Asymptomatic Urinary Tract infection in Pregnant and Non-pregnant Women in the Limbe Health District of Cameroon: A Phenotypic and Biochemical analytic study

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Abstract

Background

Asymptomatic bacteriuria in pregnancy is associated with significant maternal and fetal risks. However, there seem to be a dearth of information of its prevalence in our community. This study investigated the prevalence of the etiology and antibiotic susceptibility profile of bacterial agents incriminated in urinary tract infection (UTI) in pregnant and non-pregnant women. The association between leukocyte esterase, nitrite, serum creatinine and asymptomatic urinary tract infection (UTI) was assessed.

Methods

A total of 150 urine and blood samples were collected (75 pregnant and 75 non-pregnant) and analyzed using standard microbiological and clinical chemistry methods for detection of isolates and their antibiotic sensitivity and serum creatinine level, respectively. Data obtained was analyzed using the SPSS data package; the Chi-square (χ^2) test was used to examine significant differences at 5% significance level.

Results

The overall prevalence of asymptomatic UTI was 30%, while the prevalence in pregnant and non-pregnant women was 29.3% and 30.7%, respectively. Gram positive organisms were more prevalent (77.6%) than gram negative organisms (22.4%). The most prevalent organism was *S. aureus* (20.1%), while Enterococci (2.1%) was the least prevalent. No statistically significant association existed between serum creatinine and asymptomatic UTI. Leukocyte esterase was a common indicator for UTI and was statistically significant in non-pregnant women ($P=0.009$). Ciprofloxacin, ceftriaxone and amoxiclav were the most effective antibiotics (100%) against most isolates. However, some isolates demonstrated high resistance to cefixime and ampicillin, respectively (100%).

Conclusion

Asymptomatic UTI is a problem in pregnant and non-pregnant women in our environment, with a majority of the cases attributed to Gram positive bacteria. Resistance to some of the antibiotics currently employed in the treatment of this infection was remarkable. The use of biochemical indicators in the diagnosis of UTI in our environment should be done with caution. These findings, therefore, have epidemiological and clinical significance, and calls for more attention in the management of UTI.

Background

Bacteriuria is the presence and growth of bacteria in the urinary tract. This may lead to urinary tract infection (UTI) [1]. UTI is a predisposing factor to adverse pregnancy outcomes and presents with some effects on renal function [2]. Hormonal and immunological changes in pregnancy, including high level of progesterone secretion, which could lead to stasis and decrease immunity are contributing factors to UTI [3]. Asymptomatic bacteriuria occurs in 2 to 7% of pregnant women. However, this prevalence can be up to 30% in developing countries [4]. A study on bacteriuria in pregnant women in three hospitals in Douala, Cameroon reported a significant prevalence of symptomatic bacteriuria of 9.9% and asymptomatic bacteriuria of 5.7% [1]. In another study in Buea, Cameroon, the prevalence of bacteriuria in pregnant women was 23.5% and 7.8% [5], for symptomatic and asymptomatic cases, respectively.

Escherichia coli and *Staphylococcus saprophyticus* account for about 80% of community-acquired UTI [6]. Other Gram-negative pathogens, including *Pseudomonas spp*, *Enterobacter spp*, *Citrobacter spp*, and *Klebsiella spp*, are also incriminated in urinary tract infection [6]. Gram-positive bacteria, including *Enterococcus spp* and *Staphylococcus spp* [7] can cause nosocomial UTI due to selective pressure from the antimicrobial agents used in hospitalized patients. The relatively high prevalence of UTIs during pregnancy, and the significant consequences for women and the pregnancy outcome justify screening of pregnant women for UTIs [8].

In a urinalysis, the presence of leukocyte esterase suggests the presence of white blood cells while nitrite test detects the products of nitrate reductase, an enzyme produced by many gram negative bacteria. Serum creatinine concentration remains the standard, single-point assessment for kidney function in pregnant populations, yet a normal range in pregnancy has not been established [9]. However, routine analysis of urine in pregnant women is generally limited to just two parameters; glucose and albumin; whereas UTI and kidney malfunction are certainly dangers to pregnancy as it may affect the general health of the mother and fetus in the future.

Biochemical indicators of UTI such as the presence of leukocyte esterase and nitrite are obviously rapid laboratory procedures, which can be incorporated into the testing panel of pregnant women in resource limited settings such as Cameroon, where these tests are available and affordable. Uncomplicated UTIs are generally self-limiting, but the social stigma associated to UTI is of concern to patients and, as such, they engage in unorthodox treatment or self-medication using antibiotics [10].

Without treatment, up to 20 to 30% of pregnant women with asymptomatic bacteriuria will develop a symptomatic urinary tract infection [11]. In a previous study [12], *E. coli*, *S. aureus*, Beta haemolytic *Streptococci*, coagulase negative Staphylococci and Group B Streptococci were reported to be 100% susceptible to nitrofurantoin. In another study, *E. coli* reported 91.7% and 88.3% susceptibility to ciprofloxacin and ceftriaxone, respectively [8]. Chloramphenicol was also found to be successful in the treatment of UTI, being 100% effective against *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *E. coli* [13].

Bacterial resistance to antibiotics is one of the most challenging global health threats faced in modern medicine, and resistance of urinary tract pathogens has been reported to increase worldwide with a pattern which may vary over short periods, and depends on site of isolation and environmental conditions [14]. Clinicians are challenged in selecting appropriate treatment due to increase antibiotic resistance by these pathogens [15, 16]. Studies have demonstrated geographic variation in etiologic characteristics of UTI and their resistance patterns to antibiotics [10].

In our environment, urine culture is not routinely done for antenatal patients. The dip stick test is the most widely used method for assessing urine in pregnant women. Consequently, the microbiological picture of such urine specimen cannot be fully ascertained, as the strip cannot quantify the extent of infection as well as provide indication for antimicrobial chemotherapy. To ensure appropriate therapy for these infections, current knowledge of the etiologic agents, their biochemical indicators and antibiotic susceptibility profile of isolates are necessary for improved intervention. The aim of this study was to isolate and identify the bacterial agents causing asymptomatic UTI in pregnant and non-pregnant women in Limbe Health District, assess their association with certain biochemical indicators, serum creatinine level and their antibiotic susceptibility profile.

Methods

Study Design

This was a hospital based cross sectional study, in which asymptomatic, 75 pregnant and 75 non-pregnant women, aged 13-44 years consulting in some health care facilities in the Limbe Health District, were investigated for asymptomatic UTI and kidney function. It was based on laboratory investigations with urine and blood samples obtained from the women. Data on sociodemographic characteristics of participants was obtained using a questionnaire.

Study site and participants

This study was conducted in health facilities representing the key health areas in the Limbe Health District, along the coast line of South West Region, Cameroon. Limbe is a cosmopolitan town, made of people from different tribes, cultures and background. This district has a population of about 118,210 inhabitants with a total of 60,228 women and 8,712 women of child bearing age [17]. Most of these women are sexually active, under educated, and make use of public toilets. Those who live around the beaches mostly use seawater and springs for personal hygiene, which can predispose them to UTIs.

Ethical Considerations

All methods were carried out in accordance with relevant local and national regulations. An administrative authorization was obtained from the South West Regional Delegation of Public Health; ethical approval obtained from the Institutional Review Board (IRB), Faculty of Health Sciences, University of Buea with reference number 2020/1038-12/UB/SG/IRB/FHS. A verbal authorization was obtained from the different participating health facilities. An informed consent was signed by all subjects and an informed consent by parent or guardian of subjects less than 21 years.

Sample Collection

Samples were collected from all pregnant and non-pregnant women who gave their consent, without obvious clinical signs of UTI and/or kidney malfunction, and were not on antibiotics for the last 72 hours. Urine and blood samples were collected for culture, urine dipstick, and creatinine tests respectively. About fifteen milliliters (15 mL) of clean-catch midstream urine was collected in sterile, dry, wide-mouth, leak-proof container. The women were instructed to clean the area around the urethral opening with absorbent paper, and collect urine while holding the labia apart. Five milliliters of blood were collected aseptically from each study participant by venipuncture and transferred to a sterile vacutainer tube without anticoagulant. Samples were transported on ice to the Microbiology Laboratory (urine) and to the Clinical Chemistry Laboratory (blood), of the District Hospital, Limbe, for analysis. In situations where delay was anticipated before analysis, samples were preserved in a refrigerator at 2-8 °C for at most 4 hours (urine samples) and 24 hours (blood).

Determination of Creatinine Level

Blood samples were allowed to clot by leaving them undisturbed at room temperature for 45 min, dislodged and centrifuged between 2200-2500 rpm (revolution per minute) for 10 minutes and approximately 2 mL of serum harvested. Creatinine test was performed, using the creatinine test kit (Cypress Diagnostic, Belgium) as described by Cheesbrough (2000) [18], and according to manufacturer's instruction. Briefly, reagent 1 (R1) and reagent 2 (R2) were reconstituted in equal proportion for use within seven days, stored at 2-8 °C. Quality control (QC) runs of normal and pathological commercial control sera (CYPRESS DIAGNOSTICS Belgium) were performed daily before analysis. Briefly, 1000 µ/L of the reagent was pipetted into each of five sterile glass test tubes and labeled as follows: test tube 1 (blank), test tube 2 (standard), test tube 3 (pathological control), test tube 4 (normal control) and test tube 5 (subject code). Exactly 100 µ/L of the standard, pathological control, normal control and 100 µ/L of participants' serum were added sequentially into respective reagent tubes. The content of each tube was mixed, its absorbance and concentration measured and recorded by spectrophotometry (Sinothinker China, at wavelength 630 nm) before reconstituting the next tube. Results for normal and pathological controls were recorded and verified to fall within +/- 2SD before samples were tested, results accepted and recorded.

Urine Dipstick test

Urine samples were tested with the rapid urine dipstick (ACON Laboratories, Inc., China) by dipping the strip into urine and results for leucocyte esterase and nitrite obtained within 30 seconds to 1 minute [19]. Strips were dipped in the well-mixed urine for no longer than four seconds and excess urine, along the rim of strips, wiped. After 1 minute, the colour change in the strips was compared with the color scales provided with the kit. A violet colour produced was considered as leucocyte esterase positive, and pink colour as nitrate positive. Colour changes appearing only along the edges of the test patches or developing after more than 2 minutes were considered negative. Most gram negative uropathogens produce nitrate reducing enzymes that reduce nitrate to nitrites. Nitrite reacts with an amine impregnated on the dipstick pad to form a diazonium compound resulting in a pink colour reaction within 60 seconds. An enzyme produced by inflammatory cells, leukocyte esterase, reacts with the chloroacetate stain impregnated in a dipstick pad resulting in an iodoxy moiety that is oxidized by room air and produces a colour change in 1-2 minutes.

Urine culture test

Following standard procedure, exactly 0.01 mL of urine samples were inoculated aseptically on CLED agar (HiMedia Laboratories Pvt. Ltd., India) by streaking technique. The culture plates were incubated aerobically at 37 °C for 24-48 hours, after which they were observed for growth. Colonies were counted and the counts multiplied by 100 to have the Colony Forming Units per milliliter (CFU/mL) of urine. Plates with growth or colonies less than 1000 CFU/mL were incubated for another 24 hours. Only plates with counts >10⁵ CFU/mL were considered as significant bacteriuria [20]. The isolates were further identified on the basis of colonial morphology, cultural characteristics, Gram's staining and a battery of biochemical reactions [1,5].

Antimicrobial Susceptibility Testing

The susceptibility pattern of isolates to antibiotics employed in the empiric treatment of UTI was studied. Antimicrobial susceptibility tests were performed on bacterial isolates from midstream urine using antimicrobial discs, according to Clinical and Laboratory Standards Institute (CLSI) on Muller Hinton agar [21].

Briefly, 2-3 colonies from the pure culture were suspended in a vial of 7 mL physiological saline to obtain a turbidity equivalent to 0.5 McFarland. A sterile swab was dipped into the suspension and excess moisture removed by pressing the swab against the side of the tube. The surface of Mueller-Hinton agar was swabbed completely and allowed to dry for about 5 minutes. The following antibiotics from Fluka Industry, United Kingdom, were used: Amoxiclav (30 µg), Flucloxacillin (5 µg), Ampicillin (10µg), Ceftriaxone (30 µg), Cefixime (5 µg), Levofloxacin (5 µg), Ciprofloxacin (30µg), Ofloxacin (5 µg) and Gentamicin (10 µg). Antibiotic disks were placed on the agar keeping at least a distance of 24 mm between them. About 15 minutes after disks were applied, the plates were inverted and incubated at 37 °C for 18-24 hours. Results were reported as sensitive or resistant based on the specific diameter of zone of inhibition for the different antibiotics [22].

Statistical analysis

Data obtained was entered into excel spread sheet and analysed using SPSS statistical package. Descriptive statistics was used to determine the prevalence of asymptomatic UTI. The frequencies, percentages and measures of central tendencies were obtained. The Pearson's Chi-square was used to determine if there was correlation between the different variables. To compare the means, the student t-test was used. Statistical significance was taken at the level $p < 0.05$.

Results

Socio-demographic Characteristics

A total of 150 women were enrolled in this study with age range of 13–44 years and a mean (standard deviation) of 26.97 (±6.17) years. Seventy-five pregnant women with age range 16-38 years and a mean age (standard deviation) of 26.53 (±5.16) years, and 75 non-pregnant women with age range 13-44 years and a mean age (standard deviation) of 27.41 (±7.05) years participated in this study. Among the pregnant subjects, 28 (37.3 %) were between the ages 13-24 years, 44(58.7 %) between 25-34 years, and 3 (4.0 %) between 35-38 years (Table 1). Amongst the non-pregnant cases, 26 (34.7 %) were between the ages 13-24 years, and 12 (16.0 %) between 35-44 years. Based on parity, 56 (37.3 %), 38 (25.3 %), and 56 (37.3 %) were nulliparous, mono parous, multiparous, respectively (Table 1).

Among the 75 pregnant subjects, 36 (48.0 %), 32 (42.7 %) and 7(9.3 %) were in their 3rd, 2nd and 1st trimester of pregnancy, respectively. Overall, 39 (26.0 %) of study subjects had a history of previous urinary tract infection. This comprised 20 (26.7 %) and 19 (25.3 %) of the pregnant and non-pregnant women, respectively.

Table 1: Socio-demographic characteristics of study participants

Characteristic	Pregnant n (%)	Non pregnant n (%)	TOTAL
Most sexually active Age group			
13-24	28(37.3)	26(34.7)	54(36.0)
25-34	44(58.7)	37(49.3)	81(54.0)
35-44	3(4.0)	12(16.0)	15(10.0)
Residence			
Sea port	3(4.0)	4(5.3)	7(4.7)
Town	47(62.7)	48(64.0)	95(63.3)
West coast	25(33.3)	23(30.7)	48(32.0)
Number of children			
0	25(33.3)	31(41.3)	56(37.3)
1	23(30.7)	15(20.0)	38(25.3)
2	16(21.3)	17(22.7)	33(22.0)
3	10(13.3)	10(13.3)	20(13.3)
>4	1(1.3)	2(2.6)	3(2.0)
Trimester of gestation			
1 st	7(9.3)	/	7(9.3)
2 nd	32(42.7)	/	32(42.7)
3 rd	36(48.0)	/	36(48.0)

Prevalence of asymptomatic UTI

The overall asymptomatic UTI prevalence in the study population was 30%. The prevalence in non-pregnant and pregnant women was 29.3% and 30.7%, respectively (Table 2). However, the difference was not statistically significant (P=0.859).

Table 2: Distribution of asymptomatic UTI cases across study participant

Asymptomatic UTI			
	Pregnant n (%)	Non-Pregnant n (%)	Total n (%)
Negative	52 (69.3%)	53 (70.7%)	105(70%)
Positive	23 (30.7%)	22 (29.3%)	45(30%)
	75	75	150
			Chi square=0.032
			P-value=0.859

Etiologic profile of asymptomatic UTI

Among the identified isolates, *S. aureus* recorded a dominant 20.1 % prevalence, with 17.4 % (4) and 22.8 % (5) in pregnant and non-pregnant women, respectively. This was followed by *S. saprophyticus* with an overall prevalence of 15.4 %, higher in pregnant women (26.2 %, 6/23) than in non-pregnant women (4.5 %, 1/22). *Escherichia coli* had a prevalence of 11.1% (5) occurring more (13.6 %, 3/22) in non-pregnant than in pregnant women (8.7 %, 2/23). *Lactobacillus* and Enterococci were detected only in pregnant women, each with a prevalence of 2.2 % (1). Unidentified gram positive rods were observed with

a prevalence of 31.2 %. Their prevalence in this study population was similar in pregnant 30.5 % (7) and in non-pregnant 31.9 % (7) women. *Shigella species* (2.3 %) and *Citrobacter diversus* (2.3 %) were detected only in non-pregnant women. There was no significant difference of bacterial isolates in pregnant and non-pregnant women (P=0.482) (Table 3).

Table 3: Prevalence of bacterial pathogens in cases of asymptomatic UTI

Bacterial Isolates	Pregnant n (%)	Non-pregnant n (%)	Total n (%)
<u>Gram positive</u>			
Enterococci	1 (4.3)	0 (0.0)	1(2.1)
<i>Lactobacillus</i>	1 (4.3)	0 (0.0)	1(2.1)
Positive rods	7 (30.5)	7 (31.9)	14(31.2)
<i>S. aureus</i>	4 (17.4)	5 (22.8)	9(20.1)
<i>S. epidermidis</i>	1 (4.3)	2(9.1)	3(6.7)
<i>S. saprophyticus</i>	6 (26.2)	1 (4.5)	7(15.4)
<u>Gram negative</u>			
<i>Neisseria gonorrhoeae</i>	1 (4.3)	2 (9.1)	3(6.7)
<i>Shigella species</i>	0 (0.0)	1 (4.5)	1(2.3)
<i>E. coli</i>	2 (8.7)	3 (13.6)	5(11.1)
<i>Citrobacter diversus</i>	0 (0.0)	1 (4.5)	1(2.3)
Total	23(30.7)	22(29.3)	45(30.0)
Chi square= 8.531			
P-value=0.482			

Association between leukocyte esterase/nitrite and Asymptomatic UTI

There was no significant association between leukocyte esterase and nitrite with asymptomatic UTI in pregnant women. In non-pregnant women, leukocyte esterase was significantly associated to asymptomatic UTI (P= 0.009) (Table 4). Furthermore, women who presented with positive leukocyte esterase were 25.3 % (19) and 9.3 % (7) pregnant and non-pregnant, respectively.

Table 4: Association between leucocyte esterase and asymptomatic UTI

	Asymptomatic UTI	
	Pregnant (n=75)	Non-pregnant (n=75)
Leukocyte (cells/uL)		
0 (0)	56 (74.7)	68 (90.7)
15 (+1)	11 (14.7)	2 (2.7)
50 (+2)	0 (0)	1 (1.3)
70(+3)	7 (9.3)	3 (4.0)
125(+4)	1 (1.3)	1 (1.3)
Total for positive Leukocyte	19(25.3)	7(9.3)
	Chi square= 1.215	Chi square= 13.492
	P value= 0.749	P value= 0.009*

Nitrite was only present in a single case 1 (4.5%) (non-pregnant) and had no statistically significant association (P=0.118) (Table 5).

Table 5: Association between nitrite and Asymptomatic UTI

Asymptomatic UTI		
	Pregnant (n=75)	Non-pregnant (n=75)
Nitrite		
Negative	75 (100)	74 (95.5)
Positive	0(0)	1(4.5)
Total	75 (100)	75 (100)
	Chi square, P-value	Chi square= 2.442,
	Not applicable	P value = 0.118

Relationship between Asymptomatic UTI and serum creatinine

Abnormal creatinine levels (≥ 1.4 mg/dL) were found more in pregnant women 10(13.3 %) than in non-pregnant women 9(12.0 %). However, there was no statistically significant association between serum creatinine level and asymptomatic UTI in pregnant (P=0.154) and non-pregnant women (P=0.617) (Table 6).

Table 6: Association between asymptomatic UTI and serum creatinine

Asymptomatic UTI		
	Pregnant (n=75)	Non-pregnant (n=75)
Abnormal creatinine	10(13.3)	9(12.0)
Normal creatinine	65(86.7)	66 (88.0)
Totals	75(100)	75(100)
	Chi square=2.028,	Chi square=0.249,
	P-value= 0.154	P-value= 0.617

Antimicrobial Sensitivity

Enterococci, Lactobacilli, *S. aureus*, *Citrobacter diversus* and gram positive rods were markedly (100 %) susceptible to ceftriaxone. *Neisseria gonorrhoeae*, *Citrobacter diversus*, *Shigella species*, gram positive rods and Enterococci, *S. epidermidis* were markedly susceptible (100 %) to ciprofloxacin and amoxiclav, respectively. *N. gonorrhoeae*, *S. aureus*, and *S. epidermidis* were moderately susceptible with 66.7 %, 88.9 % and 66.7 % to ceftriaxone, respectively. *E. coli*, *S. aureus* and *S. epidermidis* were also moderately susceptible with 80 %, 77.8 % and 66.7 % to ciprofloxacin, respectively. Most isolates were markedly resistant (100 %) to cefixime, ampicillin, gentamicin and flocoxacillin (Table7).

Table 7: Antimicrobial sensitivity reaction for all isolates

Antibiotic (µg)	Isolate													
	<i>E. coli</i> n=5(%)		Enterococci n=1(%)		<i>Lactobacillus</i> n=1(%)		<i>Neisseria gonorrhoeae</i> n=3(%)		<i>Positive Rod</i> n=14(%)		<i>S. aureus</i> n=9(%)		<i>S. epidemidis</i> n=3(%)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
AMC 30	2 (40.0)	3 (60.0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (33.3)	2 (66.7)	9 (64.3)	5 (35.7)	1 (11.1)	8 (88.9)	3 (100)	0 (0)
FXL 10	0 (0)	5 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	4 (28.6)	10 (71.4)	3 (33.3)	6 (66.7)	0 (0)	3 (100)
AMP 10	0 (0)	5 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	4 (28.6)	10 (71.4)	1 (11.1)	8 (88.9)	0 (0)	3 (100)
CTX 30	2 (40.0)	3 (60.0)	1 (100)	0 (0)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	8 (100)	0 (0)	8 (88.9)	1 (11.1)	2 (66.7)	1 (33.3)
CXM 5	1 (20.0)	4 (80.0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	14 (100)	0 (0)	9 (100)	0 (0)	3 (100)
LEV 5	3 (60.0)	2 (40.0)	0(0)	1(100)	0 (0)	1 (100)	1 (33.3)	2 (66.7)	4 (28.6)	10 (71.4)	3 (33.3)	6 (66.7)	0 (0)	3 (100)
CIP 30	4 (80.0)	1 (20.0)	0(0)	1 (100)	0 (0)	1 (100)	3 (100)	0 (0)	14 (100)	0 (0)	7 (77.8)	2 (22.2)	2 (66.7)	1 (33.3)
OFL 5	2(40.0)	3 (60.0)	0(0)	1(100)	0(0)	1(100)	0 (0)	3(100)	1 (7.1)	13 (92.9)	6 (66.7)	3 (33.3)	1(33.3)	2 (66.7)
GEN 10	2(40.0)	3 (60.0)	0(0)	1(100)	0(0)	1(100)	0(0)	3 (100)	2 (14.3)	12 (85.7)	2 (22.2)	7 (77.8)	1(33.3)	2 (66.7)

AMC=Amoxiclav, FXL=Flucoxacillin, AMP=Ampicillin, CTX=Ceftriaxone, CXM=Cefixime, LEV=Levofloxacin, CIP=Ciprofloxacin, OFL=Ofloxacin, GEN=Gentamicin

Discussion

Prevalence and etiological profile

Results of this study indicate that there is a relatively high prevalence of asymptomatic UTI (30.0 %) in pregnant and non-pregnant women in the Limbe Health District, with a higher occurrence of gram positive bacteria. The prevalence of 30.7 % in pregnant women is higher than 5.7 %, 7.8 %, 8.4 % and 9.0 %, respectively reported by other investigators [1,5,8,13], close to 28.7 % [23] and lower than 38.0 % reported by another [24]. The high prevalence in our study may be due to differences in sample size and methods of isolation, and to the fact that UTIs are one of the most frequent and recurrent bacterial infections in women. Furthermore, the prevalence in non-pregnant women (29.3 %) was lower than 75.0 % [24]. However, they studied a very small symptomatic non-pregnant population (n=24), a possible reason for their very high prevalence. Generally, women suffer from urinary tract infections more often because bacteria can reach the bladder easily because of the close proximity of the vagina and anus to the urethra [6], and difficulty with hygiene due to a distended pregnant belly. During sexual activity, bacteria can be pushed into the urethra. The use of diaphragms could also be associated with the increase prevalence of asymptomatic UTI, as urine that stays in the bladder is more likely to grow bacteria and cause infection. Though we did not investigate this, it has been reported as a predisposing factor for UTI [6].

Gram-positive organisms were the most prevalent pathogens in both pregnant 87.0 % (n=23) and non-pregnant 68.3 % (n=22) women. This corroborates the findings in another study [24], which reported a gram positive bacterial prevalence of 60.0 % in pregnant and 55.5 % in non-pregnant women; another study [8], also documented a prevalence of 80.95 % in pregnant women. Other researchers reported gram positive bacteria as frequent etiologic agents of UTI [7]. However, contrary to these findings, Alemu *et al* (67.5 %) [13] and Ngong *et al* (85.4 %) [23], identified gram negative bacteria as the most prevalent urinary tract pathogen in pregnant women.

From our findings, there was no significant difference in the etiologic profile of asymptomatic UTI in pregnant and non-pregnant women. This may imply that UTI in women of child-bearing age, can be caused by same organisms in pregnant and non-pregnant women. Though we did not investigate the role of immunosuppression in relation to infection, we are constrained to think that, immunosuppression in pregnancy, as has been previously reported [1], may not have significantly influenced occurrence of UTI in this study. *S. saprophyticus* occurred more in pregnant (26.2 %) than in non-pregnant women (4.5 %), while *S. aureus* occurred more in non-pregnant (22.8 %) than in pregnant women (17.4 %). *E. coli* occurred more in non-pregnant (13.6 %) than in the pregnant (8.7 %) population. The dominant isolate in this study population was *S. aureus* which is similar to a previous report [24]. They reported over 45.0 % and 38.9 % prevalence in pregnant and non-pregnant population. *S. saprophyticus* was the most common bacteria isolated in pregnant women; however, this does not agree with other studies [1,5,8,13,23], who reported *E. coli* as the leading cause of UTI in pregnant women.

The occurrence of *N. gonorrhoeae* was noticed in pregnant (4.3 %, 1/23) and non-pregnant (9.1 %, 2/22) women. This could have been acquired through multiple mucosal sites in the lower genital tract, including the urethra, cervix, Bartholin's and Skene's glands [25]. Enterococci, *Shigella* and *Citrobacter* were among the least isolated microbes (2.2 % each) in the study population. The low prevalence of these isolates could be a result of the sample size and the detection method used in this study.

Association between Leucocyte esterase/Nitrite and asymptomatic UTI

The occurrence of Leucocyte esterase in pregnant women (25.3 %) was higher than in non-pregnant women (9.3 %). Within the pregnant population, the association between Leucocyte esterase and asymptomatic UTI was not statistically significant ($p = 0.749$), while a significant association was noticed in non-pregnant women ($p = 0.009$). Being an indicator of immune response, its occurrence is remarkable. However, sterile pyuria is common in pregnancy, usually due to contamination by physiological vaginal discharge [26]. Sterile pyuria is a condition where a urinary dipstick test is leukocyte esterase positive with no associated positive urinary culture [27]. Pyuria could also occur as a result of the presence of non-detected bacterial and viral infections [28]. Moreover, about 58.7 % (44/75) of the pregnant population was within the most sexually active group (25-35years) which could be a predisposing factor to UTI. Over 49.3 % of the non-pregnant population also fell within this high sexually active group; a possible predisposing factor that could have accounted for significance in association between Leucocyte esterase and asymptomatic UTI, though Ngong *et al* [23] reported no statistically significant association between UTI and sexual activity.

Nitrite was virtually absent as an indicator of asymptomatic UTI in both study groups, probably because it is produced by specific gram negative microbes, notably *E. coli* [29], whose occurrence was low in this study. The absence of nitrite may be related to the low frequency of such specific isolates. This study reported a dominance of gram positive microbes, which mostly do not metabolize nitrate to nitrite. More so, microbial population density is also a factor related to the concentration of this waste product; and such population density may hardly occur in asymptomatic UTI cases [27].

Association between asymptomatic UTI and serum creatinine level

Serum creatinine levels in asymptomatic bacteriuria in this study population ranged from 0.2 to 1.1mg/dL and 0.6 to 1.5mg/dL in pregnant and non-pregnant women, respectively. The physiologic increase in glomerular filtration rate during pregnancy results in a decrease in concentration of serum creatinine, which falls by an average of 0.4 mg/dL. [30]. Low serum creatinine level could also be due to low muscular activity [31]. More (13.3 %) of the pregnant women had abnormal values of serum creatinine according to biological reference range compared to non-pregnant women (12.0 %) with abnormal results. These abnormal serum creatinine values in pregnant women could be due to renal physiologic changes that occur during pregnancy, and not necessarily a UTI [32]. This finding maybe indicative of the effect of non-bacterial factors associated with renal malfunction such as immune mediated mechanisms [33].

Antimicrobial Sensitivity

Antimicrobial resistance has increased worldwide and susceptibility patterns show substantial geographic variation as well as differences in population and environment [34]. Treatment decreases the risk of pyelonephritis, low birth weight infants, and preterm births. In our study, most isolates were sensitive to

ceftriazone and ciprofloxacin. *E. coli* was highly sensitive (80.0 %) to ciprofloxacin, in line with another study [35]. All three isolates of *N. gonorrhoeae* (1 pregnant and 2 non-pregnant women), were 100 % sensitive to ciprofloxacin, and 66.7 % to ceftriazone.

S. aureus was 88.9 % susceptible to ceftriazone and 77.8 % to ciprofloxacin. Susceptibility to ciprofloxacin is similar to another finding [36] in Nigeria, where uropathogenic *S. aureus* isolates were reported to have a 60-70 % susceptibility to this antimicrobial agent.

Most isolates were completely resistant (100 %) to cefixime. This high resistance to cefixime contradicts a previous study [5], in which cefixime was reported as the most effective antimicrobial agent against UTI pathogens in pregnant women. However, their most predominant isolate was *E. coli*, a gram negative bacilli, unlike in this study, where gram positive organisms were more prevalent. This may indicate that cefixime could be more effective against gram negative bacteria than gram positive, though the few gram negative isolates in this study were still resistant to cefixime. *N. gonorrhoeae* has progressively developed resistance to antimicrobials used for the treatment of gonorrhea. Declining susceptibility to cefixime resulted in a change to the CDC treatment guidelines in 2012, to implement dual therapy with ceftriaxone and azithromycin used for treatment of gonorrhea [37]. In our study, *N. gonorrhoeae* was completely resistant to cefixime, ampicillin, flucloxacillin, ofloxacin and gentamicin. In another study [38] in Vientiane- Laos, this isolate was reported to be highly resistant (84.8 %) to ciprofloxacin.

Conclusions

The prevalence of asymptomatic UTI was 30.7% and 29.3% in pregnant and non-pregnant women, respectively, with gram positive bacteria as the most prevalent bacteria identified. *Neisseria gonorrhoeae*, *Citrobacter diversus* and *Shigella species* were most susceptible to ciprofloxacin (100%), while Enterococci, *Lactobacillus* and *Citrobacter diversus* were most susceptible to ceftriaxone (100%). Asymptomatic UTI had no significant effect on serum creatinine level in the study population. Leukocyte esterase was a significant indicator of asymptomatic UTI in non-pregnant women, while nitrite was not a significant indicator of asymptomatic UTI in both study groups. The use of biochemical indicators in the diagnosis of UTI in our setting should be used with caution.

Abbreviations

UTI- Urinary Tract Infection

IRB - Institutional Review Board

CLED - Cystine-Lactose Electrolyte Deficient

QC - Quality Control

CLSI - Clinical and Laboratory Standards Institute

CDC - Centers for Disease Control

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with relevant local and national regulations. An administrative authorization was obtained from the South West Regional Delegation of Public Health; ethical approval obtained from the Institutional Review Board (IRB), Faculty of Health Sciences, University of Buea with reference number 2020/1038-12/UB/SG/IRB/FHS. A verbal authorization was obtained from the different participating health facilities. An informed consent was signed by all subjects and an informed consent by parent or guardian of subjects less than 21 years.

Consent for publication

Not applicable

Availability of data and material

All data generated and analysed during this study are included in this article

Competing interest

The authors declared that they have no competing interests

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Author's contributions

CEN collected samples, carried out laboratory investigations, and together with FAN analyzed and interpreted data, and drafted the manuscript.

J-FTKA, designed and supervised the study, interpreted data and reviewed the manuscript

LMN, interpreted data and reviewed manuscript

SFE, interpreted data and reviewed manuscript

FAN, analyzed and interpreted data

RNN, designed and supervised the study, interpreted data and reviewed the manuscript

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