Asymptomatic Bacteriuria and Candida colonization among Pregnant Women in a District Hospital in Eastern Uganda.

Abstract

Background: Urinary tract infection (UTI) is the most frequently encountered infection worldwide besides those that are of intestinal origin. It is the most common reason for which antimicrobials are prescribed in pregnancy Worldwide. A greater proportion of females is affected, to as high as fourteen times more than their male counterparts. This study aimed to determine the prevalence of asymptomatic bacteriuria, Candida colonization and antimicrobial susceptibility patterns among pregnant women attending antenatal in a District Hospital in Eastern Uganda.

Materials and methods: A descriptive cross sectional study was conducted in which pregnant mothers who had come for routine antenatal care were counseled and their consents obtained before taking off urine samples for laboratory diagnosis. For those samples found to have pus cells, culture and sensitivity test was done in Busitema University Microbiology Laboratory to identify the organisms and determine susceptibility to particular antibiotics and antifungal agents.

Results: Both gram positive and gram negative bacteria were isolated with gram positives (41.7%) showing higher frequency than the gram negatives (29.6%) while the remaining percentage was accounted for by Candida spp (28.8%).Gram negative isolates were more sensitive to meropenem (100%), and ciprofloxacin (93.8%) but less sensitive to trimethoprim/sulphurmethoxazole (20%), Ceftazidime (7%), and Cefepem (6%). Gram positive isolates were more sensitive to vancomycin (100%), meropenem (87%) and linezolid (88.1%) but less sensitive to Cefotaxime (31%) and Trimethoprim/sulphurmethoxazole (14%). All bacteria isolated in this study were resistant to three or more classes of antibiotics therefore referred to as multi-drug resistant (MDR). All Candida isolates were susceptible to Econazole and Nystatin whereas all isolates were resistant to Griseofulvin. High susceptibility to Clotrimazole (95%-100%) and Ketoconazole (81%-100%) was noted among all isolates whereas high resistance to Amphotericin B, Fluconazole and Voriconazole was noted.

Conclusion: Asymptomatic bacteriuria is highly prevalent in Butaleja district with many of the bacteria isolated exhibiting resistance to the commonly used antibiotics. Antifungal resistance was common in this study. *In vitro* antifungal susceptibility testing is important in

guiding therapeutic decision making, as an aid in drug development studies, and as a means of tracking the development of antifungal resistance in epidemiological studies.

Introduction

Background

Urinary tract infection (UTI) is the most frequently encountered infection worldwide besides those that are of intestinal origin (1). Globally, it has been estimated that about 150 million people are diagnosed with a UTI per year (2). Urinary Tract infections can be classified on the basis of presentation that can be lower UTI (urethra and urinary bladder affected) or upper urinary tract (kidneys affected) or whether a pregnant woman presents with (symptomatic) or without symptoms (asymptomatic). Although UTIs affect individuals of all ages, the females are fourteen times likely to be affected than men and 50-60% will suffer an episode of UTI once in their life time since incidence increases by 10% for every decade of life above 20yrs of age and this is due to their shorter urethra and close proximity of the anus to the genital area. Amongst women too, some groups are more susceptible than others such as the sexually active, elderly and pregnant women. These infections affect individuals of all age groups but show greater occurrence in particular groups like women that are sexually active, the pregnant and the elderly. A greater proportion of females is affected, to as high as fourteen times more than their male counterparts (3) and 50-60% of women will suffer from a UTI at least once in their lifetime (4).

UTIs are more prevalent in pregnancy due to the physiological changes of pregnancy. An estimated 25% of the pregnant women develop UTI in developing countries and it is the most common cause of admission in obstetric wards. This figure is much lower in developed countries (2-10%) (5).

The prevalence of UTI among pregnant women in African countries revolves around 14% as shown by researches carried out in Sudan (14.0%), Tanzania (14.6%), and Ethiopia (11.6%). These figures do not regard the women's age, parity and gestational age. However, studies point out *E. coli* as the commonest isolated organism with multi resistance toward different antibiotics (6).

Asymptomatic bacteriuria is common among ante-natal mothers in Uganda (7). Asymptomatic bacteriuria in pregnancy is more likely to cause adverse effects that could lead to maternal and perinatal morbidity and mortality. Since screening and treatment has been shown to be beneficial for both maternal and fetal wellbeing especially where prevalence

exceeds 2%, treatment reduces the prevalence of pyelonephritis by 75%, it is important to know the dominant uropathogens and the sensitivity patterns. This study aimed to determine theprevalence of asymptomatic Bacteriuria, Candida colonization and antimicrobial susceptibility patterns among Pregnant Women in a District Hospital in Eastern Uganda

MATERIALS AND METHODS

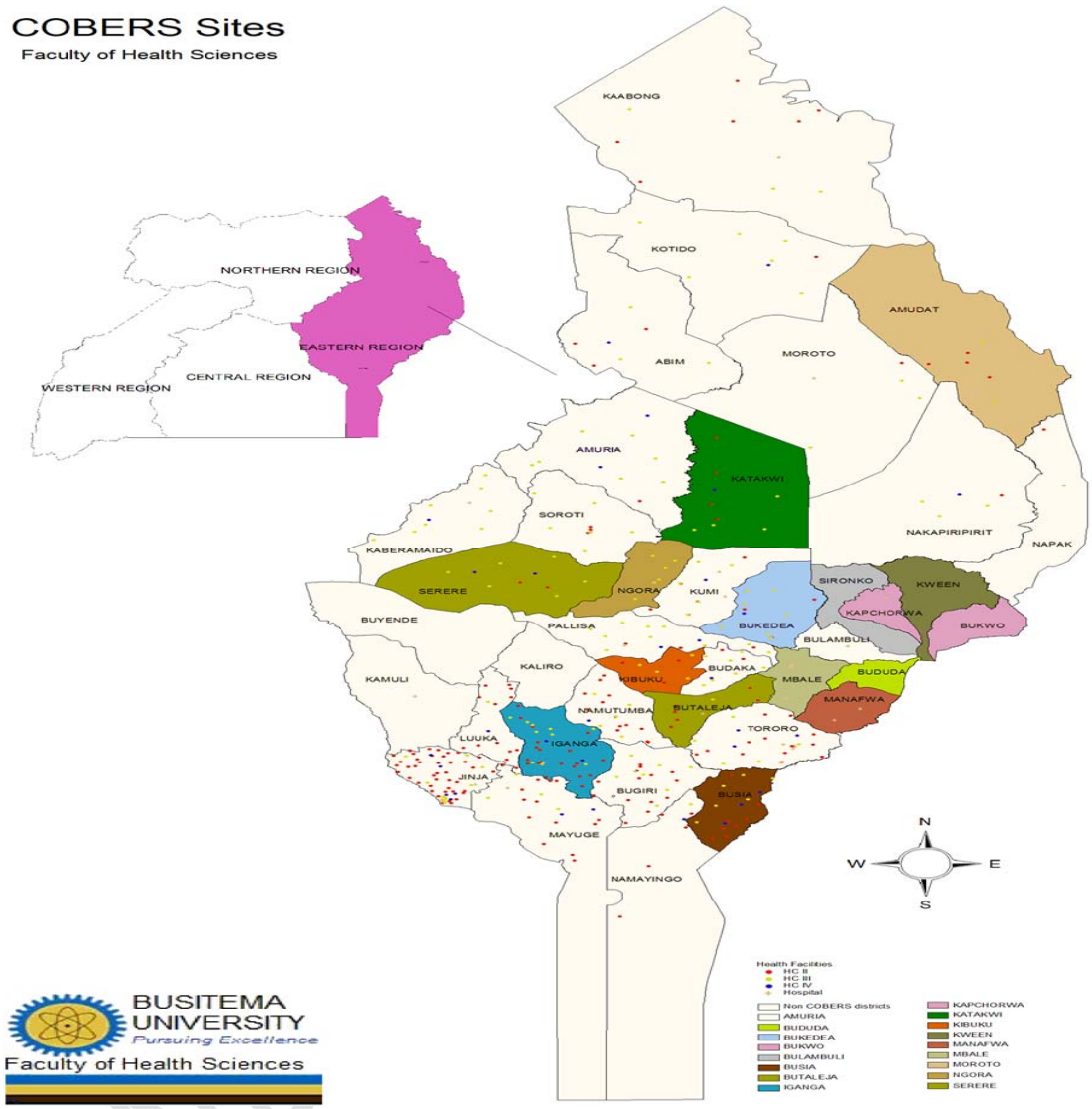
Study design.

A descriptive cross sectional study was conducted in which asymptomatic pregnant mothers who had come for routine antenatal care were counseled and their consents obtained before taking off urine samples for laboratory diagnosis. For those samples found to have pus cells, culture and sensitivity test was done in the microbiology laboratory of Busitema University Faculty of Health Sciences to identify the organisms and determine susceptibility to particular antibiotics.

Study area

This study was conducted at Busolwe Hospital; the district hospital of Butaleja district, which serves the districts of Butaleja, Namutumba, Budaka and some people from Tororo. Busolwe hospital is one of the Community Based Education, Research and Services (COBERS) sites of Busitema University Faculty of Health Sciences.

Fig. 1: Map of Uganda showing the distribution of COBERS sites in Eastern Uganda



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Study population

The study involved testing asymptomatic mothers attending antenatal services in Busolwe Hospital maternity department during the month of April, 2017. The population in this area is predominantly Banyole (85%), a Bantu tribe whose language is called Lunyole. Other tribes in the district (15%) include the Japadhola, Bagisu, Basoga, Iteso, Karimojong and Bagwere; making a total population of 245,873 as per the census report 2014.

Sampling techniques and sample collection

The mothers were sampled consecutively as they came to the antenatal clinic to give equal chance for participation in the study. The sample collection procedure was explained to all mothers and written informed consent was obtained from the mothers who accepted to participate in the study. The mother was availed with a labeled urine sample container, clean cotton, and water after explaining the procedure for sample collection to collect mid-stream urine.

Laboratory procedures

Urine dipsticks were used to screen for presence of pus cells in urine indicated by leucocyte positivity. Samples that were positive for leucocytes were transported to Busitema University microbiology laboratory for culture and sensitivity testing. Quantitative urine cultures were done in the laboratory, that is; Urine was gently shaken, tipped to slant and with a sterile pipette, 100µml of urine was transferred onto the CLED, MacConkey agar and chocolate agar. The urine was spread evenly across the plates with a sterile glass rod and allowed to soak in. The plates were incubated at 37°C for 18-24hrs and read for growth. Plates which showed no growth at 24hrs were incubated for another 24hrs to allow for detection of slow growers. The plates that were negative after 48 hours were reported as no significant growth. Growth of more than three colony types typically indicated contamination. Bacterial counts of ≥10□Cfu/ml were indicative of an infection and counts below 10^4 Cfu/ml was taken to indicate contamination and further tests would not be performed unless the organisms were Enterobacteriaceae.

Morphological and biochemical identification of the bacteria

The bacteria was identified by colony morphology on culture plates and the microscopic appearance on Gram stain. Biochemical identification of bacterial isolates was done using standard methods (8-10)Briefly, the tests employed were catalase, free and bound coagulase, DNAase, Mannital Salt Agar (Oxoid),oxidase, motility test using motility indole urea medium, reactions on triple sugar iron agar (TSI), urease, nitrate reduction, indole, methyl red (MR), Voges Proskauer (VP), citrate utilization, lysine decarboxylase, and sugar fermentation tests.

Drug susceptibility testing (DST) was performed using the Kirby-Bour disc diffusion method on Mueller Hinton Agar (MHA) (Oxoid, Hampshire, United Kingdom) plates as

recommended by the clinical laboratory standards institute (11). Bacterial colonies were emulsified into sterile saline and the turbidity of the suspension adjusted to the 0.5 McFarland standard.

Candida species were identified by Gram stain, colony morphology on Sabouraud Dextrose Agar (SDA) and color changes on Candida chromogenic Agar. Susceptibility to the antifungals was done on SDA and interpreted using the CLSI guidelines (11).

Data management and analysis.

Findings wereentered in excel, exported to STATA v14 for analysis and presented in form of tables and figures.

Ethical Considerations.

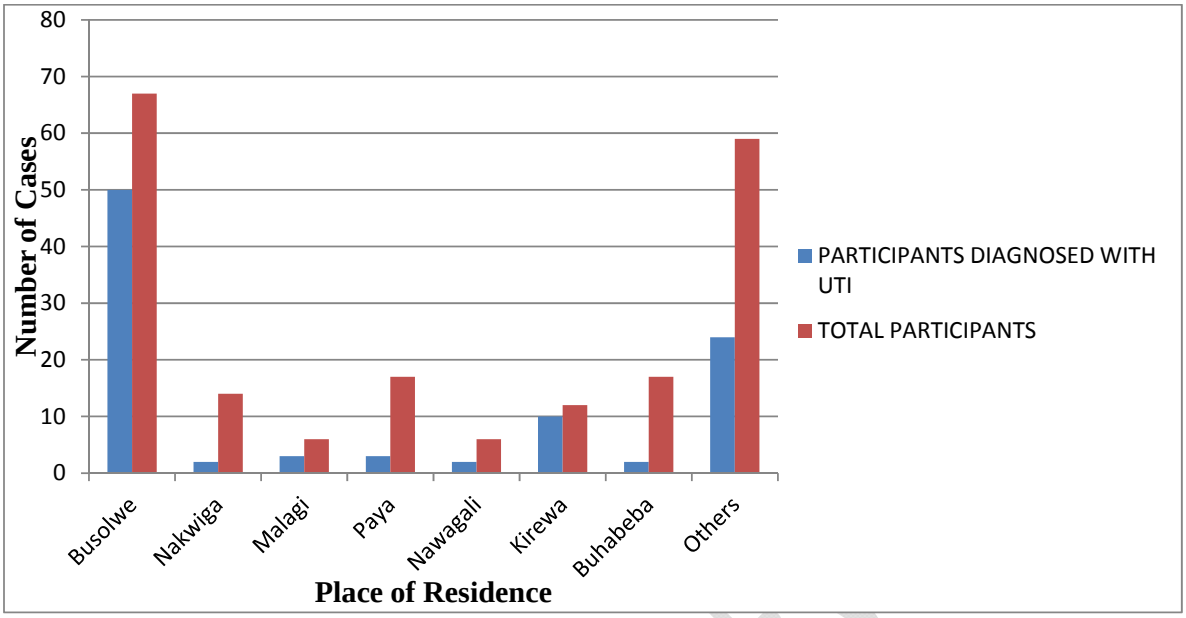
Ethical clearance was obtained from Mbale Regional Referral Hospital Research and Ethics committee, the District Health Office, Medical superintendent and the administration of Busolwe Hospital gave administrative clearance before commencement of the study. Participating mothers consented to participate in the study and research information was accessible to only the research team. Serial numbers were used instead of names to maintain confidentiality.

RESULTS

Demographics characteristics and the prevalence of asymptomatic bacteriuria

The prevalence of asymptomatic bacteriuria among pregnant women in Butaleja district was 32.8%. A majority of the pregnant women with asymptomatic bacteriuria were between the ages of 18-30 (77.1%) followed by those above 35 years (16.7%) and the least were those below 18 years (6.2%). Asymptomatic bacteriuria was most prevalent among the prime gravidae (44.8%) and least prevalent among the grand multi-gravidae (14.6%). Busolwe accounted for the largest number of participants in this study with 67 participants, 50 of whom showed growths on culture. The rest of the participants were from a number other parishes (Fig.2).

Fig.2: Distribution of asymptomatic bacteriuria by place of residence



Microbial etiology of asymptomatic bacteriuria

Both gram positive and gram negative bacteria were isolated with gram positives (41.7%) showing higher frequency than the gram negatives (29.6%) while the remaining percentage was accounted for by Candida spp (28.8%).*Staphylococcus aureus* (35.6%), was the most commonly isolated among the gram positive cocci whereas *Escherichia coli* (12.9%) was the most isolated gram negative bacilli.

Sensitivity to antibiotics

Antibiotic susceptibility to the commonly used antibiotics used in our setting was carried out using the Kirby-Bour disc diffusion method on Mueller-Hinton agar. The antibiotic discs used included; Amoxicillin/clavulanic acid (20/10µg), Ciprofloxacin (5µg), Gentamicin (10µg), Meropenem (10µg), Chloramphenical (30µg), Trimethoprim/Sulphurmethoxazole (1.25/23.75µg), Cefotaxime (30µg), Cefepime (30µg), Ceftazidime (30µg), Clindamycin (2µg), Vancomycin (30µg), Nitrofurantoin (300µg) and Linezolid (30µg).

Gram negative isolates were more sensitive to meropenem (100%), and ciprofloxacin (93.8%) but less sensitive to trimethoprim/sulphurmethoxazole (20%), Ceftazidime (7%), and Cefepem (6%). Gram positive isolates were more sensitive to vancomycin 100%, meropenem 87% and linezolid 88.1% but less sensitive to Cefotaxime (31%) and Trimethoprim/sulphurmethoxazole (14%).All bacteria isolated in this study were resistant to

three or more classes of antibiotics therefore referred to as multi-drug resistant (MDR) (Table 1).

Sensitivity to the antifungals

Commonly used antifungal agents in our setting were used to determine the antifungal susceptibility pattern using the Kirby-Bour disc diffusion method on Sabourauds Dextrose Agar (SDA). The antifungal agents used were Voriconazole (1µg), Ketoconazole (10µg), Amphotericin B (20u), Griseofulvin (10µg), Itraconazole (10µg), Fluconazole (25µg), Clotrimazole (10µg), Econazole (50µg), and Nystatin (100µg).

Candida albicans, Candida parasilosis and *Candida glabrata* isolated were subjected to antifungal susceptibility to the commonly used antifungals. All Candida isolates were susceptible to Econazole and Nystatin whereas all isolates were resistant to Griseofulvin. High susceptibility to Clotrimazole (95%-100%) and Ketoconazole (81%-100%) was noted among all isolates whereas high resistance to Amphotericin B, Fluconazole and Voriconazole was noted.

Type of isolate	Antimicrobial resistance profile of the isolates, frequency (%)										
Gram negative rods	**Profile**	**AMC**	**CIP**	**C**	**CN**	**MEM**	**SXT**	**CTX**	**FEP**	**CAZ**	**MDR**
E. coli (n=17)	**S**	0(0)	14(82)	5(29)	8(47)	16(94)	3(18)	12(71)	0(0)	1(6)	
	R	17(100)	3(18)	12(71)	9(52)	1(6)	14(82)	5(29)	17(100)	16(94)	17(100)
Klebsiella spp	**S**	0(0)	8(80)	1(10)	6(60)	10(100)	2(20)	5(50)	1(10)	0(0.0)	
(n=10)	**R**	10(100)	2(20)	9(90)	4(40)	0(0)	8(80)	5(50)	9(90)	10(100)	10(100)
Enterobacter spp	**S**	5(42)	9(75)	8(67)	5(42)	12(100)	4(33)	5(42)	1(8)	2(17)	
(n=12)	**R**	7(58)	3(25)	4(33)	7(58)	0(0)	6(50)	7(58)	11(92)	10(83)	12(100)

Type of isolate	Antimicrobial resistance profile of the isolates, frequency (%)												
Gram Positive cocci	**Profile**	**CIP**	**C**	**CN**	**DA**	**MEM**	**SXT**	**CTX**	**P**	**VA**	**F**	**LNZ**	**MDR**
S. aureus (n=47)	**S**	15(32)	29(62)	39(83)	40(85)	45(96)	13(28)	17(36)	25(53)	47(100)	45(96)	45(96)	
	R	32(68)	18(38)	8(17)	7(15)	2(4)	34(72)	30(64)	22(47)	0(0)	2(4)	2(4)	7(100)
Enterococcus spp	**S**	7(88)	3(38)	2(25)	3(38)	8(100)	0(0)	2(25)	7(88)	8(100)	7(88)	7(88)	
(n=8)	**R**	1(13)	5(63)	6(75)	5(63)	0(0)	8(100)	6(75)	1(13)	0(0)	1(13)	1(13)	8(100)

Table 1: Antimicrobial susceptibility pattern of bacterial isolates from asymptomatic bacteriuria among pregnant women in Butaleja District.
Key:S- Sensitive, **R**- Resistant, **AMC**- Amoxicillin/Clavulanic acid, **CIP**-Ciprofloxacin, **C**-Chloramphenical, **CN**-Gentamycin, **DA**-Clindamycin, **MEP**-Meropenem, **SXT**- Sulfamethoxazole/Trimethoprim, **CTX**-Cefotaxime, **VA**-Vancomycin, **F**-Nitrofurantoin, **LNZ**- Linezolid, **P**-Penicillin, **FEP**-Cefepem, **CAZ**-Ceftazidime**.**

Isolate	*Antifungal susceptibility pattern, frequency (%)*									
	Profile	**VOR**	**KTC**	**AMB**	**GRS**	**ITR**	**FLU**	**CLT**	**ECO**	**NY**
C. albicans	**S**	13(62)	17(81)	13(63)	0(0)	15(71)	13(62)	20(95)	21(100)	21(100)
	R	8(38)	4(19)	8(38)	21(100)	6(29)	8(38)	1(5)	0(0)	0(0)
C. Parasilosis	**S**	4(100)	4(100)	1(25)	0(0)	1(25)	2(50)	4(100)	4(100)	4(100)
	R	0(0)	0(0)	3(75)	4(100)	3(75)	2(50)	0(0)	0(0)	0(0)
C. glabrata	**S**	0(0)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)
	R	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)

Table. 2: Antifungal susceptibility patterns of Candida Species Isolated

Key: VOR-Voriconazole, KTC-Ketoconazole, AMB-Amphotericin B, GRS-Griseofulvin, ITR-Itraconazole, FLU-Fluconazole, CLT-clotrimazole, ECO-Econazole, NY-Nystatin

DISCUSSION

Prevalence of asymptomatic bacteriuria in Butaleja District

The prevalence of asymptomatic bacteriuria among pregnant women in Butaleja district was 32.8%.This is higher than the 13.3% reported in a similar study in Uganda (7). It's also higher than the specified split prevalence for symptomatic and asymptomatic bacteriuria recorded in Mwanza, Tanzania at 17.9% and 13.0% respectively (12), 21.5% in Nairobi, Kenya (13) and elsewhere (14, 15). There has generally been a varying prevalence of asymptomatic bacteriuria across Africa though the high prevalence in this study may be attributed to the observed low socioeconomic status in Busolwe district. Other studies elsewhere have indicated that low social economic status is associated with high prevalence of asymptomatic bacteriuria (16) Some studies in Africa have similarly documented a high prevalence of asymptomatic bacteriuria among pregnant women (17) with Benin City recording a prevalence of over 50% (18).

A majority of the pregnant women with asymptomatic bacteriuria were between the ages of 18-30 (77.1%) and the least were those below 18 years (6.2%). Similar studies have associated age group 18-34 with a high prevalence of asymptomatic bacteriuria (14). The association of the above age range with asymptomatic bacteriuria may be due to high rate of sexual activity expected in this age range. Some studies have however not associated the prevalence of asymptomatic bacteriuria with age (13, 19).

Asymptomatic bacteriuria was most prevalent among the prime gravidae (44.8%) and least prevalent among the grand multi-gravidae (14.6%), though there was no association between gravidity and asymptomatic bacteriuria. Another study in Nigeria did not find a significant association between gravidity and asymptomatic bacteriuria (19). Busolwe accounted for the largest number of participants in this study with 67 participants, 50 of whom showed growths on culture. The rest of the participants were from a number other parishes. The large number of participant from Busolwe sub-county could be due to the location of Busolwe hospital and this paints a picture that there is a lot of undiagnosed asymptomatic bacteriuria from communities far away from health facilities.

Microbial etiology of symptomatic bacteriuria in Butaleja District.

Staphylococcus aureus (35.6%), was the most commonly isolated among the gram positive cocci whereas *Escherichia coli* (12.9%) was the most isolated gram negative bacilli. Whereas many studies in Uganda and elsewhere agree with the predominance of *E. coli* in Urinary

tract infections, (7, 15, 16, 18, 20-23). Other studies have demonstrated different bacterial species as dominant uropathogen like Klebsiella (24). Similar studies have demonstrated the rise in numbers of gram positive cocci in urinary tract infections for example, a study in North East Ethiopia, Gram-positive isolates were more prevalent (n=37/58: 63.8%) than Gram-negative bacteria (n=21/58; 36.2%) and the most commonly isolated bacteria were *S. aureus* (n=18; 31%) and *E. coli* (n=18; 31%) (25). Other studies have also demonstrated *S. aureus* as the most commonly isolated uropathogen ((19, 26). *Enterococcus spp* was the commonly isolated organism in a study in Ghana, followed by *Proteus mirabilis* and then *Escherichia coli* (14).

Co-infection with Candida was found in 28.8% of the asymptomatic bacteriuria cases with *Candida albicans* being the dominantly isolated yeast. The other Candida species isolated were *Candida parasilosis* and *Candida glabrata.* Candiduria has also been documented in coexistence with bacteriuria in other studies (16, 27). The co-existence of bacteriuria and candiduria may lead to works effects in pregnant women.

Sensitivity of the antimicrobials

Antibacterial susceptibility

While the range of bacteria causing asymptomatic bacteriuria is relatively constant, the susceptibility of the same to the antibiotics varies a lot by geographical location. Gram negative isolates in this study were more sensitive to meropenem, and ciprofloxacin with sensitivity patterns ranging from 75%-100%. There was no carbapenem resistance detected among the gram negative isolates. Similar patterns of sensitivity to the carbapenems have been noted by studies in neighboring Kenya (12, 13, 28, 29).

Gram positive isolates were highly susceptible to Gentamicin, Vancomycin, Meropenem Nitrofurantoin and linezolid. Similar studies have reported high susceptibility to Nitrofurantoin which indicates that Nitrofurantoin is still a useful drug for management of bacteriuria (14, 18). Its high susceptibility may though be due to is less frequent use as compared to other antibiotics in the management of urinary tract infections in Uganda. High susceptibility to Gentamicin is worth celebrating because it is relatively cheap and can be afforded by the rural poor. On the other hand, drugs like Vancomycin, Meropenem and Linezolid which show high susceptibility in this study are very expensive and with invasive modes of administration.

Sulfamethoxazole-trimethoprim (SXT), Cefotaxime, Ceftazidime and Cefepem had the lowest sensitivities to both gram positive and gram negative isolates in our study. This correlates with findings that have documented high resistance to the first line antimicrobial drugs such as cotrimoxazole (13, 30). Our findings are also in agreement with reports from other regions of Uganda which showed that the commonly used antibiotics are non-effective (7). Cefotaxime is second line antimicrobial agent in the third generation of cephalosporins. A high resistance to cefotaxime, ceftazidime and cefepem in this study calls for an urgent need for surveillance of antimicrobial resistance among pregnant women with asymptomatic bacteriuria. The resistance to cotrimoxazole in our study may be attributed to its widespread over-the-counter use in our locality and its use for prophylaxis against opportunistic infections among people living with HIV. All bacteria isolated in this study were resistant to three or more classes of antibiotics therefore referred to as multi-drug resistant (MDR)

Antifungal susceptibility

The range of Candida species associated with candidiuria is relatively constant. In this study, *Candida albicans, Candida parasilosis* and *Candida glabrata* were isolated and subjected to antifungal susceptibility to the commonly used antifungals. A similar study in Mbarara Isolated similar species of Candida (31). *In vitro* antifungal susceptibility testing now plays an increasingly important role in guiding therapeutic decision making, as an aid in drug development studies, and as a means of tracking the development of antifungal resistance in epidemiological studies (32). All Candida isolated in this study were susceptible to Econazole and Nystatin whereas all isolates were resistant to Griseofulvin. This compares with another study done in Mbarara, South-Western Uganda which showed 100% susceptibility of Candida isolates to Nystatin (31) and similar results were obtained in Argentina (33). High susceptibility to Clotrimazole (95%-100%) and Ketoconazole (81%-100%) was noted among all isolates whereas high resistance to Amphotericin B, Fluconazole and Voriconazole was noted. Our findings differ from the study in South-Western Uganda which showed good susceptibility to fluconazole.

Conclusion

Asymptomatic bacteriuria is highly prevalent among pregnant women in Butaleja District with a number of bacteria exhibiting multi-drug resistance. The common etiological agents of asymptomatic bacteriuria in Butaleja district were *S. aureus*, and *E. coli* whereas Enterococcus spp was the list commonly isolated. The susceptibility patterns shown in this

study highlight the need for sensitivity studies before initiating treatment for a UTI, a challenge that is prevalent in the local health facilities like Busolwe Hospital. The prevalence of Candida colonization was also high with notable resistance to the commonly used antifungals.

Recommendations.

We recommend regular screening of pregnant women for urinary tract infections whether asymptomatic or not so that those with bacteriuria get timely treatment. We also recommend continuous surveillance for antimicrobial resistance in this community. Antifungal susceptibility for Candida isolated should be encouraged to promote rational use of antifungal agents.

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