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Demographic Characteristics and Susceptibility Profiles of *Proteus Mirabilis* and *Escherichia Coli* Isolates from Urine Samples of Asymptomatic Pregnant Women within Ilorin Metropolis

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Abstract

Urinary tract infection (UTI) is one of the most prevalent infections among pregnant women which can have major consequences if left untreated. This study was aimed at determining the demographics and susceptibility profile of *Proteus mirabilis* and *Escherichia coli* isolates among asymptomatic pregnant women attending three ante-natal clinics within Ilorin, Nigeria. A total of 120 pregnant women between the ages of 17and 50 years attending ante-natal clinics in selected hospitals in Ilorin participated in the study. Clean catch mid-stream urine samples were collected and cultured on Blood and Eosin Methylene Blue (EMB) agar plates, incubated aerobically at 37 °C for 24 hours. The presence of significant bacteriuria ($> 10^5$cfu/mL)) was determined using the plate count method. Antibiotic susceptibility testing was done using Kirby-Bauer disk diffusion technique. Of the120 urine samples collected, 45% of them were found positive on culture. *Proteus mirabilis* was the most prevalent isolated organism (57%). Isolated bacteria were resistant to at least one antibiotic with the high resistance rate of 94.4% to ceftriaxone, 91% to cefepime, 83.3% to cefotaxime, 85.2% to ceftazidime, and 72.2% to levofloxacin. Imipenem 31.5% and tobramycin 29.6% have the lowest rate of bacterial resistance. Of the isolates, 98.1% showed Multi-Antibiotic Resistance Index (MARI ≥ 0.3). High prevalence of bacteriuria in asymptomatic pregnant women was observed in the study areas. Therefore, early diagnosis and treatment of UTI during pregnancy can ensure safety of the mother and child, likewise reducing the risk of antimicrobial resistance.

Keywords: Urinary tract infection; Asymptomatic pregnant women; *Proteus mirabilis*: *Escherichia coli*: Antibiotic susceptibility; Antibiotic resistance

1. Introduction

Urinary tract infections (UTIs) are the inflammatory disorders caused by the existence and abnormal growth of pathogens anywhere within the urinary system (Odoki *et al.*, 2019; Johnson *et al.*, 2021). It occurs in any part of the kidney, ureters, bladder and urethra, with most infections involving the lower urinary tract namely the bladder and the urethra. Being diagnosed in over 150 million people yearly, UTIs are the second most common bacterial infection and a common pathology in women (Rozwadowski & Gawel, 2022). The infections are among the most frequent

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clinical bacterial infections affecting 2%-10% of pregnancies (Olufadi-Ahmed *et al.,* 2019; Mirzaei *et al.,* 2021), ranging from asymptomatic bacteriuria, to symptomatic acute cystitis, to the most serious, pyelonephritis (Olufadi-Ahmed *et al.,* 2019).

Asymptomatic bacteriuria (ASB) is defined as the presence of at least 100,000 organisms per milliliter of urine, or more than 100 organisms/mL of urine with associated pyuria in a symptomatic patient (Olufadi-Ahmed *et al.,* 2019). The causative agents of UTI's include *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis,* Group B *streptococcus (GBS), Staphylococcus saprophyticus* (Dube *et al.,* 2022). Although rarely caused by fungi and viruses, *Candida albicans,* cytomegalovirus, herpes simplex, type 1 human polyomavirus are the common causes of fungal and viral urinary tract infection (Flores-Mireles *et al.,* 2015).

Escherichia coli and *Proteus mirabilis* are opportunistic pathogens and a natural component of the bacterial flora in the intestinal tract of both humans and animals, common in soil, water, and clinical settings (Shaaban *et al.,* 2022; Shelenkov *et al.,* 2020). They are responsible for majority of complicated and uncomplicated urinary tract, wound, respiratory, otitis media and blood infections. The complicated urinary tract infection commonly caused by *Proteus mirabilis* is mostly associated with the presence of urinary catheters, and likewise conditions like pregnancy (Mirzaei *et al.,* 2021). The increasing prevalence of multidrug resistant *E. coli* and *P. mirabilis* isolates, particularly those carrying extended spectrum β-lactamases (ESBLs) and plasmid-mediated colistin resistance genes, poses a severe threat to the management of nosocomial infectious diseases (Banda *et al.,* 2020). These enzymes and genes decreases the therapeutic efficacy of antimicrobial treatment and limits the management of its pathogenicity (Colgan *et al.,* 2020; Inwang *et al.,* 2021; Onemu *et al.,* 2024).

Antimicrobial Resistance (AMR) is the phenomenon where bacteria, viruses, fungi, and parasites evolve over time to become resistant to medicines, making infections harder to treat and increasing the risk of disease spread, severe illness, and death (WHO, 2023). Now, it has been identified by the World Health Organization as one of the biggest threats to global health (Tamma *et al.,* 2023). Infections with resistant bacteria leads to serious illnesses, increased medical cost, the use of second-line drugs, prolonged hospital admissions, therapeutic failures and increased mortality (Li *et al.,* 2022; Tamma *et al.,* 2023). The misuse and overuse of antimicrobials are the main drivers in the development of drug resistant pathogens (WHO, 2023). Also, self-medication for UTI is quickly becoming a common practice during pregnancy, especially in communities where education on the discriminatory use of medicines for various health conditions is lacking (Adedze-Kpodo *et al.,* 2022). These factors pose a major health problem in the treatment of UTI caused by uropathogens in pregnant women. There is a need for targeted interventions such as antibiotic stewardship and surveillance to prevent further escalation on antibiotic resistance. This study was conducted to determine the prevalence of *Escherichia coli* and *Proteus mirabilis* and their susceptibility profile among asymptomatic pregnant women attending three antenatal clinics in Ilorin, Nigeria.

2. Materials and Method

The study was conducted in the antenatal clinics of Civil Service Hospital, Okelele Primary healthcare center, and Sobi Specialist Hospital in Ilorin, the capital city of Kwara state, Nigeria, over a period of 3 months (March to May, 2023). The research took the form of a descriptive cross-sectional study of pregnant women who were present at the antenatal clinics. A total of 120 asymptomatic pregnant women of age ranges between 17 and 50 years were consecutively recruited over the period of the study. The inclusion criteria included pregnant women (asymptomatic) at the study area and those who gave consent to participate in the study. Pregnant women who could not give informed consent, patients who had symptoms that were suggestive of UTI, and those who had taken antibiotics within the last 2 weeks, at the same time of collection were excluded.

Ethical consideration:
Ethical approval was obtained from the Ethical Review Committee of the Ministry of Health, Ilorin, with identification number ERC/MOH/2023/03/094 and written informed consent was obtained from the participants before inclusion in the study.

Data and Sample collection:
A pretested structured questionnaire was used to interview the patients. Information sought on the questionnaire included: age of the patient, marital status, educational level, history of suggestive urinary tract infection, ingestion of antibiotics in the preceding two weeks and toilet usage.
After seeking and obtaining consent, appropriately labelled sterile disposable universal bottles was given to each participant for collection of clean midstream urine specimen following explanation of the procedure for such collection. The samples were immediately transported to the department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin for analysis.

Viable bacterial count determination
Each urine sample was shaken to allow for homogeneity. Using a pipette, 1 mL of each sample was diluted in 9 mL of saline water and a ten-fold serial dilution was performed in eight sterile tubes. 100 µL of each dilution was then transferred onto sterile nutrient agar plates and spread evenly. All plates were incubated at 37ºC for 18 hours and plates that showed colonies between 30 – 300 were counted and colony forming unit/mL was calculated. Significant bacteriuria was determined from the calculation when there was $\geq 10^5$ colony forming units per milliliter. Colonies of urine culture with significant bacteriuria were aseptically sub-cultured on already prepared sterile nutrient agar slants for storage.

Isolation and Identification of bacteria
Pure cultures were transferred from nutrient agar slants into already sterilized nutrient broth and incubated for 18 hours and thereafter a loopful of each sample was aseptically inoculated onto Blood and Eosin Methylene Blue (EMB) agar plates. The plates were incubated aerobically at 37°C for 24 hours, and examined macroscopically for bacterial growth. Bacterial Gram staining and biochemical identification were further carried out.

Antimicrobial susceptibility testing (AST):
Using the modified Kirby-Bauer disk diffusion method, 3 pure colonies of the isolates were selected, and emulsified in sterile saline solution, thoroughly mixed to prepare bacterial inoculum that was standardized by matching the turbidity of the inoculum with 0.5 McFarland standard. A sterile nontoxic dry swab stick was dipped into the inoculum and gently squeezed against the inside of the tube in order to remove excess fluid in the swab. The swab was used to inoculate sterile Mueller-Hinton (MH) agar plate, which was left to dry for about 5 mins. Sterile forceps were then used to place the antibiotics disks on the surface of the agar plate, which was incubated at 37°C for 24 hours. The diameter of zones of inhibition of the isolates to each antibiotic were measured with a calibrated ruler, and results interpreted as sensitive or resistant according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. The following antibiotic discs (Mastgroup Ltd, Merseyside, UK) were used; Ceftriaxone (30 µg), Cefepime (30 µg), Cefotaxime (30 µg), Amoxicillin/Clavulanic acid (20/10 µg), Ceftazidime (30 µg), Tobramycin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Kanamycin (30 µg), Ampicillin/Sulbactam (10/10 µg).

Data analysis and presentation
All generated data in the study including results from the laboratory analysis and specimens were coded and fed into a computer using the IBM Statistical package for social sciences (SPSS) version 22.0. Qualitative variables were summarized in frequencies and percentages.

3. Results and Discussion

Table 1 shows the socio-demographic features of the asymptomatic pregnant women in this study. Most respondents totaling 42 (35%) were within 25-29 years, 26 (21%) were within 30-39 years old, 24 (20%) within 18-24 years old, 3 (2.5%) within 40-50 years old and 1 (0.8%) respondent was less than 18 years of age. From the total pool of respondents, 102 women were married, 65 had a history of UTI, 55 had a lower level of education and 76 women frequently used public toilets. Figure 1 shows the distribution of samples collected from the study areas, and the highest number of samples were gotten from Civil Service Hospital while the lowest number of samples were gotten from Sobi Specialist Hospital. The highest number of isolates observed in this study was obtained from Okelele Primary Healthcare center, where 19 (82.6%) *E. coli* isolates and 26 (83.9%) *P. mirabilis* isolates were obtained (Figure 2). While 2 (8.7%; 8.7%) isolates each of *E. coli* were obtained from Civil Service Hospital and Sobi Specialist Hospital. Only 5 (16.1%) isolates of *P. mirabilis* were obtained from Civil Service Hospital. However, there was no *P. mirabilis* obtained from Sobi Specialist Hospital (Figure 2).

Table 2 shows the percentage distribution of asymptomatic bacteriuria (ASB) among women with respect to their demographic characteristics. A higher percentage of women with ASB were in the 25-29 years age bracket, were married, have lower level of education, previous history of UTI and frequently use public toilets.

Figure 3 shows the percentage resistance patterns of 31 *Proteus mirabilis* and 23 *Escherichia coli* isolates. *Escherichia coli* showed highest resistance to ceftriaxone (95.7%), cefepime (91.3%), ceftazidime (82%), cefotaxime (82%), and Amoxicillin/clavulanic acid (69.6%); while *Proteus mirabilis isolates* showed highest resistance to ceftriaxone (93.5%), levofloxacin (90.3%),

cefepime (90.3%), ceftazidime (87.1%), cefotaxime (83.9%) and kanamycin (67.7%). The multiple antibiotic resistance index (MARI) of the isolates showed that a higher percentage of the *E. coli* and *P. mirabilis* isolates were multi-drug resistant. Among the 23 *Escherichia coli* 95.6% (22/23) of the isolates showed MARI ≥ 0.3 while 100% (31/31) of the *Proteus mirabilis* isolates showed MARI ≥ 0.3 (Table 3).

Table 1: Socio-demographic distribution of asymptomatic pregnant women attending selected hospitals in Ilorin

Demographic characteristics	**Frequency**	**Percent (%)**
Age		
<18	3	2.5
18-24	28	23.3
25-29	50	41.7
30-39	36	30
40-50	3	2.5
Total	**120**	**100**
Marital Status		
Single	18	15
Married	102	85
Total	**120**	**100**
UTI history		
Previous UTI	65	54.2
No previous UTI	45	37.5
Null	10	8.3
Total	**120**	**100**
Type of Toilet used		
Public Toilet	76	63.3
Private toilet	44	36.7
Total	**120**	**100**
Level of education		
Lower educational level	68	56.7
Higher educational level	52	43.3
Total	**120**	**100**

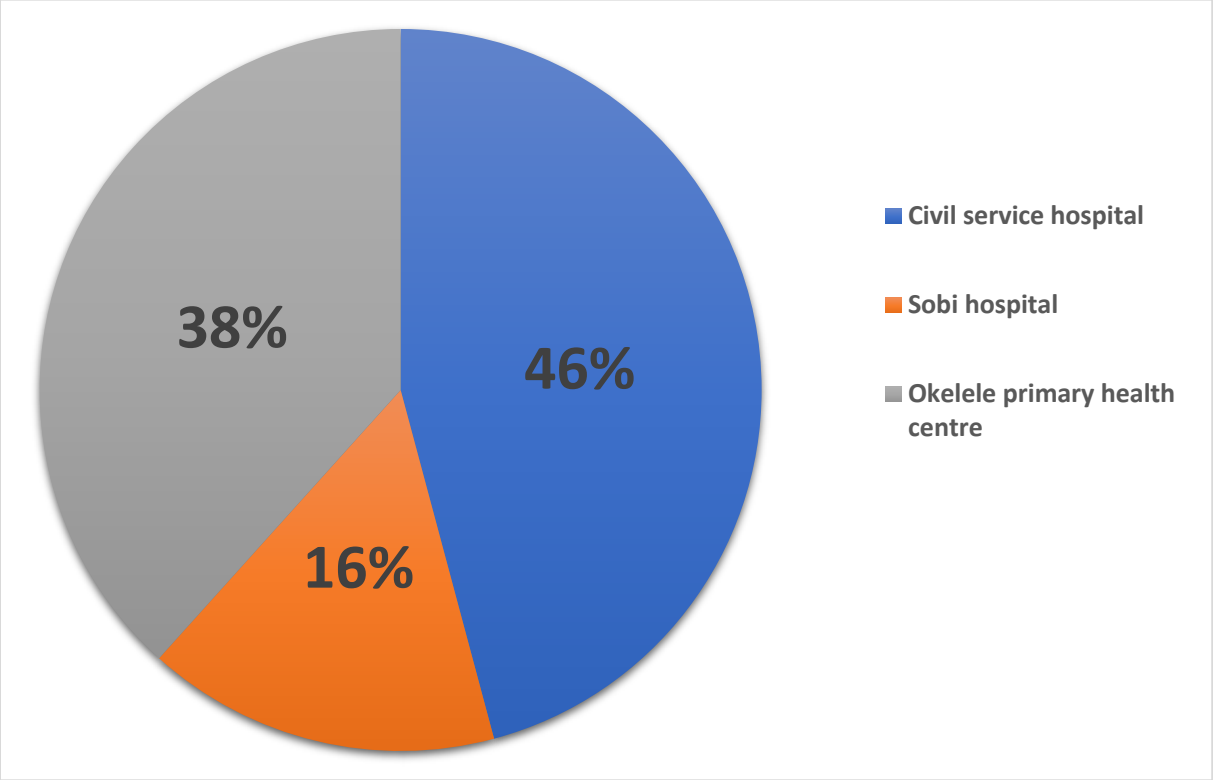

Figure 1: Percentage distribution of asymptomatic pregnant women among the selected hospitals in Ilorin

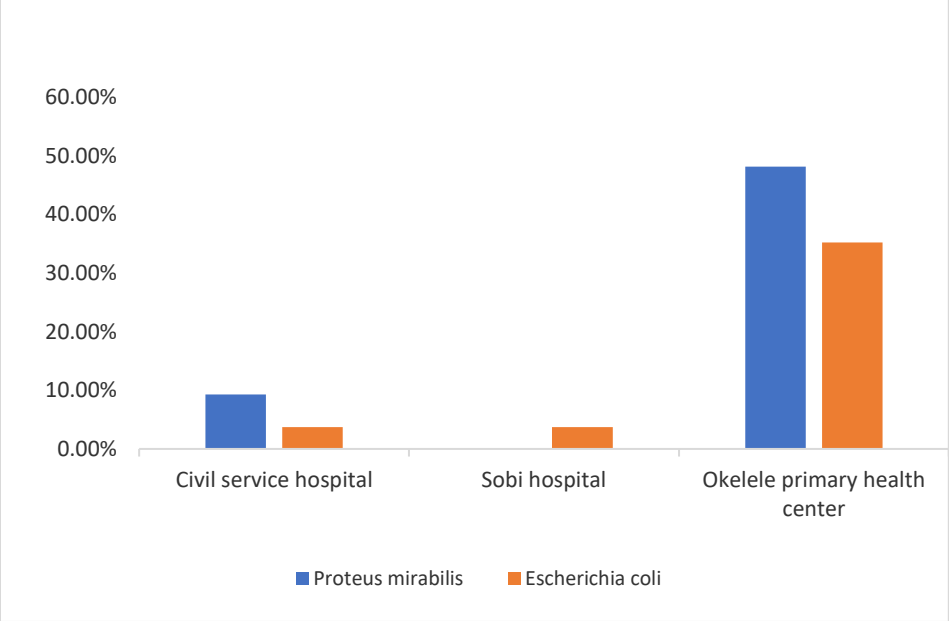

Figure 2: Percentage distribution of bacteria isolated from asymptomatic pregnant women among the selected hospitals in Ilorin.

Table 2: Association between demographic characteristics of pregnant women and presence of asymptomatic bacteriuria

Socio-demographics	**Significant bacteriuria**			**x^2**	***p*-value**
	Yes (%)	**No (%)**	**Total**		
	[54 (45)]	**[66 (55)]**	**n= 120**		
Age group(years)					
<18	1 (33.3)	2 (66.7)	3	225.903	0.000
18-24	7 (25)	21 (75)	28		
25-29	28 (56)	22 (44)	50		
30-39	17 (47.2)	19 (57.8)	36		
40-50	1 (33.3)	2 (66.7)	3		
Marital status					
Single	7 (38.9)	11 (61.1)	18	69.58	0.000
Married	47 (46.1)	55 (53.9)	102		
UTI history					
Previous UTI	34 (52.3)	31 (47.7)	65	199.041	0.000
No previous UTI	18 (40.0)	27 (60.0)	45		
Null	2 (20.0)	8 (80.0)	10		
Type of Toilet used					
Public toilet	40 (52.6)	36 (47.4)	76	76.022	0.000
Private toilet	14 (31.8)	30 (68.2)	44		
Level of education					
Lower level of education	36 (53.0)	32 (47.0)	68	98.775	0.000
Higher level of education	18 (35.0)	34 (65.0)	52		

X^2 = Chi square; p-value; CI = 95% Confidence interval

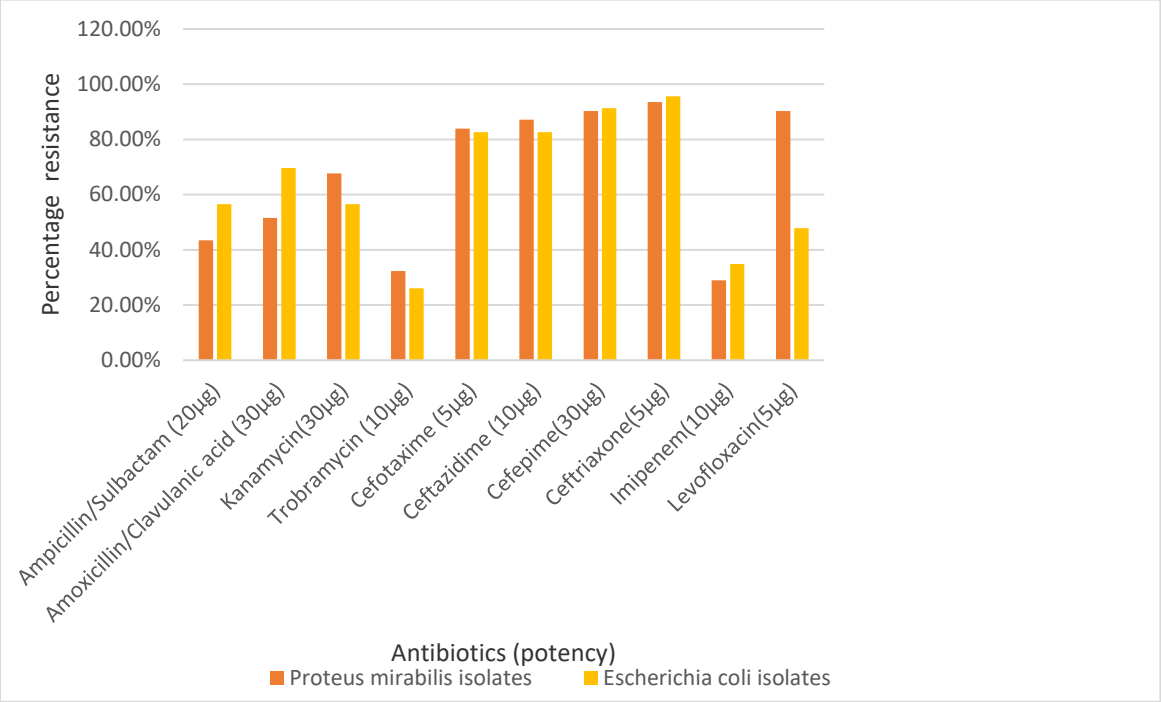

Figure 3: Antibiotic resistance patterns of bacterial isolates obtained from asymptomatic pregnant women attending antenatal clinics in the selected hospitals in Ilorin, Nigeria.

Table 4: Multiple Antibiotic Resistance Indices of bacterial Isolates

MARI	***Proteus mirabilis***	***Escherichia coli***	**TOTAL (n=54)**
0	0	0	0
0.1	0	0	0
0.2	0	1	1
0.3	2	0	2
0.4	5	1	6
0.5	4	3	7
0.6	8	8	16
0.7	6	3	9
0.8	2	3	5
0.9	3	4	7
1	1	0	1

The prevalence of asymptomatic bacteriuria among the pregnant women in this study was 45%, which is not in concordance with the global rates of 2% -10% reported in previous studies (Colgan *et al.*, 2020; Inwang *et al.*, 2021; Kebede, 2023; Onemu *et al.*, 2024); but similar to prevalence rate

of 40% reported by Ajayi *et al*., 2012, and 45% reported by Imade *et al*., 2010 in studies conducted in Ilorin and Benin respectively. These prevalence rates are however, in contrast to 83.3% which was reported by Akerele *et al*., 2001in Benin city, Edo state and 74.1% reported by Onemu *et al*., 2024 in Ondo state. Other studies conducted within Nigeria recorded lower prevalence rates as compared to these studies, Olufadi- Ahmed *et al*., 2019, Baba *et al*., 2023, Banda *et al*., 2020 and Inwang *et al*., 2021 all reported prevalence rates of 14.5%, 27.9%, 10.3% and 9.1% respectively. However, similar to 40% and 45.3% rates reported in previous studies in Ilorin (Ajayi *et al.,* 2012) and Benin (Imade *et al.,* 2010), lower than 74.1% reported in Ondo (Onemu *et al.,* 2024) and 83.3% in Benin (Akerele *et al.,* 2001), higher than 14.5% and 27.9% rates reported in other studies conducted in Ilorin (Olufadi-Ahmed *et al.,* 2019; Baba *et al.,* 2023), and far greater than 10.3% and 9.1% reported in Jos and Uyo, Nigeria respectively (Banda *et al.,* 2020; Inwang *et al.,* 2021). The differences in prevalence rates may be due to differences in sample size, study setting (hospital, community or primary care based), geographical variation, and socioeconomic status (Inwang *et al.,* 2021; Bale *et al.,* 2023). Okelele Primary health care center had the highest number (45/54) of bacterial isolates (83.3%) and 98.7% (45/46) of the respondents were observed to asymptomatically carry one organism This finding could possibly be due to the fact that Okelele primary health care center is located in a communal setting. Also, lifestyle practices, socio-economic status and personal hygiene may be possible factors for these observations.

Proteus mirabilis was the most predominant causative agent of ASB in the present study accounting for 31/54 (57.4%) of the uropathogens and *Escherichia coli* with 23/54 (42.6%). A similar finding was reported in 2012 by Ajayi *et al* who documented *Staphylococcus aureus* and Proteus species as the most predominant organisms isolated in a study carried out in Ilorin. Gram positive and gram- negative bacteria are predominantly responsible for asymptomatic bacteriuria in pregnancy (Abu *et al*., 2021), and the most common uropathogens documented to cause UTIs include *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae* and *Staphylococcus aureus* among others. This could possibly be the reason for the isolation of *Proteus mirabilis* and *Escherichia coli* in this study, although *Proteus mirabilis* is known both as a common bacterium and second most common cause of Urinary tract infections after *Escherichia coli.* The second most predominant organism isolated in this study was *Escherichia coli, this report contrasts other documented studies that showed E. coli as the* most prevalent uropathogen isolated in their studies (Banda *et al.,* 2020; Imade *et al.,* 2010; Inwang *et al.,* 2021; Obirikorang *et al.,* 2012; Okonko *et al.,* 2009).

This study established a statistically significant relationship between asymptomatic bacteriuria (ASB) and maternal age, level of education, marital status and history of UTIs. The highest frequency of bacteriuria was seen among pregnant women of age group 25-29 years (56%), This was similarly reported in other studies (Imade *et al.,* 2010; Olufadi-Ahmed *et al.,* 2019; Banda *et al.,* 2020; Inwang *et al.,* 2021; Baba *et al.,* 2023) but contrast to previous studies which reported a higher prevalence among women aged 35-39 years (Amadi *et al.,* 2007; Turpin *et al.,* 2007), although the result of the highest frequency was closely followed by age group 30-39 years with 47.2%. Women in this age groups (25-39 years) with the highest prevalence tend to engage in greater sexual activity which increases the risk of urinary tract infection (Banda *et al.,* 2020; Colgan *et al.,* 2020; Inwang *et al.,* 2021). Although, higher number of participants tested were married (102), past studies have described a relationship between high sexual activity and incidence of UTI (Banda *et al.,* 2020). Pregnant women who had recent sexual intercourse of three

or more per week were more likely to have UTI than women who had less than three intercourses per week, likely as a result of the bacteria being pushed into the urethra (Okonko *et al.,* 2009). Therefore, it is considered that married women have regular sexual intercourse than single pregnant women, hence predisposing them to UTI (Okonko *et al.,* 2009). This finding is also in agreement with previous studies in Ghana and Iran (Amiri *et al.,* 2009; Banda *et al.,* 2020). The significant association of ASB in this study with participants level of education revealed that the higher the level of education, the lower the prevalence of ASB. Existing literature also revealed findings in agreement with the study (Azami *et al.*, 2019; Ahiatrogah *et al.*, 2021; Zhu *et al.*, 2021), indicating least educated women were at a higher risk of bacteriuria. On the other hand, the low prevalence of asymptomatic bacteriuria associated with higher level of education may be attributed to the knowledge base of pregnant women on personal hygiene. This result is contrary to a finding in a previous study by Labi *et al*., (2015) who reported no association between the presence ASB and educational level. Majority of the isolates were obtained from participants with past history of UTI, this finding suggests previous history of UTI can be a risk factor for developing asymptomatic bacteriuria. Similar findings were reported by Haider *et al* (2010) on UTI in Pakistan and Aseel *et al* (2009) in Qatar. Masinde *et al* (2009) also identified that past history of UTI is a risk factor for UTI during pregnancy. But absence of association was reported by Hamdan *et al* (2011) in Sudan and by Kovavisarach *et al*, (2009) in Thailand.

This study, recorded high rate of resistance for both organisms against ceftriaxone 94.4%, cefepime 91%, cefotaxime 83.3%, ceftazidime 85.2% and levofloxacin 72.2%. Moderate resistance of uropathogens to amoxicillin/clavulanic acid 59.3%, kanamycin 63% and ampicillin 52%. The high rate of resistance to cephalosporins does not correlate with studies carried out in Benin, Abakaliki and Akwa- Ibom which depicted ceftriaxone as the most susceptible and second most susceptible antibiotics respectively (Imade *et al.,* 2010; Onoh *et al.,* 2013; Umoyen *et al.,* 2021).

Proteus mirabilis isolates showed the least resistance to imipenem 29%. This finding differed from a study performed by Umoyen *et al* (2021), but correlated with a study carried out by Al-Jumaily and Zgaer (2016). The least resistance observed for *E. coli* was against Tobramycin to which 26.1% of the isolates were resistant. This finding could be due to several factors, top of which would be the unavailability of oral tobramycin and the fact that it is not among the commonly prescribed antibiotics in the environment Also tobramycin is also not readily available in most pharmacies and patent medicine stores in Ilorin so it is probably not a drug commonly abused in the study population hence the low resistance observed. The various resistance observed in this study may not be unconnected to inappropriate adherence or use of sub-therapeutic doses of antibiotics in the treatment of previous cases of infections.

The multiple antibiotic resistance index (MARI) of the isolates represented in the study showed that all isolates but one, were resistant to three or more antibiotics. Among the 23 *Escherichia coli* strains, 22/23 (95.7%) showed MARI $\geq$ 0.3 and among the 31 *Proteus mirabilis*, 31/31 (100% showed MARI $\geq$ 0.3 with one isolate showing resistance to all ten antibiotics. The Multiple Antibiotic Resistance Index (MARI), a measure of an organism's resistance to antibiotics, revealed that the majority of the isolated organisms were resistant to multiple drugs from distinct antibiotic classes, thus indicating that there is a high level of antibiotic misuse or abuse. The high rate of

Multiple Antibiotic Resistance strains among the isolates is a serious cause of concern especially in pregnant women whose treatment options are already limited given their physiological condition.

4. Conclusion

This study demonstrated the prevalence of asymptomatic UTI among pregnant women in Ilorin. The most prevalent uropathogen in the study area was *Proteus mirabilis* followed by *Escherichia coli.* This study demonstrated that demorgraphic characteristics are important factors associated with the UTIs. Therefore, early diagnosis and treatment of UTI during pregnancy can ensure safety of the mother and child, likewise reducing the risk of antimicrobial resistance.

Conflict of interest

Authors declared no conflict of interest

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