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Urinary tract infections caused by carbapenem-resistant Gram-negative bacteria among pregnant women attending antenatal care clinic at Murang'a County Referral Hospital, Kenya

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Abstract

Background: Urinary tract infections (UTIs) continue to pose a significant health risk during pregnancy, with growing antimicrobial resistance making effective management increasingly challenging. Of more significance is the emergence of carbapenem-resistant (CR) bacteria, especially in resource-limited countries where diagnostic capabilities, treatment options, and comprehensive epidemiological data are inadequate. This study aimed to describe the prevalence, antimicrobial co-resistance patterns, and factors associated with UTIs caused by CR Gram-negative bacteria (GNB) among pregnant women at Murang'a County Referral Hospital in Kenya.

Methods: A hospital-based cross-sectional study involving 365 pregnant women was conducted from May to October 2023. Participants were recruited through systematic random sampling; each underwent clinical evaluation. Demographic information was collected using a structured questionnaire, while clinical data were obtained from hospital records. Clean-catch midstream urine samples were processed in the hospital's microbiology laboratory using standard microbiological techniques.

Results: The overall prevalence of UTIs was 25.5% (93/365; 95% CI: 21.01–29.95), with GNB (76.3%, 71/93) predominating. The prevalence of UTIs caused by CR-GNB and carbapenemase-producing (CP)-GNB was 4.93% (95% CI: 2.71–7.15) and 2.7% (95% CI: 1.07–4.41), respectively. *Escherichia coli* (*E. coli)* was the predominant pathogen overall (60.3%), and among CR-GNB (72.2%) and CP-GNB (60%) UTIs. More than half (10/18, 55.6%) of CR isolates co-produced extended β-lactamases (ESBLs) and carbapenemases. No colistin resistance was detected in carbapenem-resistant isolates. Co-resistance ranged from 38.5% to 92.3%, with the lowest resistance (≤46.2) to nitrofurantoin and aztreonam. High co-resistance (≥69%) was observed for most β-lactams, cephalosporins, aminoglycosides, and trimethoprim/sulfamethoxazole. Eighty-nine percent of CR isolates, including all CP-GNB, were multidrug-resistant. Multiple antibiotic resistance indices (0.67–0.93) were highest among CP isolates. Unmarried/single women were more likely to have UTIs caused by CP-GNB (adjusted odds ratio: 8.87; 95% CI: 1.57–50.01).

Conclusion: Carbapenemase- and ESBL-co-producing GNB, predominated by *E. coli,* were present among pregnant women with UTIs. Unmarried/single status was the only variable significantly associated with CP-GNB UTIs in this study. Strengthening carbapenem stewardship and enhancing infection prevention practices are urgently needed to prevent further spread of resistant strains in the current context and beyond.

Key words: Carbapenem resistance, carbapenemase production, urinary tract infections, co-resistance, pregnant women

1.0 Introduction

Globally, urinary tract infections (UTIs) are among the most common health concerns and are the leading cause of outpatient antibiotic prescriptions [1] [2]. A recent study on the global burden of disease reported a 66.45% increase in UTIs from 1990 to 2021, reaching 4.49 billion cases in 2021 [3]. UTIs are more prevalent in women, with approximately 50–60% of adult women experiencing at least one symptomatic UTIs during their lifetime [1] [3]. The close proximity of the urethra to the rectum and vagina, which are considered reservoirs of bacteria, as well as the shorter urethra, increases the risk of UTIs in women[4] [5].

Women's vulnerability to UTIs increases during pregnancy due to hormonal changes. Pregnant women have four times higher risk of UTIs compared to those who are not pregnant [6] [7]. This higher risk comes from factors like dilation of the ureters and renal calyces, urinary stasis, relaxation of smooth muscles caused by progesterone, and a reducing bladder capacity because of

the growing fetus [5]. Conditions such as vesicoureteral reflux and increased residual urine in the bladder also contribute to the risk of UTIs during pregnancy by causing more urinary stasis [5] [8]. Although pregnant women often show no symptoms of bacteria in the urine, persistent infection can be associated with symptomatic UTIs, which can cause complications if not treated[5] [9] . However, antibiotic treatment for UTIs during pregnancy is limited due to challenges in finding options that are both safe for the fetus and effective against the infection [10].

Multidrug resistance to commonly used antibiotics during pregnancy is on the rise [6] [11] [12]. This complicates UTIs management and poses significant risks to both maternal and fetal health. Carbapenems, often seen as the last line of defense against multidrug-resistant Gram-negative bacteria (GNB), are generally safe to use in pregnancy [13]. However, the rise of carbapenem resistance is worrying, as it leaves few effective treatment options for these infections [14] [15] [16]. Infections caused by carbapenem-resistant bacteria are difficult to manage and often associated with higher rates of morbidity and mortality [14]. The traditional alternative is a triple combination of polymyxin, carbapenem, and rifampin or tigecycline, but this faces challenges due to increasing resistance and a lack of safety data for pregnant patients [13].. Similarly, the safety of newer agents like ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, imipenem-cilastatin-sulbactam, plazomicin, eravacycline, and cefiderocol has not been adequately studied in pregnant patients [17].

Untreated UTIs in pregnancy are associated with a higher risk of serious complications. These include preterm birth, premature rupture of membranes, neonatal respiratory distress syndrome, and admission to the neonatal intensive care unit [12]. This is in addition to other potential consequences of UTIs during pregnancy, such as pyelonephritis, sepsis, anemia, disseminated intravascular coagulation, low birth weight, and renal abscess formation [18]. Pregnant women with risk factors such as obesity, low socioeconomic status, younger age, first-time pregnancy, diabetes, smoking, or a history of recurrent UTIs are more likely to experience complicated UTIs [5]. Despite these risks, limited data exists on the epidemiology of UTIs to guide infection prevention efforts among pregnant women in Murang'a County, Kenya, where most clinical laboratories lack microbiological diagnostic capacity. This study, therefore, aimed to determine the prevalence, antimicrobial susceptibility patterns, and associated risk factors for UTIs caused by carbapenem-resistant bacterial UTIs among pregnant women in a hospital setting in Kenya.

2.0 Materials and Methods

2.1 Study setting, design and population

This study took place at Murang'a County Referral Hospital (MCRH) in Kiharu Sub-County, Murang'a town, Kenya (coordinates: 0°43′04″S, 37°09′40″E). The hospital serves about 1.2 million people and is the main referral center for Murang'a County and nearby areas of Kiambu, Nyeri, Kirinyaga, and Nyandarua counties. It has 285 beds, ten wards, an intensive care unit, and a renal unit. Around 500 patients visit the hospital each day, including 20

pregnant women seeking antenatal care. It is the only public hospital in the county with a microbiology laboratory.

MCRH stands out as the county's sole public hospital equipped with a microbiology laboratory. This facility holds ISO 15189 accreditation from the Kenya Accreditation Service (KENAS). Further underscoring its commitment to quality, the laboratory engages in the Kenya External Quality Assessment Scheme (KNEQAS), overseen by the Ministry of Health's National Public Health Laboratories (NPHL), as well as the External Quality Assessment for Africa (EQuAFRICA), coordinated by the African Society for Laboratory Medicine (ASLM).

A hospital-based cross-sectional study design was adopted. The sample size was calculated using the Fisher *et al.* (1998) formula with a 95% confidence interval, based on a previously reported 15.7% prevalence of UTIs among pregnant women in Kenya [19]. Between May and October 2023, 365 pregnant women were systematically selected from the antenatal care (ANC) clinic, irrespective of their presenting symptoms. The recruitment process was guided by a calculated sampling interval: with an anticipated monthly attendance of 440 pregnant women (derived from 22 working days and an average of 20 daily attendees), the total expected over the six-month period was 2,640. Dividing this figure by the target sample size of 365 yielded a sampling interval of about seven. Recruitment commenced from a randomly determined starting point, after which every seventh pregnant woman was enrolled until the desired sample size was achieved.

Pregnant women attending ANC who consented to participate and provided a clean-catch midstream urine specimen for laboratory analysis were included in the study. Eligibility criteria encompassed both women presenting with symptoms indicative of UTI, such as fever, increased frequency or urgency of urination, discomfort or a burning sensation during urination, cloudy or blood-tinged urine, pain or tenderness in the lower abdomen, back, or flanks, and urine with a pronounced odour [18] [20] [21]. Exclusion criteria comprised pregnant women who had used antibiotics within the preceding 72 hours, were unable to provide a urine specimen, declined participation, or had an alternative confirmed cause of urinary symptoms. These criteria were implemented to minimise false-negative culture results, reduce specimen contamination, and ensure that urinary findings were attributable to urinary tract infection rather than other clinical conditions.

2.2 Data collection

Participant demographics were obtained through face-to-face interviews using a structured, pretested questionnaire (Supplementary file S1), with some clinical data (including Hb levels, HIV status, and blood pressure) extracted from hospital records. The questionnaire was developed through a rigorous process to ensure validity and reliability [22]. After a literature review, items were drafted based on existing tools and study needs[19] [23] [24]. Experts reviewed and revised the draft for clarity and relevance. The questionnaire was pretested with about 10% of the target sample drawn from a separate health facility not involved in the main study, to assess clarity and flow, and

feedback was used to refine it. Internal consistency was measured using Cronbach's alpha, yielding 0.76, indicating an acceptable reliability. Results from the pilot informed revisions to improve clarity, relevance, and consistency, producing a refined tool for the main research.

Urine samples were collected using standard aseptic procedures to minimise contamination and enhance the diagnostic accuracy of urine dipstick testing, microscopy, and culture. Participants were instructed to wash their hands with soap and clean the external genitalia with water before collecting a midstream, clean-catch, freshly voided urine sample (20–30 ml). The samples were collected in sterile, leak-proof, wide-mouthed, screw-capped plastic containers (Delta Lab, Spain) labeled with a unique patient identifier, and transported to the MCRH Microbiology Laboratory in a cooler box for processing within one hour of collection [25] [26].

2.3 Bacteriuria screening

Bacteriuria was screened using both biochemical and microscopic methods. Urine dipsticks (Uristix, Qingdao Hightop Biotech, China) were used for the biochemical assessment, while Gram-stained urine smears were examined microscopically. A sample was considered positive for bacteriuria if the urine dipstick showed nitrites and/or leukocytes, accompanied by microscopic evidence of bacteria. Positive samples were then cultured to confirm UTI and isolate the causative bacteria [25] [29].

The screening algorithm employed in this study reflects routine clinical practice in Kenya rather than an ideal diagnostic approach. Urine culture

remains the gold standard for diagnosing UTIs during pregnancy. However, in many Kenyan healthcare facilities, including the study setting, routine urine culture for all pregnant women is limited by laboratory infrastructure, financial constraints, and workload. As a result, dipstick urinalysis and urine microscopy are frequently used as initial screening tools prior to culture confirmation. Previous studies in Kenya have assessed the performance of urine dipsticks compared to culture, highlighting the practical realities of UTI screening in resource-limited settings [27] [28].

2.4 Bacterial isolation, identification and antimicrobial susceptibility testing

Ten microliters (10 µl) of bacteriuria-positive urine were inoculated onto Cysteine-Lactose Electrolyte Deficient (CLED) agar (HiMedia Laboratories, India) with a sterile calibrated wire loop, following standard bacteriological methods [30] [25]. Plates were incubated aerobically overnight at 37 °C, and then colonies were counted. Significant growth was defined as 1 or 2 isolates at 10^4 CFU/mL or more in participants with UTIs symptoms, or at 10^5 CFU/mL or more in those without symptoms. Gram-stained smears were examined microscopically, and species identification was confirmed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik GmbH, Bremen, Germany).

Antibiotic susceptibility of the isolates was determined using the VITEK 2 Compact machine (bioMérieux, NS. A, Nu, Germany) with AST GN 83 cards. Antibiotic selection and result interpretation followed the 2023 Clinical

Laboratory Standards Institute (CLSI) guidelines [31]. Isolates were tested against aminoglycosides (gentamicin: 0.5-16 µg/ml, amikacin: 2-64 µg/ml), cephalosporins (cefuroxime: 1-32 µg/ml, ceftriaxone: 0.25-64 µg/ml, cefotaxime: 0.25-64 µg/ml, ceftazidime: 0.25-64 µg/ml, cefepime: 0.25-32 µg/ml), carbapenems (meropenem: 0.25-16 µg/ml), penicillins (amoxicillin/clavulanic acid: 2/1-32/16 µg/ml, piperacillin/tazobactam: 4/4-128/4 µg/ml, ampicillin: 1-32 µg/ml), monobactam (aztreonam: 0.25-64 µg/ml), fluoroquinolones (ciprofloxacin: 0.06-4 µg/ml), nitrofurans (nitrofurantoin: 16-512 µg/ml), and sulfonamides (trimethoprim/sulfamethoxazole: 0.5/9.5-8/152 µg/ml). *E. coli* American Type Culture Collection [ATCC] 25922)) and *Pseudomonas aeruginosa* (ATCC 27853) served as quality control organisms [31].

Colistin susceptibility of the study isolates, except for *Proteus mirabilis,* which is intrinsically resistant to polymyxins, was assessed using the broth microdilution (BMD) technique, following CLSI 2023 guidelines[31]. Four tubes, each containing 10 mL of cation-adjusted Muller-Hinton Broth (CAMHB), were prepared and labelled as follows: 0 µg/ml (control), 1 µg/ml, 2 µg/ml, and 4 µg/ml. To the tubes labelled 1 µg/ml, 2 µg/ml, and 4 µg/ml, 1, 2 and 4 colistic disks (10 µg each) were added, respectively. All the tubes were gently vortexed for 30 minutes to promote antibiotic elution. Each test organism was adjusted to a 0.5 McFarland standard, and 50 µl of this suspension was added to each tube. To verify purity, the original inoculum was streaked onto blood agar using a 10 µl loop. All tubes and plates were incubated at 35 °C for 16–

20 hours. The test was considered valid only if pure colonies grew on the blood agar plate and turbidity was observed in the control tube. Minimum inhibitory concentrations (MICs) were interpreted as intermediate (≤2 µg/ml) or resistant (≥4 µg/ml), in line with guideline recommendations, which do not define a "susceptible" category for colistin due to variability in test methods and clinical outcomes. *E. coli* ATCC 25922 and *P. aeruginosa ATCC* 27853 served as control strains in this study [25] [32] [31].

2.5 Carbapenemase production by carbapenem-resistant isolates

Carbapenemase production in carbapenem-resistant isolates (meropenem) was assessed using the Modified Carbapenem Inactivation Method (mCIM) following CLSI 2023 guidelines [31]. Overnight cultures of each test isolate were prepared by suspending 1 µL of Enterobacterales or 10 µL of *P. aeruginosa* in 2 mL tryptic soy broth (TSB) using a sterile wire loop. After vortexing for 10 minutes, a 10 µg meropenem disc was added to each tube and incubated at 35 °C for 4 hours. Simultaneously, *E. coli* ATCC 25922 was adjusted to a 0.5 McFarland standard in normal saline and spread onto Mueller-Hinton Agar (MHA) plates, which were air-dried for 10 minutes. After incubation, the meropenem disc was removed from the TSB with a sterile 10 µL loop and placed onto the *E. coli*-inoculated MHA plate. Plates were incubated at 35 °C for 18–24 hours.

Carbapenemase production was interpreted as a 6–15 mm inhibition zone around the disc, indicating that the test isolate hydrolyzed meropenem and, consequently, diminished its activity against *E. coli* ATCC 25922. Non-

carbapenemase production was defined as an inhibition zone ≥19 mm. In the meantime, isolates showing a 16–18 mm inhibition zone, or ≥19 mm with pinpoint colonies, were classified as indeterminate and retested. Quality control strains included *E. coli* ATCC 25922 (non-carbapenemase producer) and *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 700603 (carbapenemase producer) [25] [31].

2.7 *ESBL production by carbapenem-resistant isolates*

Isolates were screened for Extended-Spectrum Beta-Lactamase (ESBL) production using the Double Disk Synergy Test (DDST) [25] [33]. A bacterial suspension was prepared to match the 0.5 McFarland standard and then plated on Mueller–Hinton Agar (MHA), allowing the plates to air dry for 3 minutes. Antibiotic disks containing cefotaxime (30 µg), ceftazidime (30 µg), and amoxicillin/clavulanic acid (20 µg/10 µg) were placed on the agar with a 30 mm-radius spacing between them. The plates were then incubated overnight at 37°C in ambient air. If an inhibition zone appeared around cefotaxime (≤27 mm) and/or ceftazidime (≤22 mm) and extended towards the beta-lactam inhibitor, it indicated the production of ESBL. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as quality control organisms [34].

The ESBL production was confirmed by phenotypic confirmatory disc diffusion test (PCDDT) [25] [33] [31]. A homogenous suspension of the isolate equivalent to 0.5 McFarland was streaked onto Mueller-Hinton agar plate and left for 4 minutes to air-dry. Cefotaxime (30 µg), ceftazidime (30 µg),

cefotaxime/clavulanic acid (30µg/10 µg) and ceftazidime/clavulanic acid (30 µg/10 µg) discs were then placed on the plate, maintaining a 30 mm separation. The plates were incubated at 37ºC overnight. Formation of ghost zones (increase of a ≥5mm in diameter of zone of inhibition) between an inhibitor (beta lactam combination) and 3rd generation cepharosporin indicated ESBL producer. *K. pneumoniae* ATCC 700603 (ESBL positive) and *E. coli* ATCC 25922 (ESBL negative) control strains were used for ESBL detection [34].

2.8 Statistical analysis

Statistical analyses were performed using Statistical Analysis Software (STATA) version 17. Categorical variables, including percentages and frequencies, were summarized and displayed in tables and figures. Bivariate logistic regression was used to assess associations between UTIs caused by carbapenemase-producing (CP) GNB and participants' demographic and clinical characteristics. Variables with a p-value of 0.2 or less were included in the multivariate logistic regression to assess the independence of these associations. Statistical significance was defined as a p-value of 0.05 or less with a corresponding 95% confidence interval (CI). Multidrug resistance was defined as resistance to at least one antibiotic in a minimum of three antimicrobial classes [27]. Multiple antibiotic resistance indices(MARI) were calculated as a/b, where **'a'** is the number of antibiotics to which the isolate was resistant and 'b' is the total number of antibiotics tested [25] [35].

3.0 Results

3.1 Demographic and clinical characteristics of study participants

A total of 365 pregnant women participated in the study. Their ages ranged from 14 to 45 years, with a mean age of 27.4 years (SD ± 6.6). More than half (52.1%, 190/365) were between 20 and 29 years old, and half (50.7%, 185/365) were in their second trimester. The majority of the participants lived in rural areas (89.3%, 326/365), were unemployed (64.7%, 236/365), married (69.3%, 253/365), multigravida (71.5%, 261/365), and bathed in basins (84.7%, 309/365), Table 1.

Table 1: *Demographic and clinical characteristics of pregnant women attending antenatal care clinic at Murang'a County Hospital, May-October, 2024*

Variable	Frequency	Percent (%)
Age (years)		
≤19	38	10.4
20 - 29	190	52.1
30 - 39	122	33.4
40 - 49	15	4.1
Residence		
Rural	326	89.3
Urban	39	10.7
Highest level of education		
Primary	117	32.1
Secondary	187	51.2
Tertiary	61	16.7
Employment status		
Employed	129	35.3
Unemployed	236	64.7
Marital status		
Single	112	30.7
Married	253	69.3
Presenting symptoms		
Urgency urination	31	8.5
Abdominal pain	30	8.2
Incomplete voiding	20	5.5
Frequent urination	37	10.1
Back ache	20	5.5

Burning sensation	16	4.4
Discharge	21	5.8
History of abortion		
Yes	27	7.4
No	338	92.6
Gravida		
Primigravida	105	28.8
Multigravida	260	71.2
Pregnancy term		
First trimester	91	24.9
Second trimester	185	50.7
Third trimester	89	24.4
Hb level (gms/dl)		
<11	79	21.6
≥11	286	78.4
History of UTIs		
Yes	57	15.6
No	308	84.4
Prior history of catheterization		
Yes	21	5.8
No	344	94.2
History of hospitalization > 2 months to the study		
Yes	28	7.7
No	337	92.3
Presence of underlying condition (diabetes, hypertension, asthma, allergy, HIV)		
Yes	21	5.8
No	344	94.2
HIV status		
Positive	8	2.2
Negative	357	97.8
History of recurring UTIs		
Yes	20	5.5
No	345	94.5
History of antibiotic use within 6 months		
Yes	105	28.8
No	260	71.2
Previous antibiotic dose completion (n		
Yes	97	92.4
No	8	7.6
Toilet		
Pit	286	78.4
Flush	79	21.6
Mode of bathing		
Basin	309	84.7
Over-head shower	56	15.3
Fish meal per week		

Yes	2	0.5
No	363	99.5
Poultry keeping		
Yes	60	16.4
No	305	83.6

UTIs: urinary tract infection, **Hb:** hemoglobin, **HIV**: human immunodeficiency virus

3.2 Prevalence of UTIs Caused by Carbapenem-resistant Gram-negative bacteria

In this study, 25.5% (95% confidence interval (CI): 21.0- 29.9% (93/365)) of participants had UTIs, with no co-infection identified. The majority of infections were caused by GNB, accounting for 76.3% (71/93) of UTIs cases. The prevalence of UTIs caused by CR-GNB and CP-GNB was 4.9% (95% CI: 2.7-7.2% (18/365)) and 2.7% (95% CI: 1.1- 4.4% (10/365), respectively. *E. coli* was the most predominant isolate causing UTIs overall (60.3%, 57/93) and among cases caused by CR-GNB (72.2%, 13/18) and CP-GNB (60%, 6/10). More than half (10/18, 55.6%) of CR isolates co-produced ESBL and carbapenemases, Fig. 1 and 2.

Figure 1: Bacterial spectrum among pregnant women with urinary tract infections at Murang'a County Referral Hospital, May-October, 2023. **%:** percent

Figure 2: Carbapenemase-producing GNB and extended spectrum β-lactamase co-producing isolates among pregnant women with urinary tract infections at Murang'a County Referral Hospital, May-October, 2023. **CP/ESBL** carbapenemase-producing/ extended-spectrum β-lactamase producing, **%:** percent

3.3 Antibiotics co-resistance among carbapenem-resistant *E. coli*

In this study, co-resistance patterns were analyzed in 13 carbapenem-resistant *E. coli* isolates tested against 15 antibiotics. Co-resistance was not calculated for carbapenem-resistant *Pseudomonas aeruginosa* (n=1) and *K. pneumoniae* (n=4) due to the small sample sizes; their antimicrobial susceptibility results are provided in Supplementary Fig. S1.

No colistin resistance was detected in carbapenem-resistant *E. coli* (Table 2). Co-resistance rates ranged from 38.5% to 92.3%. The lowest rates were seen with nitrofurantoin (38.5%) and aztreonam (46.2%). High co-resistance (≥69%) was found for ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, ceftriaxone, cefotaxime, amikacin, gentamicin, and trimethoprim/sulfamethoxazole (Table 2).

Table 2: Antibiotics co-resistance proportions for carbapenem-resistant *E. coli recovered from* pregnant women with urinary tract infections at Murang'a County Referral Hospital, May-October, 2023.

Antibiotic class	**Antibiotic tested**	***E. coli*, n=13**
Penicillins	ampicillin	13(100)
	amoxicillin/clavulanic acid	9(69.2)
	piperacillin/tazobactam	10(76.9)
Monobactams	aztreonam	6(46.2)
Second generation cephalosporins	cefuroxime	10(76.9)
Third generation cephalosporins	ceftriaxone	9(69.2)
	cefotaxime	9(69.2)
	ceftazidime	8(61.5)
Fourth generation cephalosporins	cefepime	6(46.2)
Aminoglycosides	gentamicin	11(84.6)

	amikacin	9(69.2)
Fluoroquinolones	ciprofloxacin	7(53.8)
Nitrofurans	nitrofurantoin	5(38.5)
sulfonamides	trimethoprim/sulfamethoxazole	10(76.9)
Polymyxins	colistin	0(0.0)

n: number of isolates

3.4 Multiple antibiotic resistance indices of carbapenem-resistant isolates

Eighty-nine per cent (16/18) of CR isolates were multidrug-resistant (MDR), Table 3. This included all carbapenemase-producing (CP) isolates. Two of the non-carbapenemase-producing (non-CP) isolates were non-MDR. The number of antibiotic classes resistant was high among CP (5-7) compared with 2-6 classes for non-CP. Similarly, multiple antibiotic resistance indices (MARI) were highest among CP isolates (0.7-0.9) compared with non-CP isolates (0.2-0.7), Table 3.

Table 3: Multiple antibiotic resistance indices of carbepenem-resistant GNB isolates *causing UTIs among pregnant women attending antenatal care clinic at Murang'a County Referral Hospital, May* to *October, 2023*

#: Total number of antibiotics, **¶:** total number of antibiotics tested, **MDR:** multidrug resistance, **MARI:** multiple antibiotic resistance index, **ID:** identity, **n:** number isolates

3.5 Factors associated with urinary tract infections caused by Carbapenemase-producing Gram-negative bacteria

The findings established that single women were 9 times more likely to have UTIs caused by CP- GNB when controlling for other factors (adjusted odds ratio: 8.9 (95% CI: 1.6 - 50.0), p = 0.013 (Table 4). No other variable in this study was associated with UTIs caused by CP-GNB.

Sample	Isolate ID	# classes resistant	antibiotics resistant (¶= 15)	MARI	MDR status
Carbapenemase-producing isolates (n=10)					
222	*E. coli*	5	12	0.8	MDR
257	*E. coli*	7	13	0.9	MDR
299	*E. coli*	6	10	0.7	MDR
307,314	*E. coli*	6	13	0.9	MDR
318	*E. coli*	7	13	0.9	MDR
260, 309,321	*K. pneumoniae*	7	14	0.9	MDR
365	*P. aeruginosa*	7	13	0.9	MDR
Non-carbapenemase-producing isolates (n=8)					
137	*E. coli*	5	7	0.5	MDR
192	*E. coli*	2	5	0.3	Non-MDR
294	*E. coli*	3	6	0.4	MDR
308	*E. coli*	5	7	0.5	MDR
313	*E. coli*	5	10	0.7	MDR
319	*E. coli*	5	9	0.6	MDR
335	*E. coli*	2	3	0.2	Non-MDR
341	*K. pneumoniae*	6	11	0.7	MDR

Table 4- Factors associated with urinary tract infections caused by carbapenemase- producing GNB among pregnant women at Murang'a County Referral Hospital, May-October, 2023

Variables	Total	CP-GNB present		Bivariate		Multivariate	
		Yes	No	OR(95%CI)	P	Adjusted	P value
Age							
≤19 years	6	0	6(100)	¶			
20 - 29 years	32	5(15.6)	27(84.4)				
30 - 39 years	27	4(14.8)	23(85.2)				
40 - 49 years	6	1(16.7)	5(83.3)				
Residence							
Rural	64	10(15.6)	54(84.4)				
Urban	7	0	7(100)				
Highest level of education							
Primary	21	1(4.8)	20(95.2)	Ref			
Secondary	38	8(21.1)	30(78.9)	1.8(0.1 - 32.0)	0.683		
Tertiary	12	1(8.3)	11(91.7)	0.4(0.0 - 3.1)	0.336		
Employment status							
Employed	25	6(24.0)	19(76.0)	3.3(0.9 - 13.1)	0.08	1.6(0.3 - 8.7)	0.575
Unemployed	46	4(8.7)	42(91.3)	Ref			
Marital status							
Single	25	8(32.0)	17(68.0)	10.4(2.0- 53.8)	0.003	8.9(1.6 - 50.0)	**0.013****
Married	46	2(4.3)	44(95.7)	Ref			
Gravida							
Primigravida	21	3(14.3)	18(85.7)	1.02(0.2 - 4.4)	0.62		
Multigravida	50	7(14.0)	43(86.0)	Ref			
Trimester							
First	17	0	17(100)				
Second	32	5(15.6)	27(84.4)				
Third	22	5(22.7)	17(77.3)				
Hemoglobin							
Less than 11	17	4(23.5)	13(76.5)	2.46(0.6 - 10.0)	0.185	2.3(0.5 - 11.5)	0.324
11 or more	54	6(11.1)	48(88.9)	Ref			
History of UTIs							
Yes	8	0	8(100)				
No	63	10(15.9)	53(84.1)				
Catheterization							
Yes	2	0	2(100)				
No	69	10(14.5)	59(85.5)				
History of							
Yes	3	0	3(100)				
No	68	10(14.7)	58(85.3)				
Presence of underlying comorbidity							
Yes	5	0	5(100)	1.2(1.1 - 1.3)	0.457		
No	66	10(15.2)	56(84.8)	Ref			
HIV status							
Positive	2	0	2(100)				
Negative	69	10(14.5)	59(85.5)				
History of recurring UTIs							
Yes	3	0	3(100)				
No	68	10(14.7)	58(85.3)				
History of antibiotic use							
Yes	13	0	13(100)				

No	58	10(17.2)	48(82.8)				
Type of toilet							
Pit	50	5(10.0)	45(90.0)	0.4(0.1 - 1.4)	0.148	4.0(0.1 - 170.4)	0.467
Flush	21	5(23.8)	16(76.2)	Ref			
Bath type							
Basin	55	5(9.1)	50(90.9)	0.2(0.1 - 0.9)	0.040	0.13(0.0 - 5.3)	0.282
Over-head shower	16	5(31.3)	11(68.8)	Ref			
Poultry keeping							
Yes	10	0	10(100)				
No	61	10(16.4)	51(83.6)				

UTIs: urinary tract infections**, OD:** odds ratio**, CP-GNB:** carbapenemase-producing Gram-negative bacteria, ***Ref:*** reference category, **Significant at 0.05, ¶ bivariate analysis was not done for lack of observations (zero entry), which make it difficult to estimate the effect of the variables using maximum likelihood estimation (MLE).

4.0 Discussion

In this study, 25.5% (93/365) of pregnant women were diagnosed with urinary tract infections (UTIs). This finding aligns closely with pooled global estimates from a systematic review and meta-analysis of 27 studies involving 30,641 pregnant women, which reported an overall UTIs prevalence of 23.9% [36]. In contrast, the prevalence identified in this study is substantially higher than the 5% reported among 2,418 pregnant women in Riyadh, Saudi Arabia [37], 15.7% among pregnant women at Pumwani Maternity Hospital, Nairobi [19], and 21.5% (219/1020) among pregnant women with asymptomatic bacteriuria in selected antenatal clinics in Nairobi, Kenya [38]. However, it remains lower than the 40.7% (165/405) reported among pregnant women attending antenatal care at Wachemo University Comprehensive Specialized Hospital in Ethiopia [39]. These discrepancies likely result from differences in population characteristics, diagnostic criteria, health-seeking behaviors, and local risk factors.

Few studies focus exclusively on pregnant women to provide reliable prevalence estimates for carbapenem resistance. In this study, the prevalence of UTIs caused by CR-GNB was 4.9% (18/365), while CP-GNB was 2.7% (10/365). Our findings are similar to those of Ogban et al., who reported rates of 4.87% (17/349) for CR-GNB and 3.4% (12/349) for CP-GNB among pregnant women at the University of Calabar Teaching Hospital in Nigeria[40]. One third (6/18, 33.3%) of CR isolates in our study produced both carbapenemase and ESBLs. This shows the presence of high-risk, multidrug-resistant GNB strains that have significant clinical and public health implications [41] [42]. This co-production often results from acquiring or co-locating multiple β-lactamase genes, which are usually found on conjugative plasmids or mobile elements. This increases the risk of horizontal gene transfer between different strains and species [42].

GNB accounted for most of UTIs in our study (76.3%, 71/93). This matches local and global findings that identify GNB as the main uropathogens in pregnancy [19] [26] [43] [44] [45] [36] [6]. The dominance of GNB may be due to their cell structure, which allows them to effectively adhere stick to epithelial surfaces and penetrate tissues, leading to infection [36]. During pregnancy, this can raise the risk of pyelonephritis. Notably, uropathogens vary among studies. In our study, *E. coli* was the most common isolate overall and in cases involving CR and CP isolates. This result is consistent with studies in Africa [45], Asia, Kenya [19] [38], Riyadh, Saudi Arabia [37], and globally [36]. In contrast, some studies have identified *K. pneumoniae* [26] and

Staphylococcus species [46] [47] as the predominant isolates in pregnancy-related UTIs. The variation in findings likely results from differences in study populations, case definitions, diagnostic methods, prior antibiotic exposure, local antimicrobial pressure, and host factors such as HIV infection or diabetes. The CR isolates in this study showed high multiple antibiotic resistance (MAR) indices, with CP isolates (0.7-0.9) exceeding those of non-CP isolates (0.2-0.7). The MAR index above 0.2 suggests that these CR isolates likely originated from environments with significant antibiotic selective pressure, including clinical settings, community misuse, or agricultural sources [48]. Eighty-nine per cent (16/18) of CR-GNB, including all carbapenemase producers, in this study were MDR. Carbapenemases are enzymes that hydrolyze most β-lactam antibiotics, such as penicillins, cephalosporins, monobactams, and carbapenems. Carbapenemase genes are often located on mobile plasmids that also carry mobile genetic elements, including transposons, insertion sequences, and integrons. As a result, these plasmids frequently harbor genes conferring resistance to other antibiotic classes [49] [50], such as aminoglycosides, quinolones, colistin, and sulfonamides, as well as genes responsible for ESBL and broad-spectrum beta-lactamase production [49] [50]. This may explain the high rate of multidrug resistance observed among CP isolates in our study. The finding of the co-production of carbapenemases and ESBLs in a third of the CR isolates (6/18, 33.3%) supports this assertion. Implementing a One Health approach is recommended to address antimicrobial resistance in this context.

The most commonly used antibiotics for UTIs in pregnancy include nitrofurantoin (NIT), cephalosporins, trimethoprim-sulfamethoxazole, and fosfomycin [51] [18]. In this study, fosfomycin susceptibility was not assessed due to unavailability, and antibiotic co-resistance was calculated for *E. coli* only, as other CR isolates were few in counts. NTI showed the lowest antibiotic co-resistance (38.5%) and is a first-line treatment for asymptomatic bacteriuria and cystitis. However, NIT is prescribed only when no alternatives are available. It is contraindicated in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency due to the risk of haemolysis, and it may cause congenital disabilities if used during the first trimester of pregnancy [18]. Other first-line options for UTI in pregnancy, such as amoxicillin-clavulanic acid, ceftriaxone, and cefepime, showed high antibiotic co-resistance with carbapenems tested (46.2-69.2%).

Among second-line treatments for asymptomatic bacteriuria and cystitis, only sulfamethoxazole-trimethoprim (SXT) was included in this study because cephalexin and fosfomycin were unavailable. SXT showed a high co-resistance rate (76.9%). As a folic acid antagonist, SXT may cause congenital disabilities if used during the first trimester and is contraindicated in the third trimester due to the potential risk of fetal jaundice [18] [51]. For pyelonephritis in pregnancy, recommended antibiotics are ceftriaxone, cefepime, ampicillin with gentamicin, and aztreonam for patients with β-lactam allergy. Fosfomycin and NIT are not recommended because they do not achieve adequate kidney

tissue levels [51]. Antibiotics in this category demonstrated high co-resistance (46.2-92.3%), indicating reduced clinical value, particularly in pregnancy.

Although carbapenem-resistant (CR) isolates in this study were still susceptible to colistin, its use is limited due to toxicity, side effects, tolerability issues, and low effectiveness. Newer treatments for CR infections, such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam, cefiderocol, eravacycline, and plazomicin, also lack sufficient clinical data for use during pregnancy. Therefore, CR infections during pregnancy pose serious health risks to both the fetus and the mother. Infection prevention, antibiotic stewardship, and AMR surveillance are strongly recommended.

The present study found that single or unmarried women were nine times more likely to experience UTIs caused by CP-GNB. This association is infrequently reported in the existing literature. Most studies attribute carbapenem resistance to healthcare-related exposures, including prior antibiotic use, hospitalization, intensive care admission, preterm delivery, low birth weight, or the use of invasive devices, rather than to sociodemographic factors such as marital status [[52] [53] [54]. The strong association identified in this study may reflect residual confounding or underlying socio-behavioral factors, including differences in self-medication, sexual behavior, socioeconomic status, or antenatal care utilization. Larger, prospective studies that directly assess these potential mediators are required to determine

whether this relationship is causal, context-specific, or indicative of other unmeasured risks.

5.0 Limitations

This study has several limitations. First, its cross-sectional design limits causal inference and prevents the assessment of temporal relationships between exposures, such as antibiotic use or healthcare contact, and carbapenem-resistant infections. Second, while urine culture is the gold standard for diagnosing UTIs during pregnancy, this study initially screened participants for UTIs using dipstick urinalysis and urine microscopy before confirming with culture. This approach reflects routine clinical practice in Kenya and financial constraints. However, using this algorithm may have missed some infections, and performing urine cultures for all participants would provide more accurate diagnoses.

Third, fosfomycin susceptibility testing and several recommended first-line antibiotics (e.g., cephalexin) were unavailable, which restricted a complete evaluation of therapeutic options. Fourth, molecular characterization of resistance genes, plasmid types, or strain relatedness was not performed, limiting insight into transmission dynamics and resistance mechanisms. Fifth, antibiotic co-resistance was assessed only for *E. coli* due to the small number of other carbapenem-resistant isolates, which limits generalizability across species. Finally, self-reported data, such as prior antibiotic consumption, may be subject to recall or social desirability bias.

6.0 Conclusion

CR-GNB, including carbapenemase and ESBLs co-producers predominated by *E. coli,* were present among pregnant women with UTIs. Unmarried/single status was the only variable significantly associated with CP-GNB CP-GNB UTIs in this study. Strengthening carbapenem stewardship and enhancing infection prevention practices are urgently needed to prevent further spread of resistant strains in the current context.

Abbreviations

ANC Antenatal Care

AST Antimicrobial Susceptibility Testing

ATCC American-Type Culture Collection

CLSI Clinical and Laboratory Standards Institute

CP-GNB Carbapenemase-producing Gram-negative Bacteria

CR Carbapenem-resistant

CR-GNB Carbapenem-resistant Gram-negative Bacteria

ESBLs Extended spectrum β-lactamases

Flight Mass Spectrometry

GNB Gram-negative Bacteria

MALDI- TOF MS Matrix-Assisted Laser Desorption Ionization-Time of

MDR Multidrug-drug Resistant

MIC Minimum Inhibition Concentration

UTIs Urinary Tract Infections

WHO World Health Organization

Declarations

Human Ethics and Consent to Participate

Ethical approval for the protocol was granted by the Kenyatta University Ethical Review Committee (Protocol number: PKU/2665/E1789), and a research permit was issued by the National Commission for Science and Innovation (Ref. No. P/23/24015). Permission to collect samples from the ANC clinic was obtained from hospital management. Informed written consent was secured from each participant, and the study was conducted in accordance with the Declaration of Helsinki. For participants under 18 years of age, consent was obtained from their parents or legal guardians. Participants' well-being was prioritized, and laboratory findings were promptly communicated to the clinicians responsible for their care.

Consent for Publication

Not applicable.

Availability of data and materials

The datasets utilized in this study can be acquired from the corresponding author upon request.

Competing interests

The authors state that they do not have any conflicts of interest.

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Author contributions

CN, JM, RO and AM conceived and developed the study protocol. CN and SG collected the data supervised by JM, RO and AM. CN, SG, and AM interpreted

the data and drafted the manuscript. All the authors read and approved the final manuscript.

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