



Original Article

# Immunological Profile and Bacterial Drug Resistance in Pregnant Women: A Cross Sectional Study

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## ABSTRACT

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### Keywords:

antibiotic resistance,  
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**Objectives:** This study aimed to investigate the immunological and bacterial profiles in pregnant women of Bafang-Cameroon.

**Methods:** Stool and midstream urine were cultured using specific culture media. The disk diffusion method was used for the antimicrobial susceptibility test. T-cell lymphocyte counts (CD3, CD4 and CD8), white blood cell counts, sensitive C-reactive protein, and interleukin-6, were measured by flow cytometry, optical detection, and the enzyme-linked immunosorbent assay solid phase direct sandwich method.

**Results:** Out of 700 participants, 71.43% were pregnant, and 28.57% were non-pregnant women. The mean age was  $29.40 \pm 8.27$  and  $27.41 \pm 6.55$  years in non-pregnant and pregnant women, respectively. CD4 T-cells were not significantly lower in pregnant women compared with non-pregnant women. There were 43.65% and 56.35% bacteria isolates obtained from urine and stool samples, respectively. Bacteria were mostly isolated in patients with a CD4 T-cell count between 461 and 806 cells/ $\mu$ L. Isolates of *Klebsiella pneumoniae* and *Enterobacter aerogenes* showed 100% resistance in non-pregnant women, however all isolated bacteria were shown to be multidrug resistant in pregnant women. *Salmonella sp.* (24.3%) and *Escherichia coli* (21.51%) showed an increase in multidrug resistant phenotypes in pregnant women.

**Conclusion:** This study demonstrated that routine bacteriological analysis during pregnancy is necessary for their follow-up care.

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## Introduction

Bacterial infections involving a variety of pathogens like *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus* [1] can occur during pregnancy. Such infections are associated with an increased risk of miscarriage, stillbirth or neonatal death [2]. In pregnant women, bacterial infections can alter the implantation of the fertilized ovum during consignment and peripartum periods, or affect the fetus as well as the new-born [3]. Many women with these infections are asymptomatic, which requires both

a high level of clinical awareness and adequate screening. This situation is much more complicated when multidrug resistant bacteria are involved during pregnancy [4].

Bacterial resistance to antimicrobial agents during pregnancy is an increasing health concern [5]. Pregnancy causes numerous changes in a woman's body. During pregnancy, the body's immune system is reduced. Hormonal and mechanical changes increase the risk of urinary stasis and vesicoureteral reflux. These changes, along with an already shortened urethra and difficulties maintaining strict hygienic conditions due to a distended pregnant belly, increase the frequency of urinary

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tract infections in pregnant women [6]. There is a decrease in bacterial susceptibility to almost all available antibiotics [7]. The 2013 report of United States Centers for Disease Control and prevention declared that at least 2 million people in the USA acquired infections with resistant bacterial phenotypes, with at least 23,000 people dying yearly because of these infections [8]. It was demonstrated in 2014 that, 7.29 % ( $n = 482,917$ ) of women received outpatient care for urinary tract infections during the 90 days before the date of their last menstrual period or during pregnancy [9]. Given the potential risks associated with the use of some of these antibiotics in early pregnancy and the potential unrecognized pregnancy, women's healthcare providers should be familiar with the American College of Obstetricians and Gynecologists recommendations and consider the possibility of early pregnancy when treating women of reproductive age [10].

In Africa in general, although controlling the spread of resistant bacteria is a priority, an update to determine the extent of the resistance phenomenon in pregnant women in various regions is necessary. The objective of this work was to study the hematological and epidemiological profiles of pathogenic bacteria from the enteric and urinary tracts of pregnant women in the town of Bafang, West Region of Cameroon, while also determining the extent of antibiotic resistance during pregnancy.

## Materials and Methods

### 1. Study framework

This was a cross-sectional epidemiological study carried out over a period of 3 years (from November 2016 to September 2019) in pregnant women (without distinction of gestational age of their fetus) who attended antenatal consultation in health centers in Bafang (Western Region of Cameroon) including Adluem Banka Bafang, District Hospital, Dokovie Bafang Annex Centre. Pregnant women with hepatitis or HIV were not included in this study. Non-pregnant women who did not have HIV or hepatitis were also included in the study.

### 2. Biological material

Urine, blood, and stool samples were collected from 700 voluntary patients (500 patients were pregnant women, and 200 were not pregnant) who were hospitalized or receiving consultations whilst not receiving antibiotic therapy, and had given written informed consent. Sociodemographic information (age, marital status, gestational age, activity carried out, total number of pregnancies) was obtained, and questionnaires were administered. Duplicate questionnaires were systematically eliminated.

### 3. Collection of samples

In this study, samples (blood, urine, and stool) were collected under aseptic conditions for 700 women and were analyzed within 2 hours. There was 10 mL of blood collected directly into 3 tubes; 2 containing the anticoagulant (EDTA) and 1 tube without anticoagulant. The blood samples were sent directly for analysis of white blood cell counts, C-reactive protein (CRP), interleukin-6 (IL-6), and CD4, CD3, and CD8-T lymphocytes. Stool and urine samples were sent for microbiological analyses.

### 4. Analysis of blood samples

The blood in the EDTA tube was analyzed after being homogenized (to avoid the formation of blood clots), using a cell counter (Mindray PE 6800, PROCAN, China, Mainland) for the white blood cells which gave the total number of white blood cells, lymphocytes, monocytes, and granulocytes. The counting of CD4, CD3 and CD8 T-lymphocytes was carried out by flow cytometry using a FACS Calibur machine (Becton Dickinson, San Jose, CA, USA). Depending on the number of cells obtained, the cells were stained at room temperature for 30 minutes with 100 mL of anti-CD4 and PE-anti-CD8 monoclonal antibodies labelled with fluorescein isothiocyanate, then washed once in the FACS buffer. The CD4 and CD8 surface expression was visualized using flow cytometry. Lymphocytes were defined by the sideward and forward diffusion parameter, and they were controlled in such a way that cells in the defined window could be taken into consideration. The flow cytometry data were analyzed within 24 hours of staining using software (FlowJo). The results were expressed as percentage of positive flow cytometry for CD4 and CD8. The measurement of IL-6 was performed using Enzyme-Linked Immunosorbent Assay (ELISA) kits (R&D system, London, UK), according to the manufacturer's instructions. Blood collected in the tube without an anticoagulant was analyzed by spectrophotometry for the quantification of CRP (Actim CRP, the Medix Biochemica Company, Finland). The highly sensitive (hs)-CRP was measured in duplicate by a high sensitivity ELISA, as described above. The sensitivity of the assay was 0.2 ng/mL. The intra and the inter assay variability were measured as 3.9% and 7.4%, respectively.

### 5. Bacterial isolation

*Salmonella-Shigella* agar, Manitol salt agar, Hecktoen agar, Eosin Methylene Blue, Cystine Lactose Electrolyte Deficient, Muller Hinton agar and the MacConkey agar were used for the isolation and phenotypic identification of bacteria. The following antibiotic discs were used: amikacin (AMI, 30 µg), imipenem (IMI, 10 µg), amoxicillin + clavulanic acid (AUG, 20 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR 10 µg), ceftriaxone (CEFT, 30 µg), erythromycin (ERY, 15 µg), doxycycline (DOX, 30 µg), vancomycin (VAN, 30 µg)

chloramphenicol (CHL, 30 µg) amoxicillin (AMO, 10 µg), cefuroxime (CEFU, 30 µg), gentamicin (GEN, 30 µg), tetracycline (TET, 30 µg), nitrofurantoin (NIT, 100 µg), ofloxacin (OFL, 5 µg), and sulfamethoxazole + trimethoprim (SUL, 25 µg). Super cooled agar culture media were poured into tri-segmented petri dishes (90 mm). After the solidification of the culture media at room temperature, the liquid stool samples were directly streaked into the culture media, while the urine samples were flooded onto the culture media, and incubated at 37°C for 24 hours. Solid stools were initially dissolved in sterile physiological water. The bacterial colonies of the stool observed on the surface of the culture media after incubation, were purified by successive sub-culturing until pure colonies or clinical isolates were obtained. The conventional method of interpretation of bacterial colonies of urine (criteria defined by Kass) was used and the parameters of the urinary infection such as leukocyturia > 10<sup>4</sup> /mL, and bacteriuria in colony forming unit (CFU) per mL > 10<sup>5</sup> per mL (threshold of E coli was lowered to 10<sup>3</sup> CFU/mL and considered to be uropathogenic) were taken into consideration. After macroscopic observation of the colonies, the identification of bacteria, based on the study of biochemical and enzymatic characters, was performed using the API 20E galleries (bio Mérieux SA, Marcy L'Etoile, France).

## 6. Antibiotic susceptibility test

The susceptibility study of the different isolates was carried out using the Mueller-Hinton agar diffusion technique according to the protocol recommended by the Clinical and Laboratory Standard Institute [11] with some modifications. A volume of 2 mL of bacterial suspension (1.5 × 10<sup>6</sup> CFU/mL) was spread on the surface of a 90 mm Petri dish previously cast with Mueller-Hinton agar. This was then placed in a hot air oven for 5 minutes and the antibiotic discs selected accordingly. Clinical isolates were placed on the surface of the agar using sterile forceps. After 15 minutes, the petri dish was incubated for 24 hours at 37°C, and the interpretation was made according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2018), followed by measurement of the diameters of the inhibition zones using a graduated ruler.

## 7. Ethical approval

Ethics approval was granted by the Ethics Review and Advisory Committee of CAMBIN for this study on the 24<sup>th</sup> April 2019 (CBI/437/ERCC/CAMBIN) and approval from the director of the Bafang Health Area was granted. Information sheets detailing the purpose and process of the study were provided to each participant. Participants gave written and informed consent for voluntary participation.

## 8. Processing and statistical analysis of data

In establishing the percentages of resistance of the different bacterial species, the results were described as "intermediate," "resistant," and "susceptible." Descriptive analysis of the data was performed using SPSS Version 20 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2010. Proportions were compared using the chi-square test. Student t test was used to compare continuous variables. A value of  $p < 0.05$  was considered statistically significant.

## Results

There were 700 patients studied, 500 (71.43%) were pregnant women and 200 (28.57%) were non-pregnant women who gave stool and/or urine samples for analysis. These patients included women who were in a relationship (married, cohabiting) or single (single, separated, divorced, widowed). Table 1 shows that 324 (73.80%) pregnant women were in a relationship compared with 115 (26.20%) non-pregnant women. There were 176 (67.43%) pregnant women who were single, compared with 85 (32.57%) non-pregnant women. There was a statistically significant difference in the distribution of the age groups in the study ( $p = 0.001$ ). The mean age of the participants was higher in non-pregnant women (29.40 ± 8.265 years) compared with pregnant women (27.41 ± 6.547 years).

Table 2 shows the mean values of the blood tests obtained in the study population, as well as the white blood cell, CD4, CD3 and CD8 T-lymphocytes, hs-CRP and IL-6 levels. Results show that, CD4 T-cell counts were lower but not significantly lower in pregnant compared with non-pregnant women. Contrarily, total white blood cells ( $p = 0.782$ ), granulocytes ( $p = 0.905$ ), CD8 T-cell ( $p = 0.490$ ) and hs-CRP ( $p = 0.421$ ) were higher but non-significant in pregnant women compared to those who were non-pregnant. A total of 252 bacterial isolates were obtained in this study, of which 187 (74.20%) and 65 (25.80%) isolates were obtained from pregnant and non-pregnant women, respectively. A total of 110 (43.65%) isolates were obtained from urine samples, whereas 142 (56.35%) isolates were obtained from stool samples.

Figure 1 shows that, *Shigella sp.*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Escherichia coli* were isolated more frequently in stool samples from pregnant women with values of 26 (86.66%), 17 (68%), 13 (65%), 16 (22.54%), respectively, compared with non-pregnant women with values of 4 (13.34%), 7 (28%), 7 (35%), 4 (5.63%), respectively. On the other hand, *Escherichia coli*, *Salmonella sp.* and *Klebsiella pneumoniae* isolates were identified more in urine samples from pregnant women with 39 (54.93%), 31 (35.63%) and 1 (4%), compared with non-pregnant women with 12 (16.9%), 8 (9.2%) and 0%,

Table 1. Demographic profile of patients according to pregnancy status.

Parameters		Pregnant women n = 500	Non-pregnant women n = 200	p
Gestational trimester	First	139 (27.80)		NA
	Second	189 (37.80)	NA	
	Third	172 (34.40)		
Relationship status	Married (n = 439)	324 (73.80)	115 (26.20)	0.071
	Single (n = 261)	176 (67.43)	85 (32.57)	
Age (y)	14-24 (n = 237)	176 (74.26)	61 (25.74)	0.001
	25-34 (n = 334)	248 (74.25)	86 (25.75)	
	35-44 (n = 110)	71 (64.55)	39 (35.45)	
	45-54 (n = 19)	5 (26.32)	14 (73.68)	
Age (y) (min-max)	Total	27.41 ± 6.54 (14-51)	29.40 ± 8.26 (16-53)	0.001
	Couple	28.54 ± 6.36 (16-51)	32.12 ± 8.22 (17-53)	
	Single	25.33 ± 6.39 (14-42)	25.72 ± 6.79 (16-47)	

NA = not applicable.

Data are presented as n (%) or average ± SD.

Table 2. White blood cell count results, CD4, CD3, CD8 T-cell count, hs-CRP and IL-6 of pregnant and non-pregnant women.

Parameters	Pregnant Women (n = 500)		Non- Pregnant Women (n = 200)		p
	Average values ± SD	Range (min-max)	Average values ± SD	Range (min-max)	
Total WBC	7.86 ± 3.53	0.90-31.20	7.79 ± 3.29	1.60-28.80	0.782
Lymphocytes	2.08 ± 0.94	0.10-6.30	2.14 ± 1.69	0.30-22.20	0.537
Monocytes	0.57 ± 0.54	0.10-8	0.58 ± 0.49	0.10-5.30	0.757
Granulocytes	5.17 ± 3.07	0.10-18.8	5.14 ± 3.07	0.20-23.40	0.905
CD4 T-cell	540.70 ± 160.64	114-1056	779.66 ± 176.25	336-1479	0.063
CD3/CD4 T-cell	716.74 ± 323.50	91-1,520	742.39 ± 318.13	91-1520	0.341
CD8 T-cell	312.81 ± 223.08	10-805	299.84 ± 226.83	1-812	0.490
hs-CRP	6.26 ± 6.89	0-50.40	5.83 ± 4.86	0.10-48	0.421
IL-6	111.86 ± 86.16	25-640	120.92 ± 90.11	25-640	0.230

IL-6 = interleukine 6; hs-CRP = high sensitive C-reactive protein; WBC = white blood cells.

respectively.

Table 3 shows the frequency of bacteria isolates and their association with different blood parameter intervals. It is seen that bacteria was isolated more frequently in patients with a CD4 T-cell count between 461 and 806 cells/ $\mu$ L. Isolates of *Shigella sp.* were higher (69.23%) in pregnant women with a CD4 T-cell count between 114-460 cells/ $\mu$ L and p value of 0.317. In contrast, in CD3 and CD8 T-lymphocytes, *Shigella sp.* isolates were higher, that is, between 569-1,045 (46.15%) and

10-277 (46.15%) respectively. However, more bacteria were isolated from patients with serum IL-6 levels 25-230 (pg/mL). *Salmonella sp.* isolates were also higher (95.16%) but not significantly, in pregnant women with serum IL-6 levels 25-230 (pg/mL).

The susceptibility of the isolates obtained from 14 different antibiotics was assessed in this study. Table 4 shows the susceptibility results of the isolates to these antibiotics. It can be seen that *Salmonella sp.* and *E coli* were susceptible to IPM

Table 3. Frequency of bacterial isolates obtained in different blood parameters intervals.

Bacterial Species	CD4 T-cell count Unit/ $\mu$ L				CD3/CD4 T-cell count Unit/ $\mu$ L				CD8 T-cell count Unit/ $\mu$ L				IL-6 count (pg/mL)			hs-CRP count (mg/L)				
	114-460	461-806	807-1152	p	91-568	569-1045	1046-1522	p	10-277	278-544	545-811	p	25-230	231-435	436-641	p	0-17	17.10-34	34.10-51	p
<i>Enterobacter aerogenes</i>	1	9	3	0.534	2	6	5	0.469	5	3	5	0.469	13	0	0	0.315	13	0	0	0.336
<i>Klebsiella pneumoniae</i>	6	12	0	0.429	7	8	3	0.519	8	8	2	0.429	16	1	1	0.431	17	1	0	0.350
<i>E. coli</i>	22	28	5	0.388	12	31	12	0.944	30	11	14	0.861	47	8	0	0.624	51	3	1	0.336
<i>Shigella sp.</i>	12	12	2	0.224	8	12	6	0.370	12	6	8	0.370	24	2	0	0.606	25	1	0	0.268
<i>Salmonella sp.</i>	17	41	4	0.190	21	31	10	0.442	34	15	13	0.702	59	3	0	0.098	61	1	0	0.562

hs-CRP = high sensitive C-reactive protein.

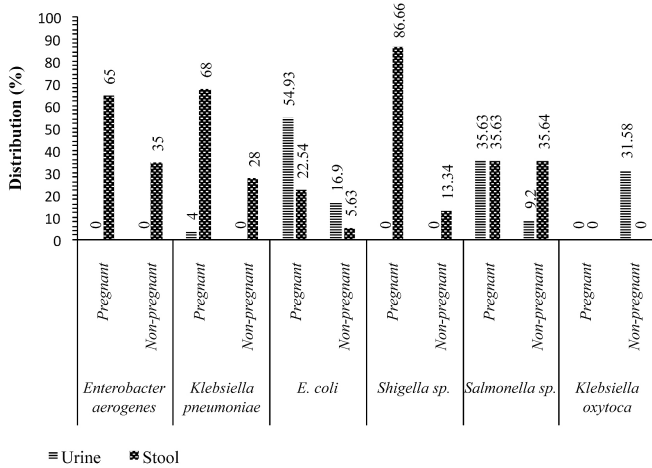


Figure 1. Distribution of bacterial pathogens amongst patients enrolled in the study.

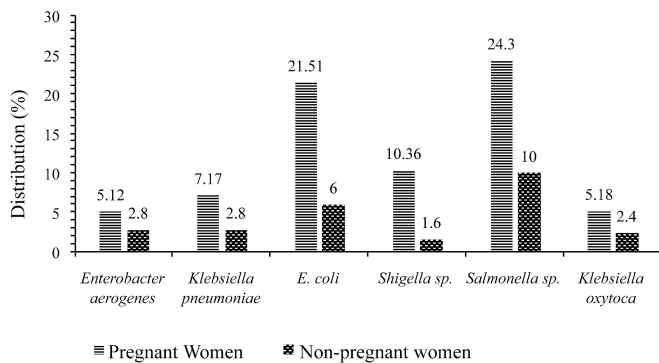


Figure 2. Frequency of occurrence of multidrug resistant bacteria isolated from pregnant and non-pregnant women.

(51.61%), (78.18%); CIP (59.68%), (70.9%); CHL (46.77%), (81.82%); AMO (72.58%), (54.55%); TET (69.35%), (74.55%); AUG (53.23%), (87.27%); and CEFT (56.45%), (76.36%); respectively, in pregnant women. On the other hand, isolates of *Klebsiella pneumoniae* and *Enterobacter aerogenes* showed 100% resistance in non-pregnant women.

Figure 2 shows the frequency of multidrug resistance of different isolates in pregnant and non-pregnant women. All bacteria show multidrug resistance in pregnant women compared to non-pregnant women. It can be seen that isolates of *Salmonella sp.* (24.3%) and *Escherichia coli* (21.51%) showed an increase multidrug resistance in pregnant women.

### Discussion

Pregnancy increases the protentional incidence of many bacterial and viral diseases by suppressing immunity. In turn this increased susceptibility may lead miscarriages caused by infections or birth defects in the fetus [10]. Pregnancy is a state in which the immune system does not mount an immune response against foreign paternal antigens of the fetus, but needs to protect the mother and the fetus from invading pathogens. In the present study, most pregnant women were in the age groups 25-34 (74.25%), and 14-24 (74.26%). This result was similar to the statistics reported by Essiben et al in 2017 [12] with an estimated pregnancy prevalence of 31.8% in 20-25 year old's in 2019. The mean pregnancy age in this current study was 27.41 ± 6.547 years, similar to that observed by Frauke et al in 2009 [13].

The high average age of clinical presentation of pregnancy in Bafang, West Cameroon can be explained by the culture of family values that prepare young boys and girls to become responsible adults, through various rituals, and close family

Table 4. Antibiotic resistance profile of bacterial isolates from pregnant and non-pregnant women.

Antibiotic		Bacterial strains in pregnant (a), non-pregnant (b) women and frequency (%)											
		<i>Enterobacter aerogenes</i>		<i>Klebsiella pneumoniae</i>		<i>E. coli</i>		<i>Shigella sp.</i>		<i>Salmonella sp.</i>		<i>Klebsiella oxytoca</i>	
		a	b	a	b	a	b	a	b	a	b	a	b
IPM	R	2 (15.38)	0 (0.0)	4 (22.22)	1 (14.29)	43 (78.18)	14 (87.5)	6 (23.08)	1 (25)	32 (51.61)	13 (52)	13 (100)	5 (83.33)
	I	2 (15.38)	0 (0.0)	2 (11.11)	0 (0.0)	6 (10.9)	1 (6.25)	8 (30.77)	0 (0.00)	7 (11.29)	4 (16)	0 (0.00)	0 (0.00)
	S	9 (69.23)	7 (100)	12 (66.66)	6 (85.71)	6 (10.9)	1 (6.25)	12 (46.15)	3 (75)	23 (37.1)	8 (32)	0 (0.00)	1 (16.67)
CIP	R	4 (30.77)	4 (57.14)	6 (33.33)	4 (57.14)	39 (70.9)	9 (56.25)	4 (15.38)	2 (50)	37 (59.68)	12 (48)	12 (92.3)	6 (100)
	I	4 (30.77)	0 (0.00)	3 (16.67)	2 (28.57)	5 (9.09)	3 (18.75)	6 (23.08)	1 (25)	6 (9.68)	1 (4)	0 (0.00)	0 (0.00)
	S	5 (38.46)	3 (42.86)	9 (50)	1 (14.29)	11 (20)	4 (25)	16 (61.54)	1 (25)	19 (30.65)	12 (48)	1 (7.69)	0 (0.00)
CHL	R	13 (100)	7 (100)	13 (72.22)	7 (100)	45 (81.82)	11(68.75)	26 (100)	4 (100)	29 (46.77)	17 (68)	1 (7.69)	0 (0.00)
	I	0 (0.00)	0 (0.00)	2 (11.11)	0 (0.00)	6 (10.9)	2 (12.5)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (23.08)	1 (16.67)
	S	0 (0.00)	0 (0.00)	3 (16.67)	0 (0.00)	4 (7.27)	3 (18.75)	0 (0.00)	0 (0.00)	29 (46.77)	17 (68)	9 (69.23)	5 (83.33)
DOX	R	10 (76.92)	7 (100)	12 (66.66)	4 (57.14)	14 (25.45)	4 (25)	23 (88.46)	3 (75)	38 (61.29)	16 (64)	3 (23.08)	3 (50)
	I	1 (7.69)	0 (0.00)	2 (11.11)	0 (0.00)	1 (1.82)	0 (0.00)	0 (0.00)	0 (0.00)	2 (3.23)	1 (4)	5 (38.46)	0 (0.00)
	S	2 (15.38)	0 (0.00)	4 (22.22)	3 (42.86)	40 (72.73)	12 (75)	3 (11.54)	1 (25)	22 (35.48)	8 (32)	5 (38.46)	3 (50)
AMO	R	12 (92.30)	6 (85.71)	16 (88.89)	6 (85.71)	30 (54.55)	9 (56.25)	23 (88.46)	4 (100)	45 (72.58)	20 (80)	1 (7.69)	1 (16.67)
	I	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (5.45)	2 (12.5)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4)	0 (0.00)	0 (0.00)
	S	1 (7.69)	1 (14.29)	2 (11.11)	1 (14.29)	22 (40)	5 (31.25)	3 (11.54)	0 (0.00)	17 (27.42)	4 (16)	12 (92.3)	5 (83.33)
GEN	R	0 (0.00)	1 (14.29)	2 (11.11)	0 (0.00)	16 (29.09)	3 (18.75)	0 (0.00)	0 (0.00)	22 (35.48)	6 (24)	7 (53.85)	3 (50)
	I	1 (7.69)	0 (0.00)	0 (0.00)	0 (0.00)	4 (7.27)	1 (6.25)	0 (0.00)	0 (0.00)	2 (3.23)	0 (0.00)	2 (15.38)	1 (16.67)
	S	12 (92.30)	6 (85.71)	16 (88.89)	7 (100)	35 (63.64)	12 (75)	26 (100)	4 (100)	38 (61.29)	19 (76)	4 (30.77)	2 (33.33)
COT	R	2 (15.38)	2 (28.57)	3 (16.67)	3 (42.86)	35 (63.64)	10 (62.5)	9 (34.62)	4 (100)	24 (38.71)	10 (40)	5 (38.46)	2 (33.33)
	I	1 (7.69)	1 (14.29)	1 (5.56)	0 (0.00)	0 (0.00)	0 (0.00)	1 (3.85)	0 (0.00)	8 (12.9)	3 (12)	4 (30.77)	3 (50)
	S	10 (76.92)	4 (57.14)	14 (77.78)	4 (57.14)	20 (36.36)	6 (37.5)	16 (61.54)	0 (0.00)	30 (48.39)	12 (48)	4 (30.77)	1 (16.67)
OFL	R	8 (61.54)	5 (71.43)	13 (72.22)	5 (71.43)	17 (30.91)	5 (31.25)	18 (69.23)	3 (75)	46 (74.19)	19 (76)	6 (46.15)	3 (50)
	I	3 (23.08)	1 (14.29)	0 (0.00)	1 (14.29)	7 (12.73)	4 (25)	4 (15.38)	0 (0.00)	6 (6.45)	0 (0.00)	0 (0.00)	2 (33.33)
	S	2 (15.38)	1 (14.29)	5 (27.78)	1 (14.29)	31 (56.36)	7 (53.85)	4 (15.38)	1 (25)	12 (19.35)	2 (24)	7 (53.85)	1 (16.67)
TET	R	7 (53.85)	4 (57.14)	14 (77.78)	3 (42.86)	41 (74.55)	11(68.75)	20 (76.92)	3 (75)	43 (69.35)	19 (76)	12 (92.3)	6 (100)
	I	0 (0.00)	0 (0.00)	1 (5.56)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (3.23)	0 (0.00)	0 (0.00)	0 (0.00)
	S	6 (46.15)	3 (42.86)	3 (16.67)	4 (57.14)	14 (25.45)	5 (31.25)	6 (23.08)	1 (25)	17 (27.42)	6 (24)	1 (7.69)	0 (0.00)
AUG	R	9 (69.23)	7 (100)	14 (77.78)	7 (100)	48 (87.27)	13 (81.25)	21 (80.77)	1 (25)	33 (53.23)	19 (76)	5 (38.46)	1 (16.67)
	I	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	10 (16.13)	2 (8)	0 (0.00)	1 (16.67)
	S	4 (30.77)	0 (0.00)	4 (22.22)	0 (0.00)	7 (12.73)	3 (18.75)	5 (19.23)	3 (75)	19 (30.65)	4 (16)	8 (61.54)	4 (66.66)
NOR	R	4 (30.77)	2 (28.57)	5 (27.78)	6 (85.71)	27 (49.1)	9 (56.25)	6 (23.08)	1 (25)	25 (40.32)	7 (28)	9 (69.23)	5 (83.33)
	I	5 (38.46)	1 (14.29)	3 (16.67)	1 (14.29)	2 (3.64)	0 (0.00)	10 (38.46)	1 (25)	7 (11.29)	3 (12)	0 (0.00)	0 (0.00)
	S	4 (30.77)	4 (57.14)	10 (55.56)	0 (0.00)	26 (47.27)	7 (53.85)	10 (38.46)	2 (50)	30 (48.39)	15 (60)	4 (30.77)	1 (16.67)
CEFT	R	8 (61.54)	5 (71.43)	9 (50)	4 (57.14)	42 (76.36)	12 (75)	14 (53.85)	2 (50)	35 (56.45)	18 (72)	12 (92.3)	6 (100)
	I	1 (7.69)	0 (0.00)	1 (5.56)	0 (0.00)	1 (1.82)	1 (6.25)	0 (0.00)	0 (0.00)	5 (8.06)	0 (0.00)	0 (0.00)	0 (0.00)
	S	4 (30.77)	2 (28.57)	8 (44.44)	3 (42.86)	12 (21.82)	3 (18.75)	12 (46.15)	2 (50)	22 (35.48)	7 (28)	1 (7.69)	0 (0.00)
CEFU	R	13 (100)	7 (100)	17 (94.44)	7 (100)	24 (43.64)	7 (53.85)	23 (88.46)	4 (100)	44 (70.97)	21 (84)	4 (30.77)	4 (66.66)
	I	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	16 (29.1)	4 (25)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	S	0 (0.00)	0 (0.00)	1 (5.56)	0 (0.00)	15 (27.27)	5 (31.25)	3 (11.54)	0 (0.00)	18 (29.03)	4 (16)	9 (69.23)	2 (33.33)
NIT	R	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	23 (41.82)	7 (53.85)	0 (0.00)	0 (0.00)	26 (41.94)	8 (100)	7 (53.85)	5 (83.33)
	I	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	3 (5.45%)	0 (0.00)	0 (0.00)	0 (0.00)	2 (3.23)	0 (0.00)	2 (15.38)	0 (0.00)
	S	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	13 (23.64)	5 (31.25)	0 (0.00)	0 (0.00)	3 (4.84)	0 (0.00)	4 (30.77)	1 (16.67)

a = pregnant women; AMI = amikacin; AMO = amoxicillin; AUG = Amoxicillin + clavulanic acid; b = Non-pregnant women; CEFT = ceftriaxone; CEFU = cefuroxime; CHL = chloramphenicol; CIP = ciprofloxacin; DOX = doxycycline; ERY = erythromycin; GEN = gentamicin; I = intermediate; IMI = imipenem; NIT = nitrofurantoin; frequency (%); OR = norfloxacin; R = resistant; s = susceptible; TET = tetracycline; VAN = vancomycin.

members who serve as guardians who prohibit premarital sex. This is especially the case for women, and ancestral doctrines discourage parental discussions with children, especially on sex issues, which greatly influences attitudes and behaviors [14].

It was observed that CD4 T-cell counts were lower, but not statistically significantly lower, in pregnant compared with non-pregnant women. In addition, levels of total white blood cells ( $p = 0.782$ ), granulocytes ( $p = 0.905$ ), CD8 ( $p = 0.490$ ) and CRP ( $p = 0.421$ ) were higher, but not statistically significantly higher, in pregnant women compared with those who were non-pregnant. These immune system cells are involved in protecting the body against infectious diseases and foreign invaders [15]. Disorders of the hematopoietic system are common throughout pregnancy [16]. In addition, the increase in the number of white blood cells during pregnancy may not always indicate an infection [16]. The absolute value of CD4 T-cells was  $499.46 \pm 181.807$  cells/ $\mu\text{L}$ , which was lower than those previously reported. Babatope et al [17] reported a mean value of 614.49 cells/ $\mu\text{L}$  in pregnant patients residing in Ekpoma, Nigeria, and Tanjong et al [18] reported a mean value of 851 cells/ $\mu\text{L}$  in pregnant women residing in Buea, Cameroon. These higher figures may be due to physiological leukocytosis resulting from repeated infections.

The etiology of enteric and/or urinary disorders in pregnant women are multifactorial. Stool and urine samples were examined in this study. Bacteria were isolated from 187 (37.4%) pregnant women and 65 (32.5%) non-pregnant women. The high isolation frequency in pregnant women than non-pregnant women may be linked to pregnancy, which weakens the defense mechanisms in pregnant women [6]. *Shigella sp.*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Escherichia coli* were isolated more frequently in stool samples of pregnant women with values 26 (86.66%), 17 (68%), 13 (65%), 16 (22.54%), respectively, than compared with non-pregnant women whose values were 4 (13.34%), 7 (28%), 7 (35%), 4 (5.63%). On the other hand, *Escherichia coli*, *Salmonella sp.* and *Klebsiella pneumoniae* isolates were more frequently isolated in urine samples from pregnant women with 39 (54.93%), 31 (35.63%) and 1 (4%), respectively, compared with non-pregnant women whose values were 12 (16.9%), 8 (9.2%) and 0%, respectively. These bacteria are among those that cause various pregnancy-related infections [19,20].

The isolation of the bacteria and their association with CD4 T-cell counts, serum hs-CRP and IL-6 levels were studied. In the present study, bacteria isolated from patients with CD4 T-cell counts between 461 and 806 cells/ $\mu\text{L}$  showed the relationship between CD4 T-cell count and the presence of bacteria in pregnant women was not significant. Isolates of *Shigella sp.* were higher (69.23%) in pregnant women with a CD4 T-cell count between 114-460 cells/ $\mu\text{L}$  but this was

not significantly different to the number of isolates in non-pregnant women with the same CD4 T-cell count ( $p = 0.317$ ). In CD3 and CD8 T-lymphocytes, *Shigella sp.* isolates were more frequently observed between 569-1,045 cells/ $\mu\text{L}$  (46.15%) and 10-277 cells/ $\mu\text{L}$  (46.15%). Bacteria were more frequently isolated from patients with serum IL-6 levels of 25-230 pg/mL. *Salmonella sp.* isolates were more frequently isolated (95.16%) in pregnant women with serum IL-6 levels 25-230 pg/mL but this observation was not significant. IL-6 is a multifunctional cytokine that plays a key role in the inflammatory response and in the direction of T-cell differentiation in adaptive immunity [21]. IL-6 is widely expressed in the female reproductive tract and gestational tissues thus, exerting regulatory functions in embryonic implantation and placental development, as well as the immune adaptations necessary to tolerate pregnancy [22].

Results of antimicrobial susceptibility testing revealed that, most isolates of *Salmonella sp.* and *E. coli* were more susceptible to IPM (51.61%), (78.18%); IPC (59.68%), (70.9%); CHL (46.77%), (81.82%); AMO (72.58%), (54.55%); TET (69.35%), (74.55%); AUG (53.23%), (87.27%) and CEFT (56.45%), (76.36%) respectively, in pregnant women. Isolates of *Klebsiella pneumoniae* and *Enterobacter aerogenes* showed 100% resistance in non-pregnant women. However, all bacteria showed multidrug resistance in pregnant women. *Salmonella sp.* (24.3%) and *Escherichia coli* (21.51%) showed multidrug resistance in pregnant women. This resistance may be a result of the high use of antibiotics by pregnant women [23].

This study was designed to understand the burden of bacterial disease in pregnancy. The representation of these findings in the entire pregnant and non-pregnant women population in the studied area was limited due to the number of participants. The cross-sectional nature of the study does not allow deduction of a cause and effect relationship between parameters and presumed etiological factors.

## Conclusion

This study documented the hematological profile and the resistance of pathogenic bacteria to antibiotics in pregnant women. The results of antimicrobial susceptibility testing revealed that most isolates of *Salmonella sp.* and *E. coli* were more susceptible to IPM, CIP, CHL, AMO, TET, AUG and CEFT in both pregnant and non-pregnant women, whereas *Klebsiella pneumoniae* and *Enterobacter aerogenes* showed a high resistance profile in both populations. All bacteria showed multidrug resistance in pregnant compared with non-pregnant women. *Salmonella sp.* and *Escherichia coli* showed an increase multidrug resistance in pregnant women. Pathogenic bacteria were more frequently isolated in participants with a CD4 T-cell count between 461 and 806 cells/ $\mu\text{L}$ . Routine bacteriological

analyses during pregnancy are recommended for prompt attention and treatment to avoid complications.

## Supplementary Materials

Actual data is available at <http://www.kcdcphrp.org>.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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