Bacteria profile associated with Bacteriuria in Pregnancy at the Yaounde University Teaching Hospital (CHUY)

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Abstract:-
Background: The increased risk of bacteriuria in pregnancy poses a threat to the mother and fetus, some of which include preterm labor and pyelonephritis. Screening for bacteriuria in pregnant women has been included as one of the most cost effective strategies for achieving the Millennium Development Goals for health in developing countries.

Findings: The overall prevalence of bacteriuria among pregnant women was 26.2%. It was higher in pregnant women with symptoms of acute UTI (16.7%) than in those without symptoms (9.5%). *E. coli* (26.7%), CoNS (26.7%), *Klebsiella pneumoniae* (13.3%), *Proteus mirabilis* (10%) and group D streptococci (10%) were common uropathogens. Pyuria was the strongest predictor of bacteriuria. However, there was no statistically significant risk factor of bacteriuria thus, limiting the possibility of targeted screening. GNB were least resistant to gentamicin and ciprofloxacin while the gram positives showed least resistance to cefuroxime. All isolates were susceptible to nitrofurantoin.

Conclusion: This study reveals that bacteriuria occurs in one of every four pregnant women and ASB occurs in one of every ten pregnant women. This is of great concern since bacteriuria is harmful to both mother and fetus. However, 15 isolates showed resistance to at least one antibiotic of the beta lactam class (50%) and 86.7% of isolated uropathogens were resistant to at least one antibiotic hence, we fail to reject the null hypothesis: Resistant bacteria are the most frequent cause of bacteriuria in pregnancy.

Keywords: *Bacteriuria, ASB, Uropathogens, Antibiotic Resistance.*

I. INTRODUCTION

Bacterial urinary tract infections (UTI) remain among the most common medical complications during pregnancy occurring in the body's urinary system which includes kidneys, ureter, bladder and urethra. UTI are most frequently caused by bacteria in the urinary tract (Bacteriuria). Further,

UTI can equally be caused by parasites like schistosomes and typically characterized by hematuria. Most of these infections are limited to the bladder (cystitis) and urethra (urethritis) but may sometimes include the kidneys (pyelonephritis) and ureter. To continue, pregnancy is a state associated with physiological, structural and functional urinary tract changes which promote ascending infections from the urethra [1]. Similarly as in non-pregnant women, bacteriuria in pregnant women could be classified as asymptomatic (when the infection is limited to bacteria growth in the urinary tract) and symptomatic. Bacteriuria is said to be symptomatic when the following are been manifested [2, 3]:

i. Hematuria (presence of blood in urine). ii. Pyuria (presence of leucocytes in urine). iii. Polyuria (increased urine volume).
iv. Dysuria (painful urination).
v. Frequency (increased urge to urinate).
vi. Pelvic and back pain.
vii. Systemic inflammatory response syndrome (fever or hypothermia, hyperleukocytosis or leukopenia, tarchycardia or tarchypnoea).

Microorganisms can reach the UT by hematogenous and lymphatic spread, but clinical and experimental evidence show that the ascent of microorganisms from the urethra is the most common pathway leading to bacteriuria, especially with microbes of enteric origin notably *Escherichia coli, Klebsiella spp., Proteus spp.* etc. This provides a logical explanation for the greater frequency of bacteriuria in women than in men, and for the increased risk of infection following bladder catheterization or instrumentation. Relatively uncommon microbes causing bacteriuria like *Staphylococcus aureus*, *Candida spp.*, *Salmonella spp.* and *Mycobacterium tuberculosis* infect the UT via the hematogenous route [4]. Bacteria can infect the urinary system of just anyone but most frequently occurs in pregnant women owing to their growing uterus which presses on the bladder, thus increasing the intravesical pressure which may result in vesico-ureteral reflux and urine retention in the bladder after micturition. Urinary stasis and impaired physiological anti-reflux mechanism create favorable conditions for bacteria growth and ascending infection. To add, bacteria colonization of the urinary system is enhanced by pregnancy-specific biochemical changes in urine including increased amounts of glucose, amino acids and hormone degradation products which increase urine PH [5, 6]. Further, increased levels of hormones associated with pregnancy (progesterone) bring about dilation of the urinary tract combined with slight hydroureter and hydronephrosis, caused partly by a reduction in smooth muscle tone with slowing of ureteral peristalsis, and urethral sphincter relaxation in about 80% of pregnant women [3, 5]. Some suggested risk factors for bacteriuria during pregnancy include lower socioeconomic status, sexual activity, older age, multiparity, anatomical urinary tract abnormalities, sickle cell disease and diabetes [3, 7-10]. Further, based on the concept of bacterial virulence or pathogenicity, not all bacteria species are equally capable of inducing infection. The more compromised the natural defense mechanisms (renal obstruction, malignancy or bladder catheterization) the fewer the virulence requirements of any bacteria strain to induce infection [4]. Also, bacteriuria prior to prenatal care initiation and a pre-pregnancy history of UTI are the strongest predictors of bacteriuria at prenatal care initiation [11].

Although the incidence of bacteriuria in most studies is only slightly higher in pregnant women than in non-pregnant women, its consequences for both the mother and fetus are very much severe with a much higher risk (up to 40%) of progression to pyelonephritis and possibly increased risk of pre-eclampsia, premature birth and low neonatal birth weight [3, 16]. Further, asymptomatic bacteriuria (ASB) is not associated with preterm delivery if it does not progress to symptomatic bacteriuria and its most serious consequence is the significant risk of acute pyelonephritis in later pregnancy [17]. Also, several observational studies have showed a relationship between maternal symptomatic bacteriuria and the risk of premature delivery and low birth weight [18, 19]. To continue, about 15-20 % of women with pyelonephritis have bacteremia [6, 20]. Equally, they might develop complications like acute kidney injury, hypertension, preeclampsia, sepsis and septic shock, hemolytic anemias, thrombocytopenia and acute respiratory distress syndrome due to renal and other tissue damage caused by bacteria endotoxins, systemic inflammatory response with endothelial injury and late initiation of treatment [21, 22]. Pyelonephritis independently increases the risk of delivery before 37 weeks gestation [12, 23].

Unlike the general population, all pregnant women should be screened for bacteriuria with urine culture and all positive cases be it symptomatic or asymptomatic for UTI be treated using an antibiotic with good maternal and fetal safety profile. Nearly all antimicrobials cross the placenta, and some of them may exert teratogenic effects. Commonly accepted antibiotics used in treating UTIs in pregnancy, regardless of the gestation period include derivatives of penicillin and cephalosporin (particularly those with low protein-binding ability).

The increased risk of bacteriuria in pregnancy poses a threat of complications to the mother and fetus, some of which include preterm labor and pyelonephritis. Screening for bacteriuria in pregnant women has been included as one of the most cost effective strategies for achieving the Millennium Development Goals for health in developing countries. Diagnosis of bacteriuria in most of our settings is via urinalysis and urine microscopy. This is principally due to the high cost and extensive turnaround time (usually 48 to 72 hours) of uroculture and antibiotic susceptibility of uropathogens. Hence, treatment of bacteriuria in pregnancy is usually empiric based on the limited available data on uropathogens and their antibiotic resistance pattern. With the rising resistance rates of uropathogens to frequently used antibiotics (fostered by indiscriminate use of empiric therapy) in treating CAUTI and the susceptibility of uropathogens to antibiotics showing significant geographical variation, studies to increase knowledge on spectrum of bacteria associated with bacteriuria in pregnant women and their resistance patterns to antibiotics at the local and national levels are very important in setting up an evidence based empiric treatment for bacteriuria in pregnant women. Recent studies in Cameroon have reported a rise in rates of drug resistance. Hence, there's an urgent need for continuous surveillance of susceptibility of uropathogens to antibiotics. To add, data on uropathogens and their susceptibility to frequently used and available antibiotics is scarce in Cameroon.

II. MATERIALS AND METHODS

2.1 Study area and period

This study was carried-out at the Yaounde University Teaching Hospital (CHUY) from 1st April to 31st May 2019. Yaounde is the political capital of Cameroon and with a population of over 2.8 million, it is the second largest and most populated city in the country. It lies in the heart of the nation at an elevation of about 750metres (2500 feet) above sea level. The city generally enjoys a high standard of living and security owing to its high-profile central structures and diplomatic services. Local residents are engaged in urban agriculture and cash crop cultivation like cocoa, coffee and sugar cane. The city also has some few industries producing diary, tobacco, glass and brewery products. Yaounde has a tropical wet and dry climate with constant temperatures throughout the year and equally has over 10 public and private hospitals with CHUY been one of the city's largest and referral hospital [24].

2.2 Study design and target population

Since this research was aimed at describing bacteriuria in pregnant women and equally collected observations from every unit (pregnant woman) in the study at a certain point in time disregarding the length of time of the study as a whole, a cross-sectional descriptive study design was used in the study. This research studied and generalized its findings to pregnant women. The sample population and sampling scheme consisted of all the pregnant women attending the antenatal clinic of the Yaounde University Teaching Hospital.

2.3 Sampling method and sample size

The convenient consecutive sampling, which is a non-probability and unrestricted sampling scheme was used in selecting participants. All units of the sample population constituted the sample size giving a 1% margin of error at a 95% confidence interval (Census). The exclusion criteria was a minimum of 48hours of no antibiotic therapy.

2.4 Collection of socio-demographic and clinical data

All data collected for the study were primary data. Structured personal interviews aided by a structured questionnaire was used to obtain socio-demographic and clinical data of participants.

2.5 Collection, transport and analysis of urine specimen

The following procedures were implemented to ensure that urine samples were collected aseptically.

i. Urine cup was first labelled with participant's code.
ii. The urethral opening and vulva was cleaned using a compress soaked in 10% demobacter solution and then with compress soaked with sterile distill water.
iii. Participants then urinated into a basin with the outer lip of the vagina held to one side.
iv. 30ml to 50ml clean catch mid-stream urine was then collected into labelled sterile urine cup, firmly closed and immediately transported to the laboratory for analysis.
v. Participant then washed their hands and the entire area and equipment was disinfected with 70% sodium hypochlorite solution.

The urine samples were transported to the laboratory in less than 30 minutes after they were collected using a biosafety box carrying the labelling "Infectious". In case of any anticipated delays, boric acid powder was added to the urine samples (0.1g/10ml of urine). Boric acid at a concentration of 10g/l (1% w/v) keep bacteria viable without multiplying them. Also, leucocytes, red cells and casts are well preserved and there's no interference in the measurement of urinary protein and glucose [2].

2.5.1 Urine Macroscopy

The color, consistency and odour of the urine samples were examined macroscopically and recorded. This was used to deduce if the participant was symptomatic or asymptomatic. Bacteriuria is usually characterized by cloudy urine with an unpleasant smell and usually contains leucocytes [2]. Equally, normal freshly passed urine is clear and pale yellow to yellow depending on its concentration [2].

2.5.2 Urine culture

Following the Macroscopic analysis, 3ul of uncentrifuged urine was inoculated onto prepared CLED and macConkey agar culture plates using a calibrated wire loop. The plates were then incubated aerobically at 37^0 C for 24hours. Media preparation was done following the manufacturer's instructions (Laboratorios Conda, S.A.). Cultures with bacteria count of ≥30 CFU/3ul of urine after 24hour incubation were considered positive [2]. According to the recommendations outlined by the Infectious Diseases Society of America (IDSA), significant bacteriuria in asymptomatic women is defined as bacterial monoculture in the quantity of $\geq 10^5$CFU/ml in 2 consecutive midstream clean catch urine specimen or $\geq 10^2$CFU/ml in urine collected from single urinary bladder catheterization [1]. Therefore, participants who had positive cultures but insignificant pyuria (<10 leucocytes/ul of urine) and showed no signs and symptoms of UTI, were recollected and tested 4days after. Those tested for the second time and were positive with the same isolate were considered positive for ASB. Growth characteristics was equally noted after 24hours incubation. Bacteriuria without pyuria may occur in diabetes, enteric fever, bacterial endocarditis or the urine contains contaminants. Equally, pyuria with sterile urine culture may be found with renal tuberculosis, gonococcal urethritis, *Chlamydia trachomatis* infection and leptospirosis or when a patient with bacteriuria has been treated with an antibiotic. Moreover, Pure bacteria isolates were inoculated into brain heart infusion plus 40% glycerol broth medium and preserved by freezing in an ultralow freezer (at -80ºC), which can keep bacteria cells viable for several years. Furthermore, daily control of reagents and equipment was done with respect to the manufacturer's instructions. A plate of every newly prepared media was subjected to 24hours incubation (sterility test) to check for contamination during process of preparation [2].

2.5.3 Urine cytology

Cytologic analysis (leucocyte and erythrocyte count) of uncentrifuged urine was done using the Kovac's slide. Kovac's slide was charged with 1ul of well mixed urine and allowed to settle for 5minutes in a damp environment (usually on cloth soaked with water), then read microscopically using the 40x objective.

2.5.4 Microscopic examination of urine sediment

15ml to 20ml of uniform urine sample was aseptically transferred into a conical tube and centrifuged at 700g for 5minutes. The supernatant was discarded and the sediment used to make a wet preparation and a smear allowed to air dry and gram stained [2]. The following can be seen in the wet preparation of a urine sediment [2]

i. Red cells (could be normal, enlarged or crenated depending on the tonicity of urine) ii. Yeast cells, pus cells and epithelia cells iii. Parasites including *Trichomonas vaginalis* and *Schistosoma heamatobium* egg iv. Casts (indicative of renal tissue damage)
v. Crystals (indicative of renal calculi).

Samples that showed the presence of yeast cells were also inoculated onto sabauroud's dextrose agar. The microscope was controlled daily using a known slide and the morning and evening temperatures of the incubator and refrigerator was recorded daily and closely monitored.

2.5.5 Biochemical analysis of urine

This was done using the urine biochemical test strips combi 11 (ACON Laboratories Inc, san diego, CA 92121, USA) which test for the following in urine: proteins, nitrites, leucocytes, blood, PH, urobilirubin, ketone, bilirubin, glucose, ascorbic acid and specific gravity. The presence of proteins in urine was further confirmed by checking for cloudiness upon addition of few drops of sulphosalicylic acid reagent. Also, the presence of leucocytes and red blood cells were confirmed upon microscopic examination of urine sediment. The combi 11 strips were controlled daily using sterile distill water and their sensitivity together with that of sulphosalicylic acid was regularly tested with a 1 in 32 diluted standard.

2.5.6 Gram control of isolates

After 24hours incubation, isolates were then subjected to gram staining. Samples which showed presence of bacteria or yeast in the gram and wet mount examination and had insignificant growth after 24 hours incubation were further incubated for 24 hours.

2.5.7 Identification of isolates

Gram positive cocci (GPC) were subjected to catalase, coagulase, bile aesculin hydrolysis and hippurate hydrolysis identification tests and gram negative bacilli (GNB) were subjected to the oxidase test and the API 20E system for identification as shown in Fig 1.

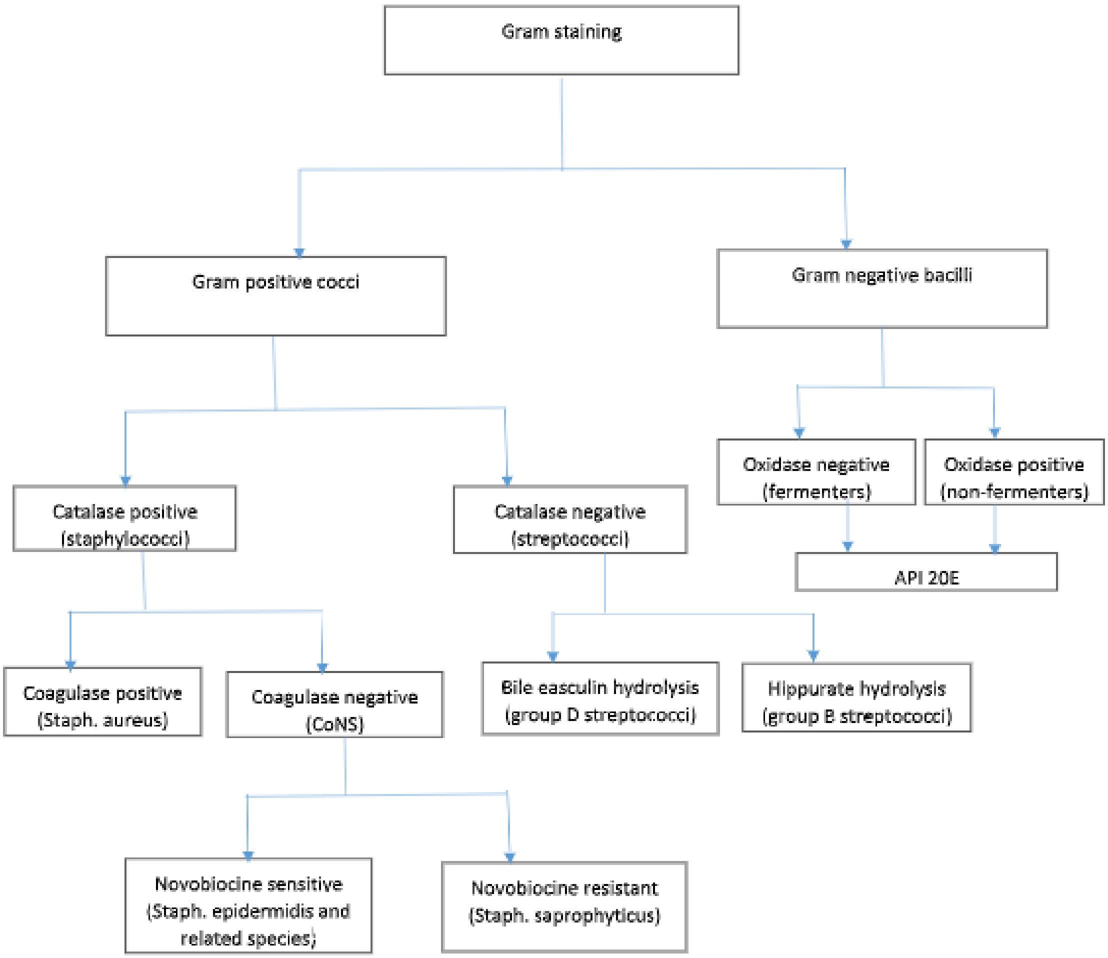

Figure 1. Identification of uropathogens

2.5.8 Antibiogram

This was done via the disk diffusion method (Kirby-Bauer method) described by the Clinical and Laboratory Standards Institute (CLSI) [25], in which a suspension (inoculum) prepared by thoroughly vortexing 2 colonies of an identified bacteria in 5ml of sterile physiologic saline with its turbidity adjusted to that of 0.5 McFarland standard by comparing the absorbance at 530nm wavelength was inoculated on a Mueller hinton media plate using a sterile swap. This suspension possesses 1-5 x 10^5 CFU/ml. Commercially available antibiotic disk was then evenly placed on the surface of the plate (maximum 6) and incubated overnight at 37^0C [25]. The antibiotic susceptibility pattern for all the bacteria isolates was determined using the following classes of antibiotics Penicillins, Cephalosporins, aminoglycosides, flouroquinolones, nitrofuran, lincosamide and carbapenem (Oxoid laboratories, London, UK). All antibiotics used in the study were of the FDA risk category B and C hence, had a good maternal and fetal safety profile [1]. The double-disk synergy test [26] in which a 30pg disc of ceftriaxone and a disk of amoxicillin clavulanate (containing 10pg of clavulanate) positioned at 30mm from center to center investigated ESBL production. However, ESBL production was shown by a clear cut enhancement of the zone of inhibition of ceftriaxone (champagne cork or keyhole) infront of clavulanate containing disc.

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2.5.9 Waste management

Urine samples were disinfected with 0.1% sodium hypochlorite solution for 10 minutes and then emptied into a draining sink. Used culture plates were autoclaved and sent to the incinerator together with other waste materials.

2.6 Statistical analysis

Research results and data were subjected to logistic regression and chi-square test using the EPI info version 7.2 statistical software (CDC/WHO, Atlanta, USA).

2.7 Ethical Consideration

Ethical approval to conduct this study was obtained from the Institutional Review Board (IRB) of the faculty of health sciences, University of Buea, Cameroon. Also, approval to carry-out the study at CHUY was granted by the director general of the institution. Participants received a full explanation of the study objectives and were reassured of the safety and confidentiality of their profile. All participants filled and signed an informed consent form. With approval from the IRB, pregnant women aged less than the legal age of consent in Cameroon (21years) and attending antenatal care services were considered emancipated minors and thus allowed to provide informed consent for themselves. Test result was given to participants exactly 4days after their sample were collected for appropriate management. All research protocols were performed in accordance with the ethical standards of committees on human experimentation laid down in the Helsinki declaration of 1964 revised in 2000 [27].

III. RESULTS

3.1 Participants' characteristics

Eighty four pregnant women attending the antenatal clinic of CHUY were selected in the study upon obtaining their informed consent. Most of the study participants were aged 21-30 years (59.21%), married (54.55%), had completed the university (62.82%), were in the third trimester (55.13%) and lived in urban settlements (95.3%). Participants without at least 2 days hospitalization and had no history of recurrent UTI were considered outpatients (96.10%). 17.11% of study participants had history of UTI, 41.03% admitted they consumed over 2l of water daily and 39.74% used non-cotton pants (Table 6).

Table 6: Participants' data and analysis

Characteristics	**Level**	**Frequency**	**Significant**	**Bivariate analysis**			
		(%)	**bacteriuria (%)**	Odds ratio	UCL	LCL	Pvalue
Age	<21	2.63	00				
	21-30	59.21	30	1.5	0.5	4.7	0.5
	31-40	38.16	24	0.8	0.3	2.5	0.7
	41-50	00	00				
Civil status	Married	54.55	27.0	1.0	0.3	3.0	1.0
	single	45.45	26.7	1.0	0.3	3.0	1.0
Level of education	Primary	2.56	00				
	Secondary	34.62	31.8	1.5	0.5	4.6	0.5
	university	62.82	24.4	0.7	0.2	2.3	0.6
Pregnancy trimester	First	12.82	50	3.5	0.9	13.8	0.07
	Second	32.05	21.1	0.7	0.2	2.4	0.5
	Third	55.13	23.1	0.7	0.2	2.0	0.5
Patient status	Inpatient	3.9	00				
	outpatient	96.1	28.1				

Table 6 continued: Participants' data and analysis

Characteristics	**Level**	**Frequency**	**Significant**	**Bivariate analysis**			
		(%)	bacteriuria (%)	Odds ratio	UCL	LCL	Pvalue
Clinical presentation	Symptomatic	64.86	30.4				
	Asymptomatic	35.14	34.6				
Bacteriuria	Yes	22.6					
	No	73.8					
Pyuria	Yes	24.59	66.7	5.7	1.6	20.0	0.004
	No	75.41					
History of UTI	Yes	17.11	23.1	0.8	0.2	3.5	0.8
	No	82.89					
Hematuria	Yes	1.32	00				
	No	98.86					
Dysuria	Yes	6.58	60	4.6	0.7	30.2	0.09

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	No	93.42					
Polyuria	Yes	27.63	38.9	2.1	0.7	6.8	0.2
	No	72.37					
Frequency	Yes	67.11	20.9	0.4	0.1	1.3	0.1
	No	32.89					
Urgency	Yes	49.33	26.5	0.9	0.3	2.6	0.8
	No	50.67					
Pelvic and suprapubic pain	Yes	11.84	33.3	1.4	0.3	6.3	0.7
	No	88.16					
Back pain	Yes	55.84	33.3	2.1	0.7	6.4	0.2
	No	44.16					
≥2l of water consumed daily	Yes	41.03	28.6	1.2	0.4	3.6	0.7
	No	58.97					
Used cotton pants	Yes	39.74	21.4	0.6	0.2	2.0	0.4
	No	60.26					
Immunocompromised	Yes	37.18	19.2	0.5	0.2	1.7	0.3
	No	62.82					
Settlement	Urban	95.3					
	Rural	4.7					

3.2 Prevalence of bacteriuria

Early morning clean catch MSU samples collected from 84 pregnant women were cultured and 22 yielded significant bacteriuria giving a 26.2% prevalence of bacteriuria. 8 participants were asymptomatic and had significant bacteriuria giving a 9.5% prevalence of ASB. The percentage of ASB among bacteriuria cases was 36.4%. The overall prevalence of symptomatic bacteriuria was 16.7% (Figure 2). Further, bacteriuria was most prevalent in participants who were 21-30 years of age, mentioned married or had completed the university.

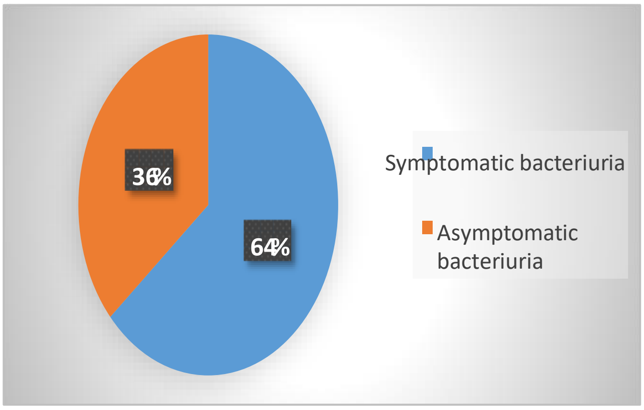

Figure 2. Occurrence of bacterial UTI

3.3 Predictors of bacteriuria

Table 6 shows the association between clinical and socio-demographic characteristics and bacteriuria in pregnant women. The bivariate analysis showed that first trimester pregnancy (OR=3.5; 95% CI=0.9-13.8; p=0.07), dysuria (OR=4.6; 95% CI=0.7-30.2; p=0.09) and pyuria (OR=5.7; 95% CI=1.6-20.0; p=0.004) had statistically significant association with bacteriuria. The strongest predictor of bacteriuria following multivariate analysis (Table 7) was pyuria (OR=6.7; 95% CI=1.4-30.8; p=0.01).

Table 7: Multivariate analysis of predictors

Predictor		**Multivariate analysis**		
	Odds ratio	Lower	Upper confidence limit	P-value
Dysuria	0.4	0.04	4.0	0.4
Pyuria	6.7	1.4	30.8	0.01

3.4 Uropathogens

Thirty bacteria isolates after significant growth were obtained and identified from 22 study participants. Monomicrobic and polymicrobic growth was seen in 12 (54.5%) and 10 (45.4%) positive cultures respectively. One isolate was equivalent to a pathogen isolated from one participant. The most commonly isolated uropathogen was *Escherichia coli (E. coli)* isolated from 8 participants (26.7% of isolates) followed by coagulase negative staphylococci (CoNS) from 8 participants (26.7%), *Klebsiella pneumoniae* from 4 participants (13.3%), *Proteus mirabilis* from 3 participants (10.0%), group D streptococci from 3 participants (10.0%), group B streptococci from 2 participants (6.7%), *Serratia marcescens* from 1 participant (3.3%) and *Staphylococcus aureus (S. aureus)* from 1 participant (3.3%) making a total of 30 bacteria isolates from the study (Table 8). Among the 8 CoNS isolates, 3 were *Staphylococcus saprophyticus* (10%). There were 16 gram negative isolates giving a 53.3% prevalence of gram negative bacterial UTI (Table 8). Among the ASB cases, the most frequently isolated uropathogen was *E. coli* isolated from 3 participants (27.3%), followed by CoNS isolated from 3 participants (27.3%), *Proteus mirabilis* isolated from 2 participants (18.2%), group D streptococci isolated from 1

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participant (9.1%), *Klebsiella pneumoniae* isolated from 1 participant (9.1%), and *serratia marcescens* isolated from 1 participant (9.1%) making a total of 11 isolates (Table 8).

The frequency of uropathogens associated with ASB is similar with that of symptomatic bacteriuria (Figure 3).

Table 8: Distribution of uropathogens in bacteriuria and ASB

Uropathogen	**Frequency in bacteriuria**		**Frequency in ASB**	
E. coli *K. pneumoniae* *P. mirabilis*	8 (26.7%) 4 (13.3%) 3 (10%)	Gram negative bacilli **N=16(53.3%)**	3 (27.3%) 1 (9.1%) 2 (18.2%)	Gram negative bacilli **N=7(63.6%)**
S. marcescens	1 (3.3%)		1 (9.1%)	
CoNS Group D streptococci	8 (26.7%) 3 (10%)	Gram positive cocci **N=14(46.7%)**	3 (27.3%) 1 (9.1%)	Gram positive cocci **N=4(36.4%)**
Group B streptococci	2 (6.7%)		00	
S. aureus	1 (3.3%)		00	
Total	**30 (100%)**		**11(100%)**	

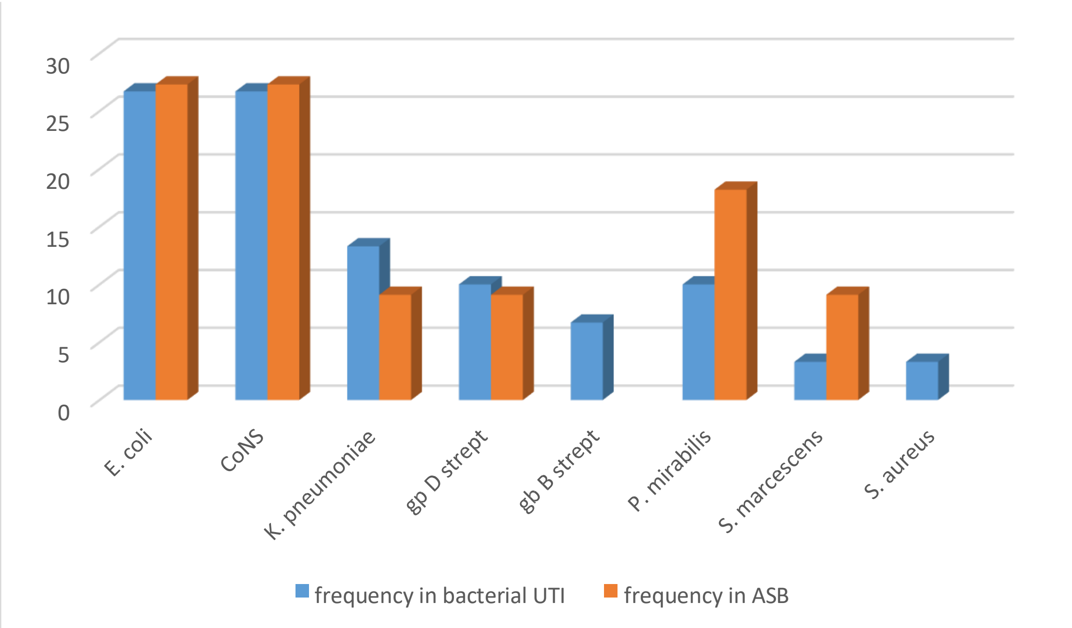

Figure 3. Frequency of bacteriuria and ASB

3.5 Antibiotic susceptibility testing

E. coli and *K. pneuminiae* isolates showed atleast 12.5% resistance to penicillin, cephalosporin and carbapenem beta-lactam antibiotic classes (Table 9). From table 9, *E. coli* and *K. pneumoniae* isolates however showed greatest resistance to amoxicillin, 75.0% and 100% respectively. More than 50% of *streptococci* isolates were resistant to gentamicin and none showed resistance to amoxicillin, imipenem and nitrofurantoin (Table 10a). Further, all *streptococcus agalatiae* isolates were resistant to ciprofloxacin while group D streptococci isolates showed a 33.3% resistance to cefuroxime (Table 10a). To continue, at least 33.3% and 20% of *staphylococci* isolates were resistant to methicillin and ciprofloxacin respectively (Table 10b). Also, no *staphylococci* isolate was resistant to nitrofurantoin and cefuroxime. However, all *S. saprophyticus* isolates were resistant to gentamicin and resistance to clindamycin was only seen with *S. saprophyticus* and *S. epidermidis* isolates (Table 10b). No drug resistance was seen with *proteus spp.* and *serratia spp*. Hence, of the 30 bacteria isolates obtained, only 4 showed no antibiotic resistance (13.3%) and 26 showed resistance to at least one antibiotic (86.7%).

Table 9: Antibiotic susceptibility for gram negative isolates

Antibiotics	*E. coli* isolates (8)		*K. pneumoniae* isolates (4)	
	Resistant isolates	% resistance	Resistant isolates	% resistance
Amoxicillinclavulanate	3	37.5	2	50.0
Amoxicillin	6	75.0	4	100.0
Ceftriaxone	3	37.5	2	50.0
Nitrofurantoin	0	00.0	0	00.0
Gentamicin	0	00.0	0	00.0
Ciprofloxacin	0	00.0	0	00.0
Imipenem	1	12.5	2	50.0

Table 10a: Antibiotic susceptibility for gram positive isolates (streptococci)

Antibiotics	Group D streptococci (3)		Group B streptococci (2)	
	Resistant isolates	% resistance	Resistant isolates	% resistance
Amoxicillin	0	00.0	0	00.0
Nitrofurantoin	0	00.0	0	00.0
Gentamicin	2	66.7	1	50.0
Cefuroxime	1	33.3	0	00.0
Ciprofloxacin	0	00.0	2	100
Imipenem	0	00.0	0	00.0

Table 10b: Antibiotic susceptibility of gram positive isolates (staphylococci)

Antibiotics	*S. saprophyticus* (3)		*S. aureus* (1)		*S. epidermidis* and other related *staphylococci* (5)	
	Resistant isolates	% resistance	Resistant isolates	% resistance	Resistant isolates	% resistance
Methicillin	1	33.3	1	100	2	40.0
Nitrofurantoin	0	00.0	0	00.0	0	00.0
Gentamicin	3	100	0	00.0	0	00.0
Cefuroxime	0	00.0	0	00.0	0	00.0
Ciprofloxacin	2	66.7	1	100	1	20.0
Clindamycin	3	100	0	00.0	1	20.0
Cephazolin	3	100	1	100	5	100
Vancomycin	0	00.0	0	00.0	0	00.0
Novobiocin	3	100	0	00.0	0	00.0

From figure 4, more than 20% of gram negative bacilli (GNB) were resistant to imipenem, amoxicillin-clavulanate, ceftriaxone and amoxicillin. However, GNB isolates were most resistant to amoxicillin (62.5%) and none was resistant to nitrofurantoin, gentamicin and ciprofloxacin. Equally, more than 20% of gram positive cocci (GPC) isolates were resistant to methicillin, gentamicin and ciprofloxacin while less than 10% of the isolates showed resistance to cefuroxime and nitrofurntoin (Figure 5). Further, the local empiric therapy for bacteriuria was gentamicin, nitrofurantoin, cefuroxime and ciprofloxacin. Six of thirty urinary isolates showed resistance to gentamicin giving a 20% percentage resistance of gentamicin to uropathogens. An overall percentage resistance of 00%, 20% and 20% was observed for nitrofurantoin, cefuroxime and ciprofloxacin respectively (Figure 6). Multidrug resistance (resistance to at least 2 or more antibiotic classes) was seen with 26 of the 30 uropathogens (86.7%). Extended-spectrum betalactamase (ESBL) production was observed in 3 of 16 gram negative isolates (18.75%). The percentage of ESBL producing GNB (Gram negative bacilli) among the isolated uropathogens was 10%. ESBL production was observed in only *E. coli* (n=1) and *klebsiella pneumoniae* (n=2). To conclude, of the 16 GNB isolates, 3 were ESBL producing (18.75%) and 13 were non-ESBL producing (81.25%). Similarly, of the 9 staphylococci isolates, 4 were methicillin resistant (44.4%) and 5 were non-methicillin resistant (55.6%).

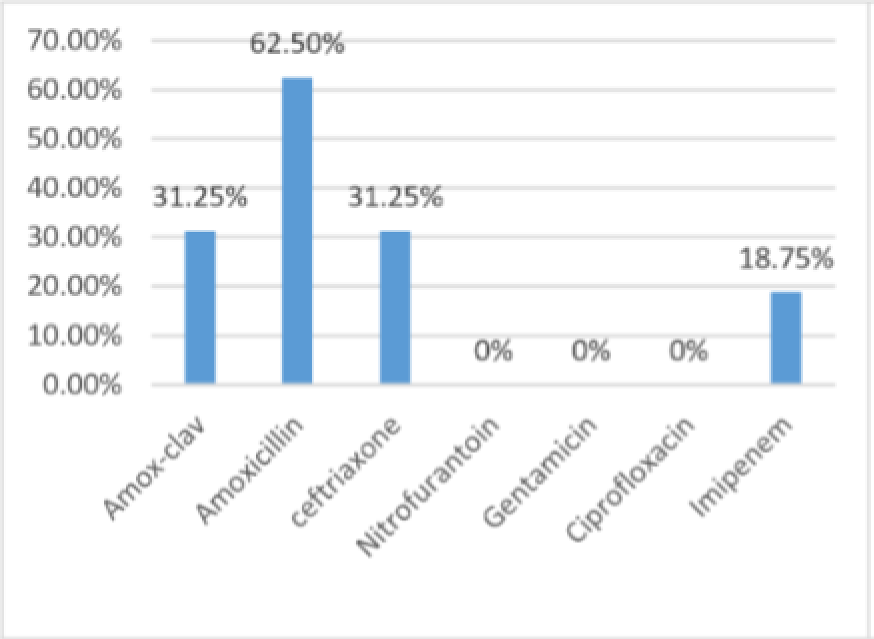

Figure 4: Percentage resistance of antibiotics to GNB

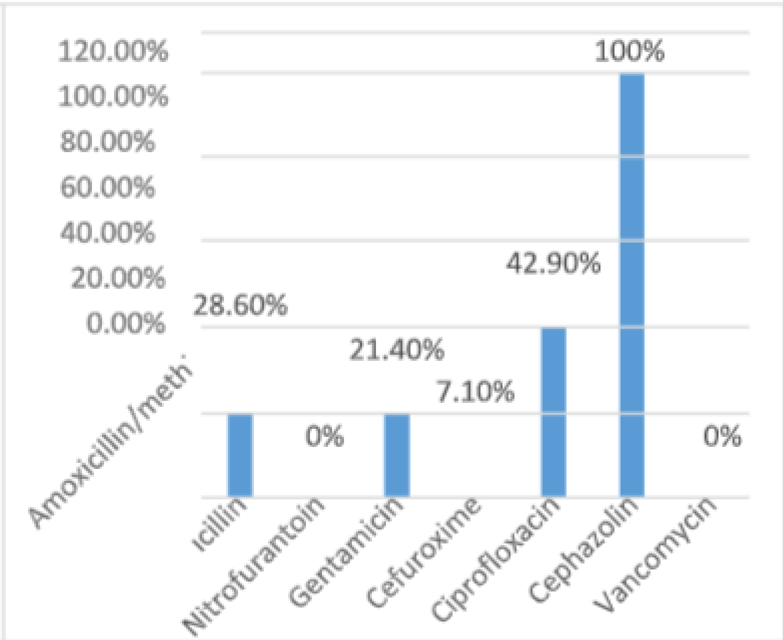

Figure 5: Percentage resistance of antibiotics to GPC

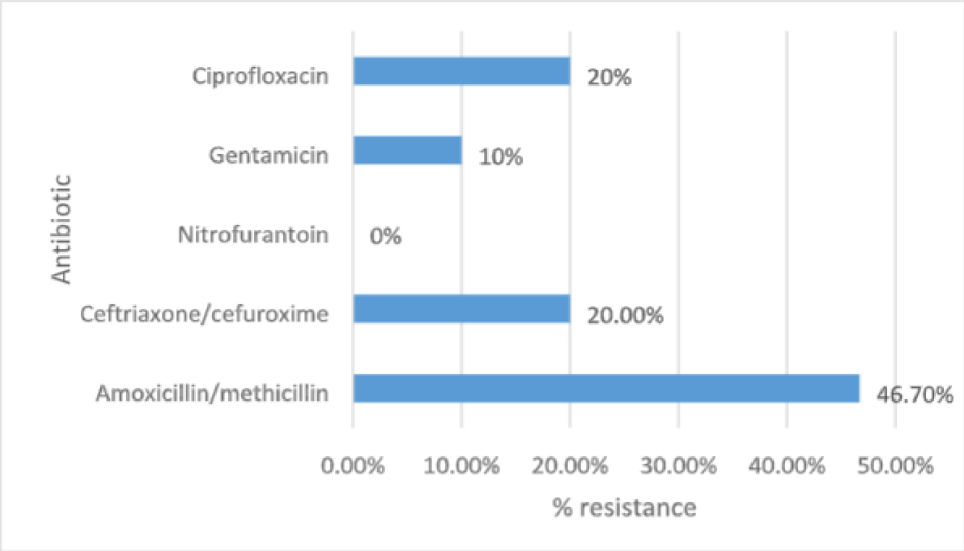

Figure 6: percentage resistance of antibiotics to uropathogens

IV. DISCUSSION

In this study, a relatively high prevalence of bacteriuria in pregnant women was recorded together with a more than 2% prevalence of ASB. *Escherichia coli* was the most frequently isolated uropathogen and nitrofurantoin was shown to be the best empiric therapy for bacteriuria in pregnancy. Following multiple logistic regression analysis, pyuria was the strongest predictor of bacteriuria. Also, 86.7% of isolated uropathogens was resistant to at least one antibiotic.

The prevalence of bacteriuria in pregnant women attending the antenatal clinic of CHUY was 26.2%. This is similar to that obtained by Morike et al. in a similar study carried out in the Buea health district (BHD), Cameroon [28]. This prevalence is higher than that obtained by Assefa et al. [29] and some other studies done in Tanzania (17.9%), Sudan (12.1%) and Saudi Arabia (12.0%) [30]. Equally, prevalence of bacteriuria in this study was lower than that reported by Okonko et al. in Nigeria. This variation may be as a result of the relatively small number of participants selected in this study. In addition, there was a 9.5% prevalence in ASB, which falls in the 2-10% range reported in other studies [29, 31]. Conversely, this prevalence of ASB is lower than that reported by authors in Kenya and elsewhere [32]. Variation in study results could be attributed to differences in gestational age, geographic location, socioeconomic status, setting of study (primary care, hospital or community) and variation in screening tests.

Risk factors associated with bacteriuria in pregnancy identified in other studies include history of UTI, age, anaemia, third trimester, level of education, low socioeconomic status, immunosuppressed persons and gravidity. In this study, the association of the following risk factors with bacteriuria was assessed: daily water consumption, history of UTI, immunocompromised state (HIV/AIDS, anemic, diabetic, cancer, undergoing radiotherapy or chemotherapy or on any immunosuppressive treatment), use of non-cotton pants, age and gestation period. None of the assessed risk factors was statistically significant in causing bacteriuria in pregnancy after simple logistic regression (bivariate) analysis. This agrees with findings made by Kehinde et al. [33] and Andabati and Byamugisha [29]. However, bivariate analysis showed the first trimester to be most likely associated with bacteriuria (OR=3.5; 95% CL=0.9-13.8; P=0.07). This correlates with findings from some Kenyan and Nigerian studies [42]. The following predictors were assessed in this study, hematuria, dysuria, pyuria, polyuria, frequency, pelvic and suprapubic pain, urgency and back pain. After the bivariate analysis, dysuria (OR=4.6; 95% CI=0.7-30.2; P=0.009) and pyuria (OR=5.7; 95% CI=1.6-20.0; P=0.004) showed significant association with bacteriuria and were subjected to multiple logistic regression (multivariate) analysis. Following the multivariate analysis, pyuria was the most significant predictor of bacteriuria in pregnant women (OR=6.7; 95% CI=1.4-30.8; P=0.01). This is in line with documented knowledge on this condition [2].

E. coli was the most common uropathogen isolated (26.7%). This correlates with findings made by Akoachere et al. [28] and Morike et al. in Buea [29], Pieboji et al. in Yaounde [34]. It is further in line with the ECOSENS report from Europe and Canada [28] and The Surveillance Network (TNS) study from the United States [28]. This may stem from the fact that *E. coli* is the most common microorganism in the vaginal and rectal area coupled with the anatomical and functional changes accompanying pregnancy and difficulty of maintaining good personal hygiene (due to stomach distension). On the other hand, a study carried-out by Akoachere et al. in Bamenda [28] found that *Klebsiella oxytoca* was the most prevalent pathogen of the UT. Variation in bacteria ecology associated with geographic location and some other host factors could account for this differences. CoNS were the second most common uropathogen isolated. This is in line with findings from Akoachere et al. [28] and Behailu et al. [30]. However, it is contrary to the low prevalence of gram positive pathogens (3.6%) obtained by Rolf et al. [34]. Further, GNB constituted the majority of etiologic agents of bacteriuria in pregnancy (53.3%). A similar finding was made in studies from Cameroon and abroad [29, 30]. In this study, contamination of urine specimen was greatly reduced by supervision of specimen collection using expounded aseptic techniques and immediate transport to the laboratory and analysis. *K. pneumoniae, P. mirabilis*, group D streptococci, group B streptococci, *S. marcescens* and *S. aureus* add up to the uropathogens outlined in this study.

Every isolate showed 100% susceptibility to nitrofurantoin making it the best empiric therapy for treating bacteriuria in pregnancy, including ASB. This correlates with findings by Okonko et al. [29], Akoachere et al. [28] and differs from those by Morike et al [29] whose isolates had poor sensitivity to nitrofurantoin. This may be as a result of regional variation in bacteriuria etiologies as well as their susceptibility to antibiotic agents. *E. coli* and *Klebsiella* isolates showed greatest resistance to penicillins (87.5% and 100% respectively). This may be due to occurrence of ESBLproducing bacteria. However, a similar resistance pattern to penicillins was observed by Rolf N. et al. in a study carried out in Yaounde, Cameroon [34]. Summarily, GNB were least resistant to gentamicin and ciprofloxacin and the gram positives showed least resistance to cefuroxime. This result is similar with findings from Behailu et al. [30]. Uncontrolled consumption of antibiotics via self-medicating, random sale and prescription of antibiotics could be the reason for high resistance to some antibiotics recorded in this study. Resistance to at least 2 or more antibiotic classes was observed with 86.7% of isolates. This was lower than the multidrug resistance level

(MDR level of 95%) reported in Gondar, Ethiopia and higher than that reported in Tikur, Ethiopia (74%) [30]. This indicates that MDR was found to be high in this study. *Staphylococcus saprophyticus* showed MDR with the following drug classes: aminoglycosides, fluoroquinolones, lincosamides and first generation cephalosporins. A similar pattern of MDR was seen in findings of Behailu et al. Antibiotic resistance has been recognized as the consequence of repeated antibiotic use and abuse [30]. Nevertheless, the reasons for these alarming phenomenon might be due to inappropriate and incorrect administration of antimicrobial agents in empiric treatment.

The identification of risk factors of bacteriuria in pregnancy in this study was limited by the relatively small sample size. Also, not all the factors which could be associated with bacteriuria in pregnancy were assessed. Factors like parity, gravidity and socioeconomic status were not assessed. Further, pregnant women were not followed up to determine their risk of developing overt bacteriuria and other adverse outcomes. However, this study was limited to just one Cameroonian city though, its findings were consistent with those from other studies [28, 29, 34]. To continue, there was no evidence to show that in vitro resistance to antibiotics was indicative of treatment failure. Moreover, a control bacteria strain wasn't implemented in the antibiotic susceptibility testing. To conclude, limited resources prevented the acquisition of a larger sample that might better represent the population of Cameroon.

V. CONCLUSION

The overall prevalence of bacteriuria among pregnant women was 26.2%. It was higher in pregnant women with symptoms of acute UTI (16.7%) than in those without symptoms (9.5%). *E. coli* (26.7%), CoNS (26.7%), *Klebsiella pneumoniae* (13.3%), *Proteus mirabilis* (10%) and group D streptococci (10%) were common uropathogens. Pyuria was the strongest predictor of bacteriuria. However, there was no statistically significant risk factor of bacteriuria thus, limiting the possibility of targeted screening. GNB were least resistant to gentamicin and ciprofloxacin while the gram positives showed least resistance to cefuroxime. All isolates were susceptible to nitrofurantoin. To add, this study reveals that bacteriuria occurs in one of every four pregnant women and ASB occurs in one of every ten pregnant women. This is of great concern since bacteriuria is harmful to both mother and fetus.

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