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Urogenital pathogens in urine samples of clinically diagnosed urinary tract infected patients in Tanzania: A laboratory based cross-sectional study

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ABSTRACT

Background: Urogenital pathogens such as *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium* and *Trichomonas vaginalis* have been reported to cause pyuria, however they are not routinely cultured from urine samples of patients clinically diagnosed to have urinary tract infections (UTI). In this study, pathogen specific PCR was done to identify the urogenital pathogens in the urine samples among clinically diagnosed UTI patients with negative routine urine culture.

Methods: A cross-sectional study was conducted involving 227 archived urine samples from clinically diagnosed UTI patients with positive leucocyte esterase but negative urine culture results. The urogenital pathogens were detected using pathogen specific singleplex PCR. Data were cleaned and analyzed using STATA version 15.

Results: The median age of patients was 31[IQR 23 – 51] years and the majority (174, 76.7%) were females. Two thirds of patients had history of antibiotic use two weeks prior to recruitment (154, 67.8%). A total of 62(27.3%) urine samples were positive for at least one urogenital pathogen. Of 62 positive samples, 9 had two urogenital pathogens and 1 had three urogenital pathogens. The most predominant urogenital pathogen detected was *Neisseria gonorrhoeae* 25(34.2%) and *Trichomonas vaginalis* 24(32.9%). Being female (aOR 2.4; 95% CI: 1.04 – 5.49; p-value 0.039) and having history of using antibiotics in the past two weeks (aOR 1.9; 95%CI: 1.04 – 3.60; p-value 0.036) was independently associated with the presence of urogenital pathogens.

Conclusion: More than a quarter of female patients with clinical symptoms of UTI and routine urine culture negative results were infected with urogenital pathogens mainly *Neisseria gonorrhoeae* and *Trichomonas vaginalis*. Further research with a larger sample set in a range of settings is required to understand the implications of these finding generally.

Introduction

Background

Sterile pyuria is a presence of greater than ten white blood cells (pus cells) per high power field in culture negative urine samples [1,2]. About 13.9% of women and 2.6% of men globally are diagnosed to have sterile pyuria [1]. Sterile pyuria has been linked with a number of causes including urogenital pathogens (like viruses, fungi, and atypical or fastidious bacteria causing sexual transmitted infections (STIs)), antibiotic use, genitourinary tuberculosis, interstitial cystitis, bladder cancer and

Abbreviations: aOR, adjusted odd ratio; CTAB, cetlytrimethyl ammonium bromide; CUHAS, Catholic University of Health and Allied Sciences; dNTP, deoxynucleotide triphosphate; HATUA, Holistic Approach to Unravel Antibacterial Resistance in East Africa; IQR, interquartile range; MSU, mid-stream urine; NIMR, National Institute for Medical Research; PCR, polymerase chain reaction; STIs, sexual transmitted infections; UTI, urinary tract infections.

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cystitis [1]. The burden of sterile pyuria varies in the population due to a number of reasons including sex, causative agents, use of antibiotics and the frequency of laboratory testing for urogenital pathogens [3]. In the general population, sterile pyuria is positively associated with pregnancy, menopause, untreated urinary tract infections (UTI), and vaginal or penile discharge [2].

Although urogenital pathogens like *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis* have been documented to cause pus cells in urine [4], they are not easily captured with routine urine culture methods [4,5]. This has led to misdiagnosis of these patients and hence delayed appropriate treatment for the underlying conditions predisposing patients to various associated complications [2,6,7]. Additionally, in resource limited settings, UTI has been the common working diagnosis for patients presenting with pyuria in community health facilities [8]. In higher health facilities where quantitative urine culture is conducted, patients with leucocyte positive and culture negative are denied from treatment due to lack of treatment guidelines to cover for this group [9]. Furthermore, treatment guidelines are silent on pyuria of unknown origin, which further delays the management of these patients, subjecting them to the consequences of self-medications, both of which may lead to a worsening of the underlying condition [2].

The pattern of urogenital pathogens among patients with pyuria but urine culture negative results is not clearly established. This study was done to establish the pattern of urogenital pathogens and factors associated with PCR positive urogenital pathogens in urine samples of patient with sterile pyuria.

Methods

Study design settings and participants

A laboratory based cross-sectional study was conducted from May to July 2021 at the Catholic University of Health and Allied Sciences (CUHAS) - Molecular biology research laboratory in Mwanza and National Institute for Medical Research (NIMR) - molecular laboratory Mwanza Centre. The study used clean catch midstream urine samples which was self-collected by the patients with clinical diagnosis of UTI from 10 different health facilities in Mwanza, Kilimanjaro and Mbeya during the Holistic Approach to Unravel Antibacterial Resistance in East Africa (HATUA) project from [10]. In HATUA project urine samples were stored under -80˚c within two hours of collection. The minimum sample size of 138 urine samples was obtained using the Kish Leslie formula (1965) with 10% prevalence of urogenital pathogens obtained among adult patients presenting with UTI symptoms [3]. However, the current study enrolled 227 archived urine samples from patients with a sign and symptoms of UTI like fever, lower abdominal pain, flank/back pain, painful urination, dysuria, hematuria, increased frequency of urination but urine culture negative results with >125 leucocyte/μl, as shown in Fig. 1. Urine leucocyte esterase was measured by urine chemistry dipstick (Siemens Healthineers AG, Pennsylvania, United States).

DNA extraction

DNA extraction was done using cetlytrimethyl ammonium bromide (CTAB) modified phenol – chloroform extraction method as described previously [11,12]. Where 10 mL of urine sample was centrifuged for 10 minutes at 2000 × g and supernatant was discarded. A total of 100μl urine deposit was suspended into 200μl of CTAB and incubated at 65°C for 30 minutes. Then 200μl of chloroform was added to each tube, capped tightly, inverted 10 times to mix and centrifuged at 13,000rpm for 5 minutes at 4°C to separate the phases.

PCR Amplification and detection

Using specific primers, samples were tested for the presence of urogenital pathogens (*C. trachomatis, M. genitalium, N. gonorrhoeae* and *T. vaginalis)* as previously described [2], detailed in Table 1. PCR amplification was performed in a 25μl PCR reaction volume master mix containing 10μl of DNA template for *C. trachomatis, M. genitalium, T. vaginalis* and 5μl of DNA template for *N. gonorrhoeae*, 0.5μl deoxynucleotide triphosphate (dNTP) mix, 2.5μl of 10X standard Taq reaction buffer, 0.5μl of forward and reverse primers, 1μl of Taq polymerase (New England BioLabs) and the remaining volume of 10μl and 15μl Nuclease free water were added up to 25μl reaction. All reactions were performed in a T-Gradient Thermoblock PCR system (Biometra, Goettingen, Germany). The cycling conditions was set at initial denaturation at 95°C for 30 seconds, denaturation at 95°C for 30 seconds, annealing for each primer set targeting 16SrRNA as indicated in Table 1 and elongation at 68°C for 1 minute, and final extension at 72°C for 5 minutes for 30 cycles of PCR [13].

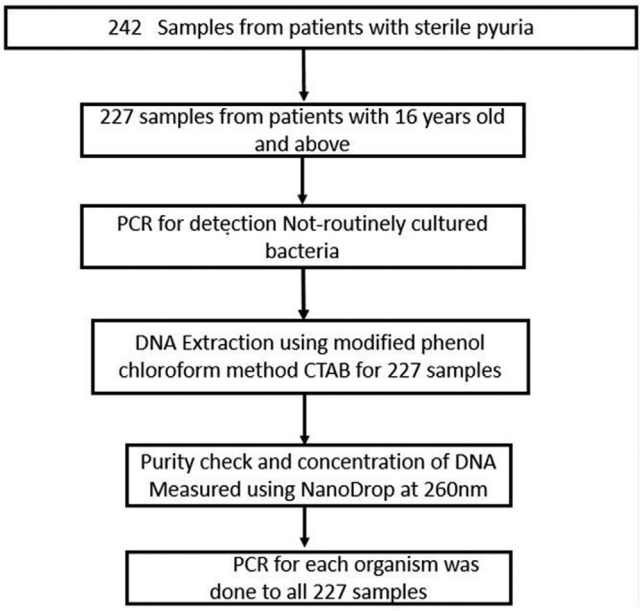

Fig. 1. Flow chart diagram for sample retrieval and processing.

Gel electrophoresis

To enhance interpretation of the band size 8μl of 100bp ladder was included in the electrophoresis of 1.2% gel. Gel electrophoresis was carried at 100V for 120 minutes (IntrogenTM, Carlsbad, CA) and visualization was done by UV-light image reader (Alpha Imager, Alpha Innotech, U.S.A) as indicated in Fig. 2 for *T. vaginalis.*

Quality control

Quality control was performed using in house-typed isolate from cervical swab sample for *C. trachomatis, T. vaginalis, N. gonorrhoeae* and *M. genitalium*.

Data analysis

Data were analyzed using STATA version 15.0. Categorical variables were summarized as proportions while continuous variables were summarized as median (interquartile range). Univariate and multivariate logistic regression models were fitted to determine the associated factors of urogenital infection. Variables with a *p-value* of less than 0.2 on univariate analysis were fitted into the multivariate logistic regression model and their odds ratios and 95% confidence intervals were reported. Variables with the *p-value* of less than 0.05 at multivariate logistic regression model were considered statistically significant.

Table 1
Details of the primer sets and expected amplicon size.

Microorganism	Primer name	Primer Sequence 5'- 3'	Amplicon size (bp)	Ta
C. trachomatis	16SrRNA-S	CGAGTCGGCATCTAATACTAT	402	44°C
	16SrRNA-AS	AAAACGACATTTCTGCCGC		
N. gonorrhoeae	16SrRNA-S	TAGCCAAGCCGCGAGGC	694	49.3°C
	16SrRNA-AS	GGCGCAGACGGTTACTTAAGCAGGA		
M. genitalium	16SrRNA-S	GTAATACATAGGTCGCAAGCGTTATC	771	51.4°C
	16SrRNA-AS	CACCACCTGTCACTCGGTTAACCTC		
T. vaginalis	*β*-tubulin gen-S	ATCGTAAAGAGCTTCGTTATCAATG	89	42°C
	β-tubulingen-AS	GCATGTTGT GCC GGA CAT AAC CAT		

Ta is annealing temperature and bp is the base pair.

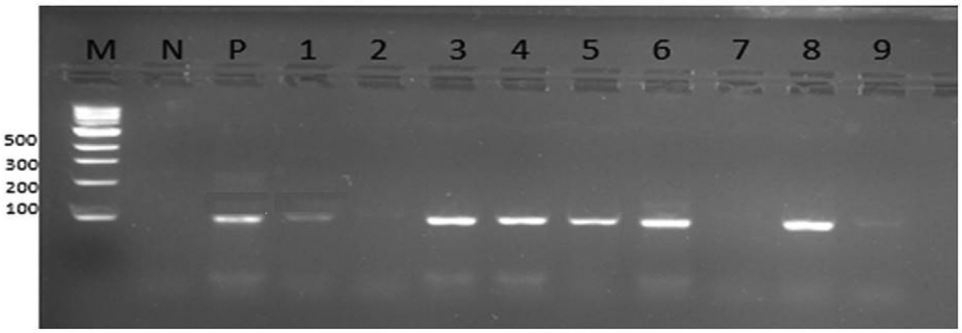

Fig. 2. Representative Images for PCR products electrophoresed on a 1.2% (wt/vol) agarose gel, stained with red safe and photographed under UV light: M=Marker 100bp, P-positive control, N- Negative control, lane number 1,3,4,5,6,8 and 9 are positive for *T. vaginalis* with 89bp.

Results

Descriptive data of study participants

In this study, the majority of samples, 174 (76.7%), were from female patients with a median age of 31 [IQR 23 – 51] years. There were 162 (71.4%) samples from married patients, 127 (55.9%) were from patients who had primary level of education and one third of samples, 73 (32.2%) were from patients who had a history of antibiotic use in the past two weeks (Table 2).

Table 2
Social demographic and clinical characteristics of 227 studied patient.

Variables	Frequency (n)	Percent (%)
Resident		
Urban health facilities	118	52
Rural health facilities	109	48
Sex		
Female	174	76.7
Male	53	23.3
Marital status		
Married	161	70.9
Not married	66	29.1
Antibiotics use in a past 2 weeks		
Yes	73	32.2
No	154	67.8
Urine characteristics		
Clear	67	29.5
Turbid	158	69.6
Blood	2	0.9
Urine pH		
>7.0	162	71.4
<7.0	65	28.6
Leucocyte		
Small	30	13.2
Moderate	118	52.0
Large	79	34.8

Small, moderate and large is when leukocyte is below 75, 120 and above 500 respectively.

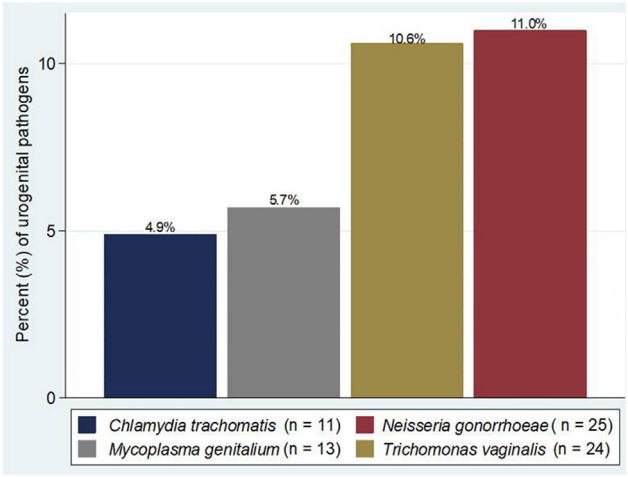

Fig. 3. Pattern of the detected urogenital pathogens.

PCR detection of urogenital pathogens (main results)

Of the 227 samples examined, 62 (27.3%) were positive for at least one urogenital pathogen. Of the 62 positive samples, nine had two urogenital pathogens and one had three, making a total of 73 urogenital pathogens detected. The most frequent urogenital pathogens detected were *Neisseria gonorrhoeae* (*n*=25, 11%) and *Trichomonas vaginalis* (n=24, 10.6), Fig. 3.

Of the 10 samples with multiple urogenital pathogens, 9/10(90%) were from female patients, 7/10 (70%) had moderate number of leucocytes (250 leucocyte/μl), Table 3.

Factors associated with PCR positive urogenital pathogens in urine samples of patient with sterile pyuria

Using univariate logistic regression analysis, being female (OR 2.5; 95% CI: 1.1 – 5.7; *p-value* = 0.026) and having a history of using antibi-

Table 3
The description of the patients with more than one urogenital pathogens.

SN	Age (years)	Sex	Macroscopic appearance	Leukocyte	Antibiotic use	Detected pathogen(s)
1.	15-35	F	Turbid	Moderate	Yes	*T. vaginalis* and *M. genitalium*
3.	15-35	F	Turbid	Moderate	Yes	*T. vaginalis* and *M. genitalium*
4.	15-35	F	Turbid	Moderate	Yes	*T. vaginalis* and *M. genitalium*
2.	36-55	M	Turbid	Moderate	Yes	*T. vaginalis* and *C. trachomatis*
6.	Above 55	F	Turbid	Moderate	Yes	*T. vaginalis* and *N. gonorrhoeae*
7.	36-55	F	Turbid	Moderate	No	*T. vaginalis* and *N. gonorrhoeae*
5.	15-35	F	Turbid	Large	Yes	*C. trachomatis* and *M. genitalium*
8.	36-45	F	Turbid	Large	Yes	*C. trachomatis* and *N. gonorrhoeae*
9.	15-35	F	Turbid	large	Yes	*C. trachomatis* and *N. gonorrhoeae*
10.	15-35	F	Turbid	Moderate	Yes	*C. trachomatis, T. vaginalis* and *M. genitalium*

Table 4
Factors associated with PCR positive urogenital pathogen.

Variable	PCR positive for urogenital infection N (%)	Univariate OR [95%CI]	*P- value*	Multivariate OR [95%CI]	*P- value*
Age (years)	31 [23 - 51]	1.0 [0.9 – 1.0]	0.156	1.0 [0.9 – 1.0]	0.961
Sex					
Male (53)	8 (15.1	1.0			
Female (174	54 (31.0	2.5 [1.1 – 5.7]	0.026	2.4 [1.0 – 5.8]	0.039
Residence					
Rural (109)	27 (22.7)	1.0			
Urban (118)	35 (32.4)	1.6 [0.9 – 2.9]	0.102	2.0 [0.9 – 3.1]	0.086
Marital status					
Not married (41)	17 (26.2)	1.0			
Married (113)	45 (27.8)	0.9 [0.5 – 1.8]	0.804	-	-
Antibiotic use in a past 2 weeks					
No (154)	35 (22.7)	1.0			
Yes (73)	27 (37.0)	2.0 [1.1 – 3.7]	0.026	2.0 [1.0 – 3.6]	0.036
Urine characteristics					
Clear (67)	13 (19.4)	1.0			
Turbid (158)	48 (30.4)	2.0 [0.9 – 3.6]	0.093	-	*
Blood (2)	1 (50.0)	4.2 [0.2 – 70.9]	0.325	-	-
Urine pH					
≥7.0 (162)	17 (26.2)	1.0			
< 7.0 (65)	45 (27.8)	0.9 [0.5 – 1.8]	0.804	-	-
Leucocyte					
Small (30)	8 (26.7)	1.0			
Moderate (118)	31 (26.3)	1.1 [0.4 – 2.9]	0.800	-	-
Large (79)	23 (29.1)	1.0 [0.4 – 2.4]	0.965	-	-

* Urine characteristics had a p-value of <0.2 (0.093) but was not included in multivariate analysis due to multicollinearity with sex.

otics in the past two weeks (OR 2.0; 95% CI: 1.1 – 3.7; *p-value* = 0.026) were factors associated with PCR positive urogenital pathogens (4). On stepwise multivariate logistic regression analysis being female (OR 2.4; 95%CI 1.0-5.8; P=0.039) and have history of using antibiotic two weeks prior to recruitment to the study (OR 2.0; 95% CI 1.0-3.6; P=0.036) indecently predicted the PCR positive urogenital pathogens (Table 4).

Discussion

Sterile pyuria in patients with sign and symptoms of UTI are of public health importance as it represents a hidden group with STI. Poor knowledge of the patients on differences of UTI and STI and stigma within the community act as barriers to treatment of STI considering that UTI is socially accepted and the treatment options is available [14,15]. Previous study conducted in Arusha Tanzania reported the seroprevalence of STI causing pathogens like *C. trachomatis, N. gonorrhoeae* and *Treponema pallidum* to be above 50% [16]. Testing for urogenital pathogens such as *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis* in urine samples of patients with pyuria and negative urine culture, is not routinely done due to lack of treatment guidelines and advanced diagnostic tools e.g., molecular techniques to detect these urogenital pathogens [4]. The current study is among the first studies to investigate the pattern of urogenital pathogens and associated factors from urine samples of patients with pyuria but negative results of urine culture for UTI diagnosis in the study setting. In this report we have documented the importance of the four urogenital pathogens (*C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis*) as causes of apparently sterile pyuria as detected by molecular techniques.

In the current study, 27.3% of urine samples were positive for at least one of the four urogenital pathogens tested. The prevalence in this study is relatively higher than the 19.5% reported among adult's patients presenting with urinary tract infection symptoms in primary health care clinic in Zimbabwe [3] and 20.8% reported from Kilifi, Kenya among pregnant women [17]. The variations in findings could be explained by the fact that the current study used urine samples from patients with clinical diagnosis of UTI and positive urine leucocytes (pyuria) while the previous study in Kilifi urine samples from pregnant women attending antenatal clinic not necessarily having pyuria [17]. The prevalence in this study is similar to the previous studies which reported a prevalence of 23.9% among adult's women in Metro Health Medical Center-USA presenting with sign of UTI [8,18].

In this study the most common urogenital pathogen was *N. gonorrhoeae* with a prevalence of 11.0%. The prevalence of *N. gonorrhoeae* is similar to the Zimbabwe study which reported a prevalence of 8.7% among adult patients presenting with urinary tract infection symptoms in primary health care clinic [3]. Moreover, the prevalence in this study is higher than that reported (6.7%) among pregnant adolescent girls in the city of Mwanza [19] and the 3.3% reported for reproductive aged women in sub-Saharan Africa [20]. Sampling differences may partly explain the variation in prevalence of urogenital pathogens observed. In

this current study, we enrolled patients with pyuria while in the previous studies in Mwanza and sub-Saharan Africa they enrolled participants without pyuria [19,20].

The prevalence of *T. vaginalis* in this study was 10.6%. The finding is comparable to 12.4%, 8.0% and 7.4% reported among pregnant adolescent girls in Mwanza Tanzania [19], adults presenting with UTI like symptoms in Harare, Zimbabwe [3] and pregnant women in Kilifi Kenya [17], respectively. However, the prevalence in the current study is slightly lower than the previous study which reported the prevalence of 16.5% among adult's women presenting at emergency department [8]. This variation in prevalence could be attributed to the difference in the study participant characteristics where previous study enrolled adult women with majority of them harbored *Trichomonas vaginalis*. This has also been proven in the current study where *Trichomonas vaginalis* was among the most common detected pathogen.

In the current study *M. genitalium* had prevalence of 5.7% similar to the previous study in Kenyan pregnant women aged between 18 and 44 years, which reported the prevalence of 6.3% [21]. Also comparable to the previous study which reported the prevalence of 3.2% among HIV patients in Kilimanjaro [22]. This can be due to the fact that *M. genitalium* is rarely associated with urogenital infections compared to the other pathogens like *N. gonorrhoeae* and *C. trachomatis.*

The prevalence of *C. trachomatis* in this study was 4.9% lower compared to the previous studies which reported the prevalence of 11.4% among pregnant adolescent girls and 36.2% among infertile women in the same setting, Mwanza [19,23]. The higher prevalence of *C. trachomatis* among infertile women may be attributable by the type of sample collected, endo-cervical swabs, whereby in the current study we collected mid-stream urine (MSU) samples. Studies have reported that endo-cervical swabs have high yields of *C. trachomatis* than MSU samples [24–26]. Although endo-cervical swab is not a convenient sample to the majority of the patients. Therefore, introduction of a convenient sample such as MSU is required.

The findings from this study shows about 1 in every 4 patients with apparent sterile pyuria is infected with urogenital pathogens not detected by the urine culture media used routinely in microbiology laboratory, which is likely to lead to report a false negative result. Failure to treat these patients will lead to return to the health care center and this has an extra burden/cost to the healthcare centres and the patients. Untreated STIs can be further transmitted to others and may lead to further complications in other individuals and may promote patients seeking over the counter medication. The use of over-the-counter medications is highly associated with antimicrobial resistance development in individual patients and community at large due to inappropriate use of antibiotics [27]. The findings cemented on the need of considering urogenital pathogens especially when leucocyte is positive while urine culture results is negative.

In this study 16.2% of the samples had coinfection of two or three urogenital pathogens. The findings are comparable to the previous study among adolescence pregnant girls in Mwanza city which reported 15% [19]. The prevalence of coinfection in the current study is relatively higher than 2.6% reported among adults presenting with UTI like symptoms [3]. In the current study, 70% of those with multiple infections were positive for *T. vaginalis* and 50% were positive for *M. genitalium* this may be due to the close symbiotic relationship among these pathogens [28].

The present study shows that female patients are more likely to be infected with urogenital pathogen, similar to the previous study which showed women are relatively highly infected with urogenital pathogens like *N. gonorrhoeae, T. vaginalis, M. genitalium* and *C. trachomatis* [29,30]. *T. vaginalis* infection among women has been associated with the shortness of urethra and its anatomical position under labia which create warm and moist environment for its survival [31]. Furthermore, the observation can partly be explained by the fact that women delay treatment due to late onset of symptoms and dependence on male partner for financial coverage of health care costs [32,33]. Moreover, the stratified squamous epithelium lining of the female urogenital tract is resistant to urogenital pathogens which subject them to asymptomatic infection leading to delayed treatment [34].

In the current study, use of antibiotics past two weeks was found to be associated with infection of the urogenital pathogens. This has also been observed previously in the study among adult patients presenting with UTI symptoms in primary health care clinics in Zimbabwe [3]. This could be due to collision in clinical signs and symptoms with UTI which lead to lower suspicious index among clinicians for STI pathogens [3]. When UTI is suspected in most cases urine culture becomes negative leading to denial for treatment. Consequently, this subjects patients to seek for self-medication with antimicrobials which does not clear the urogenital pathogens and predisposing to chronic infection and emergence of antimicrobial resistance. The findings from this study also underline the need for accurate, rapid tests for routine screening of urogenital pathogens in our setting and development of treatment guidelines as previously documented [3,30] especially for patients with pyuria but urine culture for UTI diagnosis negative.

Study limitation

In this study frozen mid-stream urine samples were used instead of first fresh voided urine which is recommended for detection of urogenital pathogens, this might lead to underestimation of the prevalence of *N. gonorrhoeae, T. vaginalis, M. genitalium* and *C. trachomatis*. Furthermore, the study did not document the non-infectious causes of sterile pyuria such as interstitial nephritis, nephrolithiasis, uroepithelial tumor, autoimmune diseases such as SLE, contamination of urine sample with vaginal leukocytes which might dilute the reported findings.

Conclusion

Our data demonstrate the importance of non-cultivated urinary pathogens *Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium* and *Chlamydia trachomatis* as a cause of sterile pyuria. Clinician should consider screening for these pathogens among patients at high risk. Further research with a larger sample set in a range of settings is required to understand the implications of these finding generally.

Declarations

Ethics approval and consent to participate

The protocol to conduct this study was approved by the joint CUHAS/BMC research ethics with certificate number CREC/4712021. The ethic committee is formed by members of the two sister institutions that's Catholic University of Health and Allied Science and Bugando Medical Centre all located in Mwanza, Tanzania. The study use data generated from HATUA study that receive all ethical permission [10]. All research participants provided written informed consent before recruitment to the project.

All data were treated as confidential.

Consent to publish

None applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interest

None declared.

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Author contribution

BM, MFM, BK, BO and SEM designed the work. BM, MFM, DNM, VS and MMM performed laboratory investigations and results interpretations. MFM, ETK, JRM, SG and SEM analyzed and interpreted the data. BM and MFM wrote the first draft of the manuscript which was critically reviewed by KK, WS, SG, AMB, AS and MH. All authors read and approved the final version of the manuscript.

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