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

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BF, MS, NP, OI, IS and NMM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NJ conducted critical reviews of the study. Authors RN and IJS managed the whole project, conducted critical reviews and approved of the final manuscript.

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ABSTRACT

Background: Urinary tract infection (UTI) is the most common reason for which antimicrobials are prescribed in pregnancy Worldwide. 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

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before taking off urine samples for laboratory diagnosis. For those samples found to have pus cells, culture and sensitivity test was done to identify the organisms and determine susceptibility to particular antibiotics and antifungal agents.

Results: Gram negative isolates were more sensitive to meropenem (100%), and ciprofloxacin (93.8%) but less sensitive to trimethoprim/sulphurmethoxazole (20%), Ceftazidime (7%), and Cefepime (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 multi-drug resistant (MDR). All *Candida* isolates were susceptible to Econazole and Nystatin whereas all isolates were resistant to Griseofulvin.

Conclusion: The prevalence of asymptomatic bacteriuria among pregnant women in Butaleja district is high with many of the bacteria isolated exhibiting resistance to the commonly used antibiotics. Antifungal resistance was common in this study.

Keywords: Asymptomatic bacteriuria; antimicrobial resistance; UTI; pregnant women.

1. INTRODUCTION

1.1 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 20 years 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 the prevalence of asymptomatic Bacteriuria, *Candida* colonization and antimicrobial susceptibility patterns among Pregnant Women in a District Hospital in Eastern Uganda.

2. MATERIALS AND METHODS

2.1 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.

2.2 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).

2.3 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.

2.4 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.

2.5 Isolation of Bacteria from Samples

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, 100 µl 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 18-24 hrs and examined for growth. Plates which showed no growth at 24 hrs were incubated for another 24 hrs to allow for detection of slow growers. The plates that were negative after 48 hrs were reported as no significant growth. Growth of more than three colony types typically indicated contamination. Bacterial counts of $\geq 10^5$CFU/ml were indicative of an infection and counts below 10^4 CFU/ml was taken to indicate contamination during collection and further tests would not be performed unless the organisms were Enterobacteriaceae.

2.6 Morphological and Biochemical Identification of the Bacteria

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-Bauer 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. The antibiotic discs used included; Amoxicillin / clavulanic acid (20/10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Meropenem (10 µg), Chloramphenical (30 µg), Trimethoprim / Sulphurmethoxazole (1.25/23.75 µg), Cefotaxime (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Clindamycin (2 µg), Vancomycin (30 µg), Nitrofurantoin (300 µg) and Linezolid (30 µg).

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 Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The antifungal

agents used were Voriconazole (1 µg), Ketoconazole (10 µg), Amphotericin B (20u), Griseofulvin (10 µg), Itraconazole (10 µg), Fluconazole (25 µg), Clotrimazole (10 µg), Econazole (50 µg), and Nystatin (100 µg).

2.7 Data Management and Analysis

Findings were entered in excel, exported to STATA v14 for analysis and presented in form of Tables and Figures.

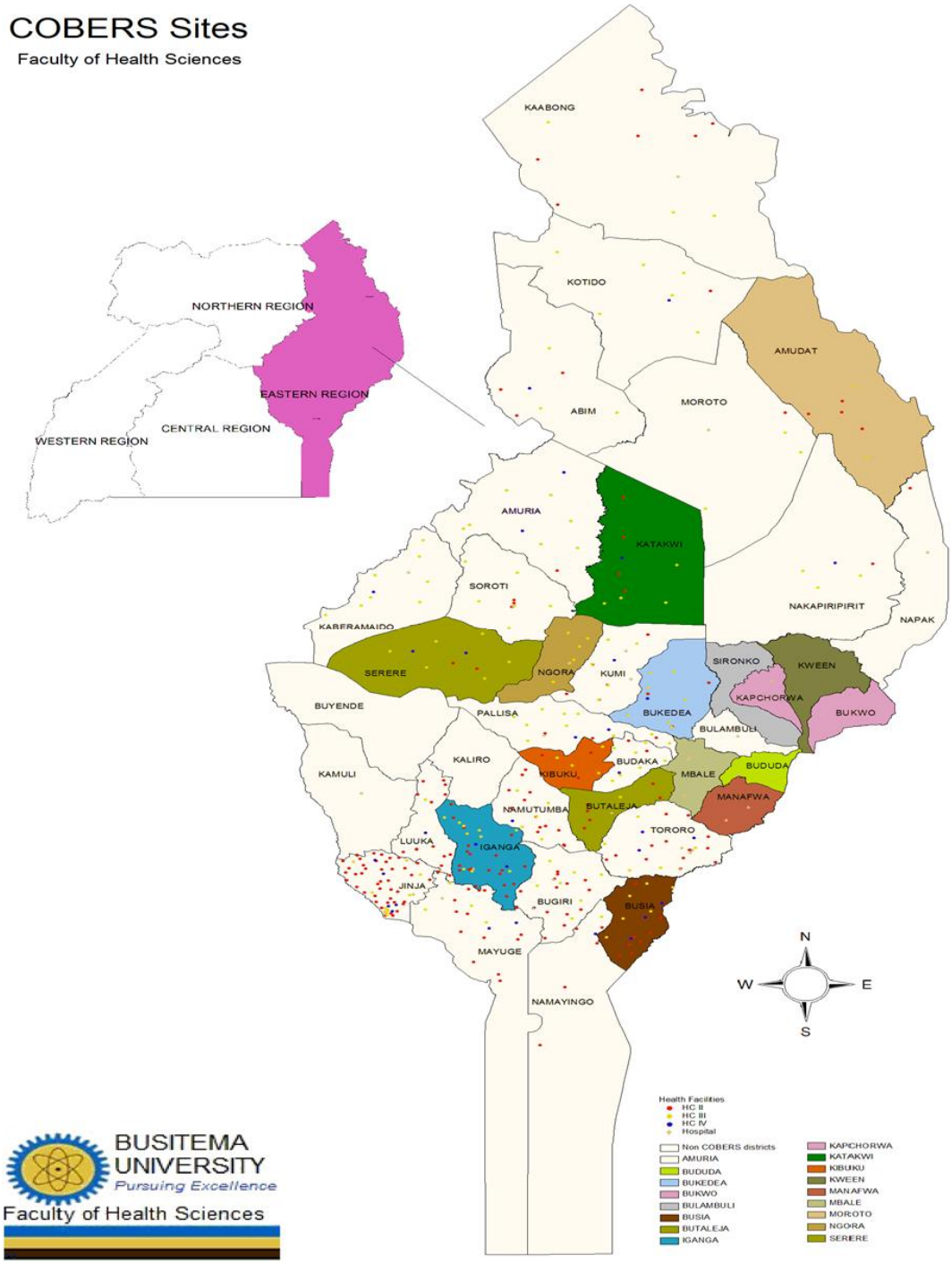

Fig. 1. Map of Uganda showing the distribution of COBERS sites in Eastern Uganda

3. RESULTS

3.1 Demographics Characteristics and the Prevalence of Asymptomatic Bacteriuria

The prevalence of asymptomatic bacteriuria among pregnant women in Butaleja district was 32.8%. 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 came from other parishes (Fig. 2).

3.2 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.

3.3 Antibiotic Sensitivity Pattern of Isolates

Antibiotic susceptibility to the commonly used antibiotics used in our setting was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Gram negative isolates were more sensitive to meropenem (100%), and ciprofloxacin (93.8%) but less sensitive to trimethoprim / sulphurmethoxazole (20%), Ceftazidime (7%), and Cefepime (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).

3.4 Sensitivity to the Antifungals

Commonly used antifungal agents in our setting were used to determine the antifungal susceptibility pattern using the Kirby- Bauer disc diffusion method on Sabourauds Dextrose Agar (SDA). *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.

4. DISCUSSION

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]. The prevalence of symptomatic bacteriuria in our study is 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].

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]. The large number of participants and cases of asymptomatic bacteriuria from Busolwe sub-county could be due to proximity to Busolwe hospital and this paints a picture that there is a lot of undiagnosed asymptomatic bacteriuria from communities far away from health facilities.

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. 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* spp [24]. Studies similar to ours have demonstrated a 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 (Table 2). 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 worse effects in pregnant women.

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 [27]. 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.

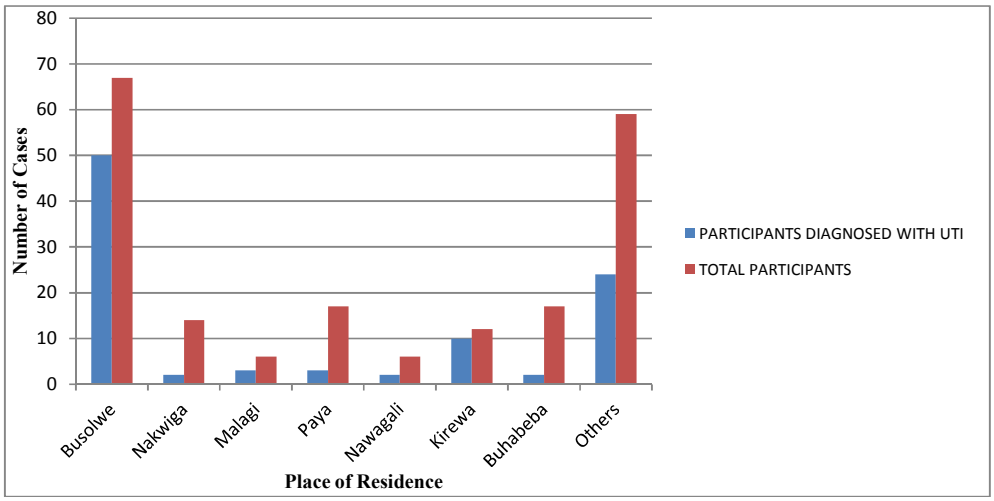

Fig. 2. Distribution of asymptomatic bacteriuria by place of residence

Table 1. Antimicrobial susceptibility pattern of bacterial isolates from asymptomatic bacteriuria among pregnant women in Butaleja District

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)

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-Cefepime, CAZ-Ceftazidime

Table 2. Antifungal susceptibility patterns of Candida species isolated

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)

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

Sulfamethoxazole-trimethoprim (SXT), Cefotaxime, Ceftazidime and Cefepime 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 cefepime 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).

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.

5. CONCLUSION

The prevalence of asymptomatic bacteriuria among pregnant women in Butaleja District is high 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.

6. 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.

CONSENT

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.

ETHICAL APPROVAL

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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