending Kam medical and diagnostic center were purposively sampled.

Sample size

The sample size was determined using the Kish and Lisle formula (1965) for cross-sectional studies.

$$n = z2p(1 - p)/d2$$

Where: z = Z score for 95% confidence interval = 1.96, p = prevalence, d = tolerable error =5%. Where z=10, p=6, d=5%. = 120. Therefore 120 samples were collected and analyze.

Sample collection and analysis

After patients had given consent, 20 ml midstream urine was collected into a sterile screw-capped, universal container and the sample containers were labeled with patient details such as name, age, laboratory number, and sex. The labeled specimens were transported to the laboratory and processed within an hour of collection. A 1.5ml aliquot of the sample was placed into a sterile cryovial labeled with patient information for microbiological analysis.

Urine dipstick

For biochemical analysis, urine samples were analyzed using the 10 parameter urine test strips. Each strip was dipped in the urine sample and analyzed according to the manufacturer's instructions. The results for the biochemical analysis were recorded awaiting culture and sensitivity results.

Microbiological culture

Cysteine Lactose Electrolyte - Deficient (CLED), blood agar was prepared using the manufacturer's instructions (Rosco Diagnostic). 15–20 ml CLED agar was poured into a sterile Petri dish (90– 100 mm diameter), allowed to solidify, and incubated at 37°C for 18 to 24 hours. After incubation, the plates were checked for sterility then inoculated with 50μl of the sample stored in the 1.5ml cryovial. The samples were inoculated using the streak plate method and then incubated for 18 to 24 hours at 37°C for growth to take place.

Isolation and identification of bacterial isolates

Following 24 hours of incubation, the plates were inspected for bacterial growth. Bacterial colony numbers less than 104/ml were considered to be insignificant and possible contamination. For colony growths considered to be significant, the bacterial colonies were further identified using colony morphology, gram reaction, and biochemical tests.

Colony morphology

The appearances of urinary pathogens on CLED agar as described by Cheesborough, 2006 was used for the primary identification of the bacterial colonies.

E. coli appear as Yellow (lactose-fermenting) opaque colonies often with a slightly deeper colored center and *S.aureus* appear as deep yellow colonies of uniform color.

Klebsiella species appear as large mucoid yellow or yellow-white colonies and *Proteus* species appear as translucent blue-grey colonies.

Aeruginosa appears as Green colonies with rough periphery (characteristic color and

Faecalis appears as Small yellow colonies

Saprophyticus and other coagulase-negative *staphylococci* appear as yellow to white colonies.

Gram stain technique

Smears of the bacterial colonies were made and analyzed using the gram staining technique and classified as gram-negative or gram-positive based on the appearance. Gram-positive bacteria stained dark purple and gram-negative bacteria stain red. Following gram staining, gram-negative bacteria such as *E. coli, Klebsiella* spp., *Proteus mirabilis, Pseudomonas spp., Enterococcus faecalis,* and *S. aureus* was confirmed using the following biochemical tests: indole, citrate, and urea. *Pseudomonas aeruginosa* was confirmed using the cytochrome oxidase test. For the gram-positive bacteria, the bacterial colonies were confirmed using the catalase and coagulase test.

Catalase test

This test as described by Cheesborough, 2006 was used to differentiate suspected *Staphylococci species* from *streptococci*. Two drops of 3 % hydrogen peroxide were put onto a sterile glass slide using a sterile Pasteur pipette. A gram-positive yellow colony suspected to be either *Staphylococcus* or *S treptococci* was picked using a sterile wooden applicator stick and placed in the hydrogen peroxide on the glass slide. If bubbling was observed the organism was considered catalase-positive and confirmed as *Staphylococcus sp.* If no bubbling

was observed the organism was considered to be catalase-negative and possibly as *Streptococcus.*

Free coagulase test

Bacterial colonies that were confirmed to be *Staphylococcus spp* were further differentiated into

S. aureus (pathogenic) from *S. albus* (non-pathogenic) using the coagulase test as described by WHO, 2013. A drop of distilled water was placed on a slide and the test colony was added and mixed to make a suspension. A drop of plasma was added to the suspensions and mixed gently. The mixture was observed for clumping after 10 seconds. If clamping was observed, the colony confirmed was as *S. aureus.*

Oxidase test

Bacterial colonies which appeared Green with the rough periphery and suspected to be *P.aeruginosa* were subjected to the oxidase test for species confirmation. A filter paper was placed in a sterile Petri dish and 2 drops of oxidase reagent were added. Using a sterile applicator stick, a colony of the test organism was picked and transferred onto the filter paper where oxidase reagent was placed. After a few seconds, the filter paper was observed for the development of a blue-purple color. If the Blue-purple color was observed the test was considered to be oxidase-positive and the bacterial colony was confirmed as *Pseudomonas spp* (WHO, 2013).

Indole

This test was used in organisms suspected to be *E. coli* and *Klebsiella*. Indole test determines the presence or absence of the tryptophanase, an enzyme that breaks down tryptophan (WHO, 2013). 1ml of 1 % Tryptone broth mixed with one suspected colony and the mixture was incubated for 12 to 18 hours. After incubation Kovac's reagent was added to the Tryptone/sample mixture, the development of the red ring at the interface was read as a positive result for *E.coli* and *Klebsiella* (Colony morphology was used for differentiation of *E.coli* and *Klebsiella*)

Citrate test

Small yellow colonies that tested gram-positive were suspected as *Enterococcus* and subjected to the citrate utilization test. Citrate test was used to test for the presence of citrate which is the sole source of carbon for bacteria (WHO, 2013). An agar slant with a synthetic medium containing small amounts of mineral salts (citrate and ammonium) was used to perform the test (WHO, 2013). Bromothymol blue (pH indicator) was added to the agar slant. Using a sterile wire loop, colonies of suspected *Enterococcus* were inoculated in the citrate agar and then incubated aerobically at 37°C for 12 to 18 hours. The media was then observed for color change. A blue color was interpreted as positive for *Enterococcus spp.*

Antimicrobial susceptibility testing

Each bacterial isolate was subjected to antimicrobial susceptibility testing using the standard Kirby Bauer's disc diffusion method (Bauer *et al.,* 2012). A sterile wire loop was used to obtain a loop full of the sample and the sample was then streaked over the Mueller Hinton agar evenly to form a surface. Antibiotic discs of Ciprofloxacin (5μg), Nalidixic acid (30μg), Nitrofurantoin (30μg), Cotrimoxazole (25μg), Ampicillin (30μg.) and imipenem (30μg), were placed onto each plate and equally spaced out using a disc dispenser. The plates were then incubated at 37°C for 18 hours. The Zones of inhibition were measured using a ruler to the nearest millimeter and compared to the standards provided by the CLSI (2009). The zones were interpreted as susceptible, intermediate or resistant.

Quality control

Standard reference Control strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922.,*Pseudomonas aeruginosa* ATCC 27853 recommended by the National Committee on Clinical Laboratory Standards (2004) was used for the quality control test.

The samples were placed sterile universal container. Isolation and culturing were done under maximum aseptic conditions like Petri dishes; media, wire loops, and forceps were all sterilized before use. The work surfaces were disinfected with 70% alcohol before and after work. All plates were checked for sterility before use by incubating at 37ºC then overnight and checking for any bacterial growths or contamination.

Inclusion criteria

Patients presenting with signs and symptoms of urinary tract infection as indicated by the clinician and not on antibiotic therapy were included in the study.

Exclusion criteria

Patients presenting with signs and symptoms of urinary tract infection but who were on antibiotic therapy were not included in the study.

2 Data analysis

Patients' identification names were not used, numbers and letters were used to label the samples. The information was entered into excel spreadsheets before being analyzed using Microsoft excel. The occurrence of UTIs caused by microbial uropathogens was calculated using Microsoft excel for microbiological analysis.

Ethical clearance

Clearance for the study was obtained from the medical superintendent of Kam Medical and Diagnostic centre.

Written informed consent was obtained from the patients before enrolling them into the study. For patients below 10 years, consent was granted by the parents or guardians. Patients were given unique identification numbers to provide confidentiality. These unique IDs were only known to the researcher and results were issued in person to the patients enrolled in the study.

3 RESULTS

Demographic of patients enrolled in the study

A total of 120 patients were enrolled in the study. Of these 97 were female and 23 were male. The youngest participant enrolled in the study was 1 year old and the oldest participant was 78 years old.

Number of patients enrolled in the study

The occurrence of urinary tract infection based on sex and age group in patients presenting with signs and symptoms at Kam medical and diagnostic center

All patients recruited in the study were confirmed positive for UTI using microbiological culture (Table 2).

Bacterial species isolated from patient urine samples

Bacterial species were isolated and identified using morphological appearance and biochemical tests. Of the bacterial species isolated, four were Gram-negative (*E.coli spp, Klebsiella spp, Proteus spp., Pseudomonas spp*.,) and two were Gram-positive (*Enterococcus spp.* and *Staphylococcus aureus*). *E.coli* was the most common bacteria isolated in 85 of the 120 patients recruited in the study followed by *S.aureus* (13/120), *Klebsiella spp* (11/120) *Pseudomonas spp* and *Enterococcus spp* 4/120, lastly *Proteus* species 3/120. This is shown in table 3

Antibiotic susceptibility pattern of isolated bacteria

The *E. coli* isolates were susceptible to imipenem followed by ciprofloxacin (Cipro), Nitrofurantoin (F), Nalidixic acid (NA), Clotrimazole (CXM), and lastly Ampicillin (Amp). The *Klebsiella spp* isolates in the study were susceptible to imipenem followed by ciprofloxacin, Nitrofurantoin, and lastly Ampicillin; *S. aureus* isolates were susceptible to Cotrimoxazole, imipenem, and Ampicillins. P. aeruginosa was more susceptible to imipenem followed by Clotrimazole Nalidixic acid, Ampicillin, and lastly Nitrofurantoin. *Proteus spp* was more susceptible to imipenem followed by Clotrimazole and lastly Nitrofurantoin. *P. aeruginosa* and *Proteus spp* were mostly susceptible to imipenem followed by Ampicillin, and ciprofloxacin.

4 Discussion

This study recruited 120 patients (97 female and 23 male) presenting with signs and symptoms of UTIs. All study participants (120/120) were confirmed positive for UTI using the microbiological culture of urine samples. The colonies from microbiological culture were subjected to morphological examination and biochemical tests to identify the bacterial species.

The most common bacterial species associated with UTIs isolated from patients in the study patients was *E.coli* in 85/120 patients followed by *S.aureus* (13/120), *Klebsiella spp* (11/12), Pseudomonas spp, and *Enterococcus spp* (4/120), and lastly Proteus spp (3/120). The findings of this study are similar National Institute for Health and Clinical Excellence-NICE (2012) where the common isolates associated with UTIs were *E. coli, S. aureus, Klebsiella spp, Pseudomonas spp, Enterococcus spp.*, and *Proteus spp*. These bacteria belong to the group *Enterobacteria* which typically are considered to be part of the gut flora and due to improper hygiene practices, there is a great risk of cross-contamination of the urinary tract with bacteria from the gastrointestinal (GI) tract. Although these bacteria are non-pathogenic in the GIT when they cross to the urinary tract they become pathogenic and cause discomfort and disease (Gupta *et al.,* 2012). *S.aureus* a normal flora on the skin was also identified as a causative agent of UTI in 13/120 patients because when *S. aureus* crosses from the skin to the uri-

Table 1. Demographic of patients enrolled in the study

Sex	Number of patients enrolled (percentage)
Males	23 (19.2%)
Female	97 (80.8%)
Total	120 (100%)

Table 2. Urinary Tract infection status among the age group that presented atKam Medical and diagnostic centre

Age Group	Number of patients	Number of patients confirmed with UTI
0- 10	7	7/7(100%)
11 20	18	18/18(100%
21 -30	43	43/43(100%)
31-40	21	21/21(100%)
41- 50	13	13/13(100%)
51 – 60	11	11/11(100%)
61 - 70	4	4/4(100%)
71 – 80	3	3/3(100%)
Total	**120**	

Table 3. Etiological bacterial agents isolated from patients confirmed with UTI

Organism Isolated	Frequency	Percentage
E coli	85	70.8 %
Klebsiella spp.	11	9.2 %
S. aureus	13	10. 8 %
Pseudomonas spp.	4	3.3 %
Proteus spp.	3	2.5 %
Enterococcus spp.	4	3.3 %
Total	**120**	**100 %**

Table 4. Isolated Bacterial pathogenssusceptability patterns to antibiotic

Bacterial spp	Susceptibility	Cipro	NA	CXM	MEM	AMP	F
E.coli spp.	S	43	35	35	83	31	40
	R	40	50	48	2	54	10
	I	2	0	2	0	0	29
Pseudomonas spp.	S	0	1	1	4	1	2
	R	4	3	3	0	3	0
	I	0	0	0	0	0	9
Klebsiella spp.	S	6	0	5	10	2	2
	R	5	11	6	1	9	0
	I	0	0	0	0	0	9
S.aureus spp.	S	1	6	9	13	8	3
	R	6	7	4	0	5	5

Table 5. Isolated Bacterial pathogenssusceptability patterns to antibiotic

	I	6	0	0	0	0	5
Proteus spp.	S	2	0	1	3	0	2
	R	1	3	2	0	3	0
	I	0	0	0	0	0	1
Enterococcus spp.	S	3	1	2	4	4	3
	R	1	3	2	0	0	0
	I	0	0	0	0	0	1

nary tract it is capable of causing a urinary tract infection.

UTI's can be caused by one or more bacteria, and in this study, there were no co-infections reported. Typically co-infections are associated with hospital-acquired infection (HAI) and immune-suppressed persons (Abu-Bakr, 2010).

The susceptibility profiles of the 120 bacterial isolates were tested against six commonly used antibiotics. The highest resistance was observed with Nalidixic acid (77/120) followed by Ampicillin (74/120) and Cotrimoxazole (65/120) regardless of bacterial species. The findings of this study are similar to the study by Antwi *et al.,* (2018), who similarly reported the highest resistance with Nalidixic acid (31.1%), Ampicillin (58.6%), and Cotrimoxazole (44.6%). These are first-line antibiotics and are often cheap and readily available hence are often misused and this leads to the emergence and spread of antibiotic resistance. Most of the isolates were susceptible to imipenem (117/120) followed by Nitrofurantoin (59/120) and ciprofloxacin (55/120). Imipenem and Nitrofurantoin are beta-lactam antibiotics that have potent activity against a wide range of Gram-positive and Gram-negative bacteria. Ciprofloxacin a second-generation broad-spectrum antibiotic of the fluoroquinolones class is costly in Uganda. Due to this ciprofloxacin is rarely misused hence it remains effective in the treatment of several bacterial infections. Imipenem a broad-spectrum beta-lactam antibiotic was also effective against most of the bacterial isolates. Imipenem was recently introduced to the Ugandan market and is expensive relative to Uganda's income per capita therefore it is rarely prescribed and hence it is not misused and abused. The finding of this study is comparable to the study by Antwi *et al.*, (2018), who reported the highest susceptibility to imipenem (24.3%) and Nitrofurantoin (21.9%).

For the bacterial species isolated in this study, antibiotic resistance was highest in *E.coli.* The *E.coli* isolates were highly resistant to Nalidixic acid (64.9%) followed by Cotrimoxazole (73.8), Ampicillin (72.9%). However, the isolates were susceptible imipenem (2.5%) and Nitrofurantoin (12.5%).

5 Conclusions and Recommendations.

6 Conclusions

The bacterial pathogens associated with UTIs in this study were *E.coli species, Staphylococcus aureus, Klebsiella, Enterococcus species, Pseudomonas species,* and *Proteus species. E.coli* was the most common isolate followed by *S.aureus, Klebsiella spp, Pseudomonas spp*, and *Enterococcus spp*, and lastly *Proteus spp.*

The highest levels of bacterial resistance were recorded against first-generation antibiotic drugs which include Nalidixic acid (64.2 %), Ampicillin (61.7 %), and clotrimazole (54.2%).

The bacterial isolates in this study were highly susceptible to broad-spectrum, second/ third generation antibiotics drugs which include imipenem, Nitrofurantoin, and ciprofloxacin. Imipenem and Nitrofurantoin was the most potent antibiotic on the organisms tested. All isolates in this study were sensitive to imipenem.

Recommendations

Culture and sensitivity should be encouraged for proper diagnosis and identification of effective drugs to clear infections and hence avoid recurrence of UTIs. This will lead to improved efficacy of treatments and hence lower treatment costs. Furthermore, this will limit the emergence of new antibiotic-resistant bacteria.

Health workers and government officials should educate the public on the effect of self-medication

and mobilize patients to ensure that they have duly completed the prescribed antimicrobial therapy because under dosage selects for resistance and as result will lead to treatment failure, increased treatment costs, and increased mortality due to lack of effective antibiotics to treat infections

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Table 6. LIST OF ABBREVIATIONS AND ACRONYMS

ASBA	Asymptomatic bacteriuria
CDC	Centre of disease control
CFU	Colony forming unit
CLED	Cysteine lactose electrolyte deficient Agar
KMDC	Kam medical and diagnostic Centre
RPM	Revolutions per minute
STI	Sexual transmitted infection
UNIK	University of Kisubi
UTI	Urinary tract infection
WHO	World Health Organization
MDR	Multi Drug Resistance
MR	Methyl red
MRSA	Multi-resistant Staphylococcus aureus
NCCLS	National committee for clinical laboratory standards
IMVIC	Indole test, Methyl red test, Voges-proskaur test and Citrate test

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