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Pattern of Bacterial Contamination of Urine in Patients Attending Rivers State University Teaching Hospital, Port Harcourt, Rivers State

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ABSTRACT

Among the great diversity of etiological agents attributed to urinary tract infections, bacteria are the major causative organisms that are responsible for more than 95% of Urinary Tract Infections (UTIs). This study aimed at investigating the pattern of bacterial contamination of urine in patients attending Rivers State University Teaching Hospital (RSUTH) Port Harcourt, Rivers State, Nigeria. A total of 64 urine samples were aseptically obtained from consented pregnant women, renal failure, sickle cell and diabetic patients attending RSUTH at monthly interval for three (3) months and analyzed for urinary tract bacteria using standard microbiological procedures. Results revealed that, of the 64 urine samples studied, 40 (62.5%) had bacterial growth. A total of 127 bacteria were isolated and identified. Species identified and frequencies were; *Bacillus cereus* (59.4%), *Escherichia coli* (25%), *Staphylococcus* species (23.4%), *Klebsiella pneumonia* (29.7%), *Micrococcus* species (17.1%), *Alcaligenes* pieces (15.6), *Seratia* species (12.5%), *Pseudomonas* species (9.4%), and *Proteus* species (6.3%). Socio-demographic information on the prevalence of bacteria within each study population showed pregnant cases recorded 24(75%) while sickle recorded 12 (58.3%), renal failure 15 (53.3%) and, diabetics 13(53.8%). Bacterial prevalence in pregnant subjects based on occupation showed business 11(84.6%), students 3(60%), and civil servant 4(66.7%); for sickle cell subjects, business 1(50%), student 6(60%) and civil servant 0(0%)., renal failure subjects showed business of 2(66.7%), student 3(60%), civil servant 3(42.8) and for diabetic subjects; business 1(33.3%), student 3(60%) and civil servant 3(60%). Pregnant subjects recorded highest prevalence 18(75%) at the occupation level. Marital status showed, single 17(80.9%%), married 1(33%), and divorced 0(0%) for pregnancy subjects, for sickle cell subjects, single 6(54.5%), married 0(0%), and divorced 1(100%)., for renal failure single 5(83.3%), married 2(25%), and divorced 1(100%), for diabetics, single 4(57.1%), married 1(25%), and divorced 2(200%). All the isolates were positive for biofilm production and haemolytic activity. These findings highlights the need for routine urine culture screening in high-risk groups (pregnant women and sickle cell patients) as early detection and treatment of UTI can significantly reduce associated complications.

Keywords: Urinary Tract Infection, Bacteria, Pregnant Women, Diabetics, Sickle Patients, Haemolysis, Biofilm.

Introduction

Urinary tract infection (UTI) is an infection in any part of the urinary system including the kidney ureters, bladder and urethra. Most infections involve the lower urinary tract- the bladder and urethra (Mayo, 2019). Urinary tract infections (UTIs) are a significant public health concern, particularly in hospital settings, where they are a leading cause of morbidity and mortality (Foxman, 2002). The increasing prevalence of antibiotic-resistant bacteria has further complicated UTI management, emphasizing the need for current data on bacterial contamination patterns (World Health Organization, 2017).

UTI is the major cause of morbidity in hospital and community settings, and it occurs in all age groups and both genders. It is one of the most common infections to plague man worldwide and causing serious health problems affecting millions of people each year (Ebie *et al*., 2001). Urinary tract infections may arise from ascending, haematogenous or lymphatic routes, following colonization or periurethral area by enteric microorganism and there are some special features possessed by the microorganism can enable them establish the infection (Moyo *et al*., 2017).

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UTIs are a major health issue in Nigeria, with a high prevalence rate reported in various studies (Iroha *et al*., 2017). The country's healthcare system faces numerous challenges, including inadequate diagnostic facilities, poor infection control practices, and limited antimicrobial stewardship programs, which contribute to the rising incidence of UTIs (Okeke *et al*., 2005). The region's tropical climate and high humidity may facilitate the growth and transmission of bacteria, exacerbating the problem (Ronald, 2002). The lack of effective infection control measures and inadequate antimicrobial therapy has led to the emergence of multidrug-resistant bacteria, making treatment even more challenging (Moyo *et al*., 2017).

The high prevalence of UTIs in Nigeria can be attributed to various factors, including poor hygiene, inadequate healthcare facilities, and limited access to healthcare services (Iroha *et al*., 2017). Additionally, the overuse and misuse of antibiotics have contributed to the emergence of antibiotic-resistant bacteria, making treatment more challenging (Okeke *et al*., 2005).

Furthermore, the lack of effective infection control measures in hospitals has led to the spread of bacteria, increasing the risk of UTIs (Ronald, 2002). The region's tropical climate and high humidity also facilitate the growth and transmission of bacteria, exacerbating the problem (Moyo *et al*., 2017).

Materials and Methods

Study Area

The study was carried out in Rivers State University Teaching Hospital (RSUTH) Port Harcourt, Rivers State, Nigeria.

Study Design

The study was a cross-sectional study, which included pregnant women, renal failure patients, Sickle cell and Diabetic patients attending Rivers State University Teaching Hospital (RSUTH), Port Harcourt.

Inclusion Criteria

Patients who gave consent and submitted urine samples for this study.

Exclusion Criteria

The patients with incomplete demographic information or contaminated samples and those who declined consent, as well as those on antibiotics.

Sample Collection

A total of sixty-four (64) urine samples were collected from consented patients attending the Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Rivers State. Consented patients were advised and instructed to submit a clean catch mid-stream urine aseptically collected into sterile dry, universal sample containers.

The procedure was done by properly educating the patients on how to collect the sample after giving them the sterile universal container. They were told to clean the area around the opening of the urethral with very clean water, dry the area and collect about 20ml of urine. This careful procedure was to avoid contamination by skin flora. A well-structured questionnaire was used to obtain their demographic data.

The samples were properly labeled using the patient's demographic information. Samples were quickly transported to the microbiology laboratory within 1 hour of collection, and analysis was conducted within 3 hours of sample collection. Sample collection and analysis was carried out at monthly interval for three (3) months.

Microbiological Analysis of Urine Samples

Inoculation Method

Streak plate method of inoculation was carried out by using a pipette to collect 0.1mL of urine and inoculating on each of the following media; Nutrient agar (NA), MacConkey Agar (MCA), Eosin methylene blue agar (EMB) and Mannitol salt agar (MSA) using a streaking plate method as described by Cheesbrough (2000).

The plates were observed for colonies after 24 hours of incubation aerobically at 37°C and also their growth characteristics were taken note of both in Nutrient agar (NA), MacConkey Agar (MCA), Eosin methylene blue agar (EMB) and Mannitol salt agar (MSA) respectively (Cheesbrough, 2000).

The different media helped to further distinguish between lactose fermenters and non- lactose fermenters. If any colonies were present it was confirmed to see if they were significant enough to indicate a clinical urinary tract infection. If any colonies were present it was confirmed to see if they were significant enough to indicate a clinical urinary tract infection. The number of colonies per ml of urine was recorded. If contaminant were present, it was ensured that the colonies of main bacteria were reported and not contaminants. The colonies were summed up in each plate which gave a total colony count. Plates containing 30-300 colonies was used to calculate the bacterial population and recorded as CFU/ml. To determine the colony counts in CFU/ml of sample, the following formula was used (Cheesbrough, 2000).

Calculation of Microbial Load in Colony forming units per milliliter (CFU/ml) =

$$\frac{\text{Number of Colonies x Dilution Factor}}{\text{Volume of Inoculum used}}$$

Results

Table 1 shows the Prevalence of bacteria among patients with different health condition in the category of pregnant women, sickle cell, Renal failure and Diabetic patients revealing that out of 64 urine samples studied, 40 (62.5%) had bacterial growth. The study also showed that the pregnant cases recorded 24(75%), while sickle recorded 12 (58.3%), renal failure 15 (53.3%) and, diabetics 13(53.8%).

Table 1: Prevalence of bacteria in urine among patients with different health conditions

Patient Category	Number studied	Number of positive with Bacteria	Prevalence (%) in population studied	Percentage (%) Prevalence within Group
Pregnancy	24	18	28.13	75
Sickle cell	12	7	10.94	58.3
Renal failure	15	8	12.5	53.3
Diabetic	13	7	10.94	53.8
Total	64	40	62.5	62.5%

Table 2 shows socio demographic data and prevalence of bacteria in urine of study population where prevalence in pregnancy subjects based on occupation shows business 11(84.6%), student 3(60%), and civil servant 4(66.7%); for sickle cell subjects, business 1(50%), student 6(60%) and civil servant 0(0%)., renal failure subjects showed business of 2(66.7%), student of 3(60%), civil servant of 3(42.8) and for diabetic subjects; business 1(33.3%), student of 3(60%) and civil servant 3(60%). The highest percentage prevalence recorded of bacterial pattern for pregnancy subjects of 18(75%) at the occupation level. The bacterial urinary prevalence in pregnant subjects based on gender female showed 18(75%) and male 0(0%); for sickle cell subjects, renal failure and for diabetic subjects showed female 3(50%), male 4 (66.7), female 3(42.8%), male 5(62.5%) and female 3(50%), male 4(57.1%). The highest percentage prevalence recorded of bacterial pattern for pregnancy subjects of 18(75%) at gender level.

The bacterial prevalence associated with pregnancy, based on marital status showed, single 17(80.9%%), married 1(33%), and divorced 0(0%) for pregnancy subjects, for sickle cell subjects, single 6(54.5%), married 0(0%), and divorced 1(100%); for renal failure single 5(83.3%), married 2(25%), and divorced 1(100%), for diabetics single 4(57.1%), married 1(25%), and divorced 2(200%). The highest percentage prevalence recorded of bacterial pattern for pregnancy subjects of 18(75%) at marital status level.

Table 3 shows prevalence of bacteria according to age ranges are; 11-20 years (Pregnancy 2(66.7), sickle cell 3(50%), renal failure 1(100%) and diabetic 1(50%), while prevalence bacteria for 21-30 years (Pregnancy 7(70%), sickle cell 3(75%), renal failure 1(50%) and diabetic 1(50%); prevalence for 31-40 years showed pregnancy to be 9(90%), sickle cell 0(0%), renal failure 2(66.7%) and diabetic 1(33.3%).

For prevalence age of 41-50 years showed pregnancy 1(100%), sickle cell 1(100%), renal failure 3(75%) and diabetic 1(50%), for 50 and above (pregnancy, sickle cell showed 0(0%), while renal failure 1(25%) and diabetic 6(46.1%). The highest percentage prevalence of subjects with bacterial based on age ranges is pregnancy of 19(79.1%).

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Table 2: Socio-Demographic data and prevalence of bacteria in urine of the study population

Demographics (n= 64)		Patient category								Total (%) Prevalence
		Pregnancy		Sickle cell		Renal failure		Diabetics		
		n	B+ (%)	n	B+ (%)	n	B+ (%)	n	B+ (%)	
Occupation										
Business	21	13	11 (84.6)	2	1(50)	3	2(66.7)	3	1(33.3)	15(71.4)
Student	25	5	3 (60)	10	6 (60)	5	3 (60)	5	3 (60)	15(60)
Civil servant	18	6	4 (66.7)	0	0(0)	7	3 (42.8)	5	3 (60)	10(55.5)
Total	64	24	18 (75)	12	7 (58.3)	15	8 (53.3)	13	7 (53.8)	40(62.5)
Gender										
Female	43	24	18 (75)	6	3 (50)	7	3 (42.8)	6	3 (50)	27(62.8)
Male	21	0	0(0)	6	4 (66.7)	8	5 (62.5)	7	4 (57.1)	13(61.9)
Total	64	24	18 (75)	12	7 (58.3)	15	8 (53.3)	13	7 (53.8)	40(62.5)
Marital status										
Single	26	21	17 (80.9)	11	6 (54.5)	6	5 (83.3)	7	4 (57.1)	32(43.4)
Married	15	3	1 (33.3)	0	0(0)	8	2 (25)	4	1 (25)	4(26.7)
Divorced	4	0	(0)	1	1 (100)	1	1 (100)	2	2 (100)	4(75)
Total	64	24	18 (75)	12	7 (58.3)	15	8 (53.3)	13	7 (53.8)	40(62.5)

Key: B+ = Number positive with Bacteria

Table 3: Presence of bacteria in urine based on age range

Age Range (Years)	Pregnant women n=24		Sickle cell n=12		Renal failure n=15		Diabetic n=13	
	No. studied	No. Positive (%)	No. studied	No. Positive (%)	No. studied	No. Positive (%)	No. studied	No. Positive (%)
11-20	3	2 (66.7)	6	3(50)	2	1(50)	2	1(50)
21-30	10	7(70)	4	3(75)	2	1(50)	2	1 (50)
31-40	10	9(90)	1	0 (0)	3	2(66.7)	3	1(33.3)
41-50	1	1(100)	1	1(100)	4	3(75)	2	1(50)
50 and Above	0	0	0	0	4	1(25)	4	2(50)
Total	24	19 (79.1)	12	7(58.3)	15	8(53.3)	13	6(46.1)

Key: %= Percentage

Figure 1 shows the distribution of bacteria among patients with different health conditions. A total of one hundred and twenty-seven (127) bacterial isolates belonging to 9 genera were identified as *Bacillus, Escherichia, Seratia, Pseudomonas, Micrococcus, Alcaligenes, Staphylococcus, Proteus* and *Klebsiella.* These isolates displayed similar biochemical and morphological characteristics with those on the ABIS data base. Results as in table 4 showed that the highest frequency occurrence of 19.7% was recorded for *Bacillus,* 18.1% for *Escherichia,* 16.5% for *Staphylococcus,* 15.0% *Klebsiella*, 9.4% for *Micrococcus,* 7.4% for *Alcaligenes,* 6.4% for *Seratia,* 4.7% for *Pseudomonas* and the lowest frequency occurrence of 3.1% was recorded for *Proteus* Spp.

Table 4 shows percentage frequency of biofilm and haemolysis production ability of the identified bacterial; *Alcaligenes* sp was 100% while *Bacillus* and *Escherichia* species were 68% positive for biofilm production while *Pseudomonas* spp. was 61.4% positive to biofilm production, *Staphylococcus* was 52.4% positivity, *Klebsiella* spp., *Micrococcus, Proteus* and *Seratia* spp. were 50% positive for biofilm production. Results for haemolytic activity demonstrated by the bacterial isolates revealed that *Seratia, Micrococcus* and *Proteus* spp were 50% positive for haemolysis. *Klebsiella* and *Staphylococcus* were 47.4% and 47.6% positive. *Bacillus* 32%, *Escherichia coli* showed 30.4%, *Pseudomonas* sp showed 38.6% positivity and *Alcaligenes* species was negative (0%) for haemolytic activity.

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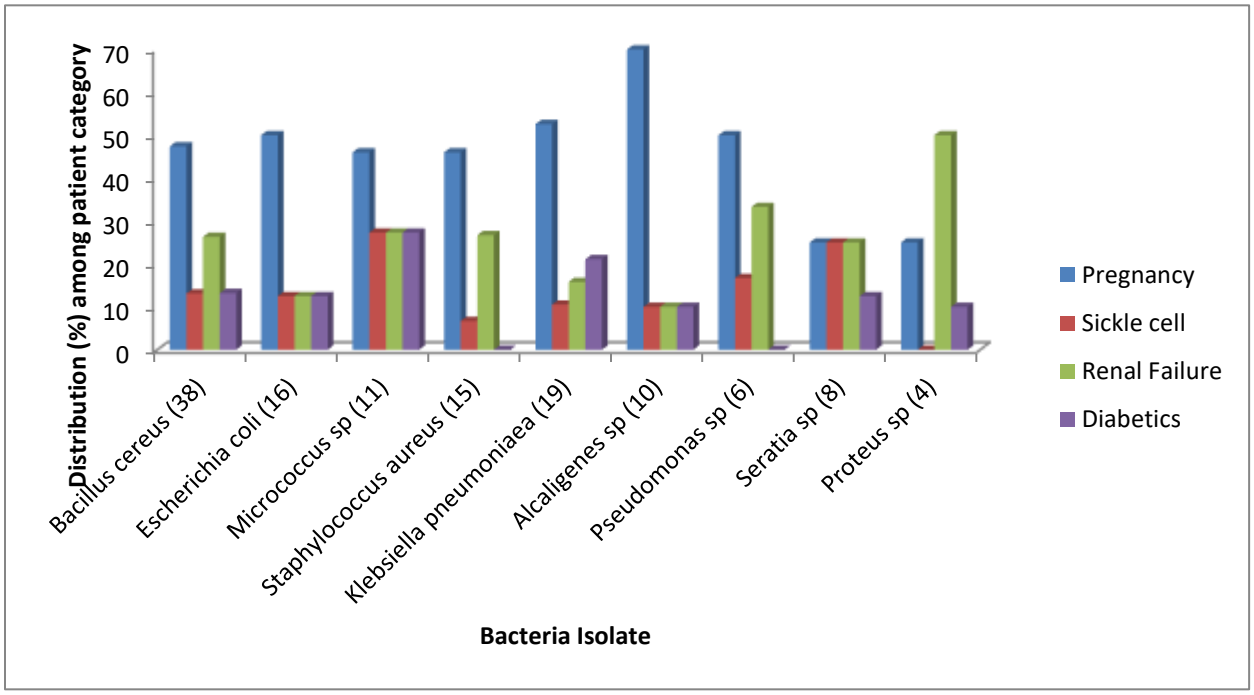

Fig. 1: Distribution (%) of bacteria among patients with different disease conditions

Table 4: Percentage frequency of biofilm and haemolytic production of the bacterial isolates

Isolates	Biofilm Test (%)	Haemolysis Test (%)
Bacillus spp (25)	17(68)	8(32)
Klebsiella spp (19)	10(52.6)	9(47.4)
Alcaligenes spp (9)	9(100)	0(0)
Micrococcus spp (12)	6(50)	6(50)
Staphylococcus spp (21)	11(52.4)	10(47.6)
Pseudomonas spp (6)	3(50)	3(50)
Proteus spp (4)	2(50)	2(50)
Escherichia spp (23)	16(69.6)	7(30.4)
Seratia spp (8)	4(50)	4(50)
Total 127	78(61.4)	49(38.6)

Key: % = Percentage

Discussion

The study provided information on the prevalence of bacterial in urine specimens of pregnancy, sickle cell, renal failure diabetic patients attending Rivers State University Teaching Hospital, Port Harcourt.

The findings revealed a high prevalence of bacterial contamination with *Bacillus cereus,* having the overall highest percentage prevalence of 29.1%, followed by *Klebsiella pneumoniae* and *Escherichia coli* with percentage prevalence of 15% and 12.6% respectively. The prevalence of the different isolated bacteria in ascending order are: *Proteus* sp (3.1%), *Pseudomonas* sp (4.7%), Seratia (6.3%), Alcaligenes (7.9%), *Micrococcus* sp (8.7%), *Staphylococcus aureus* (11.8%), *Esherichia coli* (12.6%), *Klebsiella pneumoniae* (15%) and *Bacillus cereus* (29.1%). The study is in line with the finding by Jombo *et al*., 2021 who reported similar case of *Escherichia coli* and *Bacillus cereus* of 12% and 29% as the most isolated bacterial from urine samples in Oyo State, Nigeria. This is however also contrary to the studies done by other investigators which recorded *E. coli* as the most prevalent bacterial isolate with 41.9% (Martins *et al*., 2019). The high prevalence of *Bacillus cereus* in this study agrees with the findings of Martins *et al*., 2019 that had 31.4% and Flores-Mireles *et al*. (2022), who also had bacterial prevalence of 28% isolated from different urine samples.

The results of the study on Social Demographic information and the Prevalence of bacterial among pregnant women, sickle cell, Renal failure and Diabetic revealed that out of 64 urine samples studied, 40 (62.5%) had bacterial growth

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The total percentage prevalence of Pregnancy showed bacterial growth of 18 (75%), while Sickle cell had bacterial growth percentage prevalence of 58.3%, Diabetic 53.8% and Renal failure 53.3%.

Total percentage prevalence of bacterial among pregnant women was higher than sickle cell, renal failure and diabetic patients respectively. This is however higher in contrast to the studies conducted by Mohammedaman *et al*., (2019) (63.9%), Sewify *et al*., (2016) (65%) and Jha *et al*., (2014) (44.6%) recorded among pregnancy women.

Pregnancy have a higher prevalence of bacterial as compared to other subjects sampled, this could be as a result of anatomical characteristics (shorter urethra, closer proximity of urethra to anus), hormonal changes during pregnancy, altered immunity, increased urine pH, exposure to unhygienic environment and delayed urination and incomplete bladder emptying during pregnancy period. For sickle cell diseases, individuals with sickle cell disease are at increased risk of developing UTIs due to factors such as urinary tract abnormalities and immunosuppression and other factors (Chukwuocha *et al*., 2012). Patients with renal filure are also at risk of developing urinary tract infection due to factors such as urinary retention, catheter use and immunosuppression. For diabetic patients which include depressed plymorphonuclear leucocyte functions, altered leucocyte adherence, chemotaxis, phagocytosis, impaired bactericidal activity of the antioxidant system. A higher glucose concentration in the urine may create a culture medium for pathogenic microorganisms in diabetic subjects that may result in bacterial (Stapleton 2002, Hopps *et al*., 2018).

This study demonstrated that occupation, gender, marital status and age can significant related with bacterial contamination pattern among pregnancy, sickle cell, renal failure and diabetic subjects.

The highest percentage prevalence recorded of bacterial pattern of urine contamination of patients attending RSUTH was observed for pregnancy subjects of 18(75%) at occupation level It was observed from this study that business people had the highest prevalence of 71.4% while students had 60% and the lesser bacterial pattern of contamination of 55.5% was with civil servant in the occupation category.

This could be due to various factors such as; increased stress and delayed urination among business women and men, increased frequency of sexual intercourse and delayed urination among student and also increased exposure to bacteria and inadequate hygiene practices among civil servant in this category (Martins *et al*., 2019).

The bacterial prevalence of pregnancy subjects based on level of gender female showed 18(75%) and male 0(0%); for sickle cell subjects, renal failure and for diabetic subjects showed female 3 (50%), male 4 (66.7), female 3 (42.8%), male 5 (62.5%) and female 3 (50%), male 4 (57.1%). The highest percentage prevalence recorded of bacterial pattern of contamination in the study was also recorded for pregnancy subjects of 18 (75%) at gender level. This trend also follows for the category of level of gender which female had higher prevalence a with pregnancy subjects higher than other subjects. Female increases the risks of urinary tract infection due to; anatomical characteristics (shorter urethra, closer to proximity of urethra to anus, hormonal and behavioral changes during pregnancy, increase urine pH and osmolality during pregnancy, delayed urination and incomplete bladder emptying (Brown *et al.,* 2005). This is similar to studies conducted by Kabugo *et al*., (2016); Chukwuocha *et al*., (2012)., who reported that Female gender were found to be more vulnerable to bacterial associated with UTIs than male counterpart. Studied also showed that Female gender were found to have statistical significant relationship with urinary tract infections and other related illnesses (Kabugo *et al*., 2016).

The bacterial prevalence based on marital status showed that single had a higher prevalence of bacterial contamination of 17(80.9%%) compared to married 1(33%), and divorced 0(0%). This finding may be attributed to various factors such as; increased stress and delayed urination, increased frequency of sexual intercourse and other unhygienic activity among single pregnant women. Increased frequency of sexual intercourse and delayed urination among married pregnant women, and also differences in healthcare seeking behavior and access to healthcare services among women of marital statuses (Al-Rubeaan *et al*., 2013). High percentage prevalence recorded of bacterial contamination pattern for pregnancy subjects of 18 (75%) at marital status level.

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This is as a result of physiological changes during pregnancy such as ureteral dilation which pregnancy hormones cause the ureters to dilate leading to a slower flow of urine and increased risk of bacterial growth. Other factors such as bladder expansion of uterus during pregnancy could put pressure on the bladder, reducing its capacity and leading to frequent urination can also contribute to bacterial growth and urinary tract infections. Studied showed that behavioral factors such as delayed urination, poor hygiene and increased sexual activity during pregnancy could increase the risks of bacterial growth and UTIs during pregnancy (Kibret and Abera, 2014). Studied by Kabugo *et al*., 2016, observed that factors such as pre-existing medical conditions and genetic predisposition could also lead to bacterial growth and UTIs in pregnant women.

Conclusion

The findings of this study highlights the need for continued education of members of the public and training of healthcare workers on proper hygiene practices, and the need to enhance urinary tract infection (UTIs) control measures, as well as encourage continuous research initiatives to expand knowledge on bacterial ecology and other genetic factors influencing multi-drug resistance trends.

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