



Assessment Of Nitrate Fermenting Bacteria In Various Trimesters Of Pregnant Women Attending The Antenatal Clinic In Obio Cottage Hospital

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ABSTRACT

The study examined Assessment of Nitrate Fermenting Bacteria in Various Trimesters of Pregnant Women Attending the Antenatal Clinic in Obio Cottage Hospital. A cross-sectional laboratory test to detect nitrite in mid-stream urine samples was done among 406 pregnant women amid the ages of 18 and 54 years. Laboratory testing was done with a dipstick containing a reagent that reacts with nitrites to produce a pink colour, thus suggesting the presence of bacteria that converts Nitrate to Nitrite (Nitrate fermenting bacteria). Positive Nitrate fermenters in the urine for the first trimester was 9.03% (13), the second trimester was 8.89% (12), and the third trimester was 3.15% (4). The association between the prevalence of nitrite fermenters in the urine and the various trimesters of pregnant women did not show any statistical significant difference ($p = 0.108$). Scanty *E. coli* for the first trimester was 15.38% (2), for the second trimester was 38.46% (5) and for the third trimester was 46.15% (6). Moderate growth *E. coli* for the first trimester was 16.67% (2), for the second trimester was 16.676% (2) and for the third trimester was 66.67% (8). Heavy growth *E. coli* for the first trimester was 46.15% (6), the second trimester was 66.67% (8), and for the third trimester was 50.0% (2). The prevalence of nitrite fermenters was observed to decline from first to third trimester among the women (9.0% - 3.2%). A scanty growth of the nitrate fermenters was mostly common among women in their third trimester in comparison to second and first trimester respectively. This study revealed that the first trimester of pregnancy is a high-risk window for the colonization of nitrate fermenting uropathogens.

Keywords: Nitrate Fermenting Bacteria, Trimesters, *Escherichia coli*

INTRODUCTION

Many women at some point in their lives aspire to become pregnant with the expectation a healthy baby and uncomplicated delivery (WHO, 2014). However, the desired result is not always the case due to certain factors that increase women's risk of infection in pregnancy. As indicated by Haider *et al.* (2010), he found that some pregnancies don't result in disease. However, some pregnancy-related changes in hormones, physiology, emotional states, and anatomical structures which can make them more susceptible to urinary tract infections, and increase in the size of the uterus, hemodilution, increase in

urine PH, ureteral dilation decreased bladder capacity due to compression from the gravid uterus, vesicoureteral reflux, and some progesterone-related changes are among the commonly observed conditions. (Feitosa, 2009). Despite the milestone advances in maternal care the last few years, about 295,000 women died during and following pregnancy and childbirth in 2017, and infection, among other factors, was identified as a direct cause of such death (WHO, 2019). Urinary tract infections (UTIs) are common in pregnancy (WHO, 2018). NHS (2017) defined urinary tract infections as an infection of the bladder, kidneys or the tubes connected to them. The most significant factors predisposing women to UTI in pregnancy is asymptomatic bacteriuria (ASB). Although many UTIs are easy to treat. It also has the potential to results in serious problems like pyelonephritis in 30% of the mothers. The bacteriostatic gas nitric oxide (NO) is formed when nitrite is acidified. Infections urine may contain considerable amounts of nitrate as a results of bacterial nitrate reductase activity, and the detection of nitrate in de urine is routinely used in the diagnosis of bacterial cystitis (Little *et al.*, 2009). There are few domestic reports on the prevalence of UTI to be 55% in Benin, whereas Ali and Abdallah (2019) reported a prevalence of 15.8 percent in Kano and 61 percent in Akure by Simon-Oke, Odeyemi, and Afolabi (2019). Apart from traces of leukocyte, presence of nitrates in urine is usually indication of bacterial infection/colonization (Bono and Reygaert, 2020). Nitrates are formed by breakdown of urine nitrates. Gram-negative and some Gram-positive bacteria, including *E. coli*, *Staphylococcus*, and *Klebsiella*, produce enzymes capable of fermenting urinary nitrates to nitrites and are implicated in UTIs (Tan, and Chlebicki, 2016; Ali, and Abdallah, 2019). As a result, the presence of nitrates indicates bacterial infection. Little *et al.* (2009), further explained that the bacteriostatic gas nitric oxide (NO) formed when nitrite is acidified. In essence, infection urine may contain considerable amounts of nitrate as a results of bacteria nitrate reductase activity, and the detection of nitrate in urine is routinely used in the diagnosis of bacterial cystitis. According to WHO (2018) estimates. Pyelonephritis, (infection of the kidneys) occur in 2% of pregnancies. Currently, little is known about the best way to prevent UTI in pregnancy (WHO, 2018), therefore the key remains discovering one's infection status for early management. Early detention and management is usually associates with better outcome in UTI (WHO, 2018). Due to both the high rate and potential seriousness pyelonephritis, it is recommended that all pregnant women should be screened for ASB at their first prenatal visit. This is most often done with a clean catch urine culture. The treatment of ASB reduces the rate of clinical infection to 3% or 4%. In a developing country such as Nigeria, such screening is attainable, hence the need to screen pregnant women for significant proteinuria in women with hypertension in pregnancy.

Statement of the problem

As of 2017, approximately 810 women died from preventable causes related to pregnancy and childbirth daily (WHO, 2019). UTIs are one of these causes, and they can be identified and treated by carrying out a rapid screening. The failure to activate a timely response to an obstetric emergency is associated with an increased risk of death (WHO, 2017). On the other hand, asymptomatic bacterium during pregnancy has been linked to an increased risk of preterm delivery and low birth weight (Morgan, 2017). Consequently, infections can be passed to the infant via the placenta or during delivery. When this happens, the baby is also at risk for health complications as well. Unfortunately, the probability of missing or overlooking early signs of infection in pregnancy is high because it may be difficult to recognize subtle symptoms presentation masked by pregnancy idiosyncrasy, (Morgan, 2017). When the immune system of a pregnant woman is compromised and she may have other reasons for malaise, flushes, miscellaneous aches, nausea, vomiting, and stomach discomfort, all of which could be herald infection (Morgan, 2017). According to Lee *et al.* (2019), screening coverage and management of UTIs is still limited in low-middle income countries (LMICs), whereas in advanced nations, diagnostic and treatment procedures have significantly improved due to expansive screening (Gilbert *et al.*, 2013). In essence, many cases remain untreated in these localities due to inadequate diagnostic procedures. This indicates the significance of diagnosing this condition because the difference in pregnancy outcomes lies in to obtaining adequate care that addressing issues revealed during diagnosis.

Aim and Objectives of the study

The aim of the study is to assess nitrate fermenting bacteria in various trimesters of pregnant women attending antenatal clinic at Obio cottage hospital. Specifically, to:

1. assess the presence of nitrate fermenters in the urine of first trimesters pregnant women in Obio cottage Hospital.
2. investigate the presence of nitrate fermenters in the urine of the second trimesters pregnant women in Obio cottage Hospital.
3. evaluate the presence of nitrate fermenters in the urine of the third trimesters pregnant women in Obio cottage Hospital.
4. assess the prevalence of nitrate fermenting bacteria in the urine of pregnant women across various trimesters of pregnant women in Obio cottage Hospital.

METHODOLOGY

The study is a cross-sectional laboratory test to detect nitrate in urine. The study was carried out in Obio Cottage Hospital located in Port Harcourt which is situated in the South-South Geo-Political zone, Nigeria. It is a pioneer hospital in the implementation of the community health care insurance scheme which is sponsored by the Shell Petroleum Development Company (SPDC). The average attendance over a year in the antenatal clinic is about 9,851. The sample size of 406 was resolved utilizing power analysis formula for estimating a single population proportion, mid-stream urine samples were collected from the 406 pregnant women between the ages of 18 and 54 years who visited the antenatal clinic. The instrument for data collection was a self-constructed questionnaire sectioned into two: socio-demographic information and reaction to nitrate. Laboratory testing was done with a dipstick containing a reagent that reacts with nitrites to produce a pink color, thus suggesting the presence of bacteria. Nitrite Combur10-Test M-strip was employed and the manufacturer' guidelines regarding test and interpretation was strictly adhered to. To minimize the risk of disease, study participants were required to appropriately wash their hands with cleanser and water, they were also required to wash their private parts with a swab soaked with ordinary saline. Participants were given guidelines on the best way to gather clean-catch midstream urine, and these were gathered in a clean, wide-mouthed plastic-covered container as utilized by Jido (2014). Data entry and analysis was completed utilizing the Statistical Package for Social Sciences (SPSS) Programming Version 22. Descriptive measurements such as percentages and frequencies mean and standard deviation was used to analysis the demographic data, while the t-test was utilized to compare the prevalence of nitrate across the three trimesters. Confidence interval was set at 95% (p values of ≤ 0.05 was considered statistically significant).

RESULTS

Table 1: Distribution of Trimester of Participants (n=406)

Trimester	Frequency	Percentage (%)
First	144	35.47
Second	135	33.25
Third	127	31.28

Most participants were in their first trimester, 35.47% (144), followed by those in their second trimester, 33.25% (135) and third trimester, 31.28% (127) as shown in Table 1.

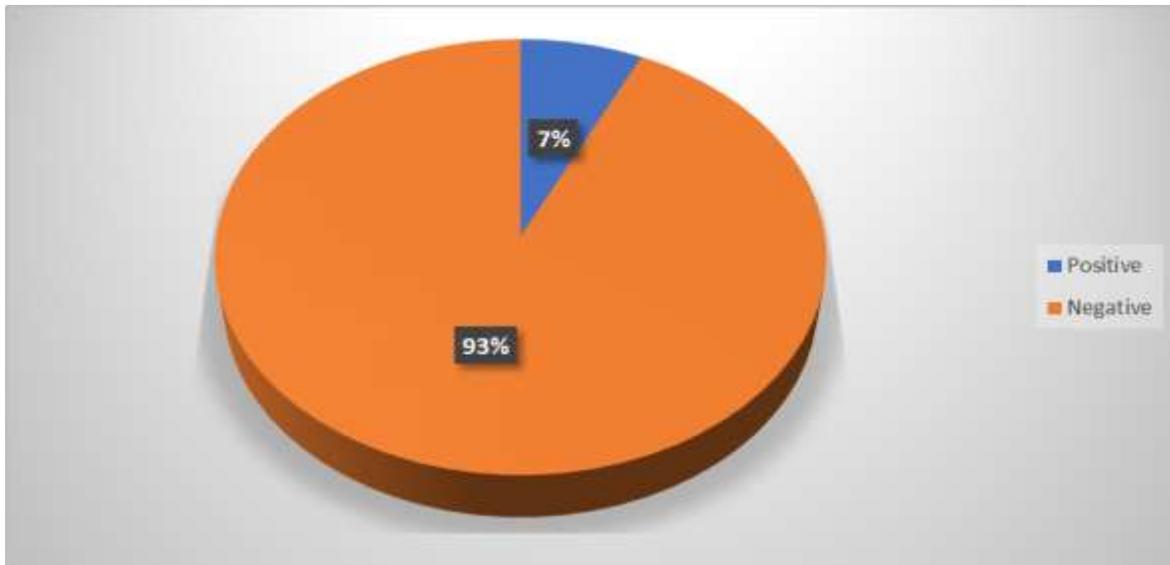


Figure 1: Prevalence of nitrate fermenters

The prevalence of nitrate fermenters in the study population is 7%.
Non nitrate fermenters =93%

Table 2: Clinical parameter of Participants (n=406)

Characteristics	Frequency	Percentage (%)
Nitrate Fermenters		
Positive	29	7.14
Negative	377	92.86
Bacteria Identified (n=29)		
Heavy growth <i>E. coli</i>	16	55.17
Moderate growth <i>E. coli</i>	8	27.59
Scanty growth <i>E. coli</i>	5	17.24
Glucose		
Positive	1	0.25
Negative	405	99.75
Protein		
Positive	15	3.69
Negative	391	96.31

Those positive for nitrate fermenters were 7.14% (29), glucose, 0.25% (1) and protein, 3.69% (15). Bacteria identified were proportionally higher in the Heavy *E. coli*, 55.17% (16), followed by Moderate *E. coli*, 27.59% (8), and Scanty *E. coli*, 17.24% (5) as shown in Table 2.

Table 3 Prevalence of nitrite fermenters in the urine of pregnant women across the various trimester of pregnancy in Obio cottage hospital.

Trimester	Nitrite fermenters in the urine		Total	Fishers exact p
	Positive n=29 Freq (%)	Negative n=377 Freq (%)		
First	13 (9.03)	131 (90.97)	144 (100.0)	0.094
Second	12 (8.89)	123 (91.11)	135 (100.0)	
Third	4 (3.15)	123 (98.85)	127 (100.0)	

Prevalence of Nitrate fermenters in the urine for the first trimester was 9.03% (13), and for the second trimester, 8.89% (12) and for the third trimester, 3.15% (4).

The link between the pervasiveness of nitrate fermenters in the pee and the various trimester of pregnant women did not show a statistically observable difference (p=0.108) as shown in Table 3

Table 4: Prevalence of nitrate fermenting bacteria in the urine of pregnant women across the various trimester of pregnancy in Obio cottage hospital.

Trimester	Nitrate fermenter bacteria in the urine			Total	Fishers exact p
	Scanty growth <i>E. coli</i> n=5	Moderate growth <i>E. coli</i> n=8	Heavy growth <i>E. coli</i> n=16		
	Freq (%)	Freq (%)	Freq (%)		
First	2 (15.38)	5 (38.46)	6 (46.15)	13 (100.0)	0.186
Second	2 (16.67)	2 (16.67)	8 (66.67)	12 (100.0)	
Third	1 (25.0)	1 (25.0)	2 (50.0)	4 (100.0)	

*Statistically significant (p<0.05), χ^2 =Chi-Square

Scanty *E. coli* for the first trimester was 15.38% (2), and for the second trimester, 38.46% (5) and for the third trimester, 46.15% (6).

Moderate *E. coli* for the first trimester was 16.67% (2), and for the second trimester, 16.67% (2) and for the third trimester, 66.67% (8).

Heavy *E. coli* for the first trimester was 46.15% (6), and for the second trimester, 66.67% (8) and for the third trimester, 50.0% (2).

The link between the pervasiveness of nitrite fermenter bacteria in the pee and the various trimester of pregnant women did not show a statistically observable difference (p=0.186) as shown in Table 4

DISCUSSION OF FINDINGS

Presence of nitrite fermenters in the urine of first trimester pregnant ladies

On account of the morphological and physiological changes that happen in the genitourinary tract during pregnancy, urinary tract infection (UTI) is a typical inconvenience in pregnancy (Thakre *et al.*, 2012). It may be either indicative or asymptomatic. Asymptomatic bacteriuria is a typical bacterial disease that effects people for the duration of their lives, especially ladies, of whom roughly half endure indications of urinary tract contaminations identified with bacteriuria sooner or later in their lives. Pregnant ladies are more helpless than men since to their life structures, which incorporates a little urethra and simple tainting of the urinary parcel with fecal microorganisms. According to the current examination, 7% of the 406

examination subjects had nitrite fermenter developments. As an outcome, the pervasiveness of nitrite fermenters in the investigation test was 7%. This is harmonious with the discoveries of Thakre *et al.* (2012), who found a 3.1 - 19.8% pervasiveness of nitrite fermenters in pregnant ladies' pee in India. In a comparative concentrate among Eritrean ladies, tracked down a 9.5% pervasiveness of nitrite fermenters, which is more prominent than the 7% found in the current examination. There was a 8% occurrence of nitrite fermenters in the pee of pregnant ladies in Lagos state in a comparative Nigerian examination. The variable predominance of nitrite fermenters has been identified with an assortment of attributes what's more, determinants, including the ladies' tested equality, race, and financial position. 9% of the 144 ladies in their first trimester showed nitrite fermenter development in their pee tests. This is in accordance with the discoveries of Nwachukwu *et al.* (2018), who tracked down an 8.9 percent pervasiveness of nitrite fermenters among pregnant ladies in their first trimester, he also discovered 9.1% nitrite fermenters in the pee of pregnant ladies in their first trimester in Ile-Ife, southern Nigeria. The power of these nitrite fermenters among these pregnant ladies in their first trimester has been connected to a higher danger of contamination in pregnant ladies, especially those in their first trimester (Simon-Oke *et al.*, 2019). Infection of the urinary system is a typical medical problem among pregnant ladies. This typically starts inside the first six weeks of conception and peaks between the 22nd and 24th days stretch of pregnancy because of an assortment of components including urethral dilatation, expanded bladder volume, and diminished bladder tone, the entirety of which add to expanded urinary balance and ureter vesical reflux. In this condition, around 70% of pregnant ladies endure glycosuria, which advances bacterial improvement in the pee. Dysuria, polyuria, suprapubic torment, and, in certain conditions, hematuria are normal clinical signs (Tula and Iyoha, 2014). Although dysuria and polyuria may show UTIs, these indications may likewise be available in pregnant ladies experiencing different ailments, such as bacterial vaginosis (Simon-Oke *et al.*, 2019). *Escherichia coli* was the most widely recognized uropathogen segregated from pee tests, with shifted levels of expansion found in the sample going from inadequate to heavy. This finding is reliable with the discoveries of different examinations (Oladeinde *et al.*, 2015; Nwachukwu *et al.*, 2018; Simon-Oke *et al.*, 2019). It is accounted for that *Escherichia coli* causes most asymptomatic bacterinuria. Other Enterobacteriaceae incorporate *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter spp.*, *Providencia stuartii*, and *Morganella morganii* (Oladeinde *et al.*, 2015; Nwachukwu *et al.*, 2018; Simon-Oke *et al.*, 2019).

Presence of nitrate fermenting bacteria in the urine of second trimester pregnant women.

The current investigation showed the prevalence of nitrate fermenters among women in second trimester of pregnancy was 8.9%. This is predictable with the reach in the report of Thakre *et al.* (2012), which noticed an 8.1 - 9.8% pervasiveness of nitrate fermenters in pee of pregnant women in their second trimester in India and also announced an 8.5% predominance of nitrate fermenters among pregnant women in their subsequent trimester. The variation of prevalence of nitrate fermenters among women in their subsequent trimester may be attributed to factors such as personal hygiene, awareness of optimal self-care during pregnancy, and exposure to previous uropathogenic infections (Ochada *et al.*, 2015; Nwachukwu *et al.*, 2018). The moist environment of the female's perineum favours microbial growth and predisposes females to bladder contamination (Nileka and Sagar, 2015). Other factors including inappropriate cleaning of the perineum, the utilization of napkins and sterile towel together with pregnancy and sex add to the higher occurrence of UTI in different women (Akobi *et al.*, 2014; Ochada *et al.*, 2015; Elzayat *et al.*, 2017). According to the current examination, most of the women 66.7% had a heavy growth of *E. coli*, demonstrating a high susceptible to the infection among the women in their second trimesters. Pregnancy has been reported to be a state of altered immune response thereby increasing susceptibility to infections (Perersen *et al.*, 2017). Placental immune responses and its tropism for specific viruses and pathogens affect the outcome of the pregnant woman's susceptibility to and severity of certain infectious disease (Elzayat *et al.*, 2017). In order for an embryo to effectively implant. Immune cells flood into the lining of the womb and cause inflammation. The heightened state of the immune system lasts for the first 12 weeks of pregnancy to allow the foetus to establish itself fully. The mother's immune system is suppressed for the next 15 weeks to allow the foetal cells to grow and develop. There is an expanded

danger of UTI in women during this time, especially when cleanliness levels are problematic (Sekikubo *et al.*, 2017; Ghouri *et al.*, 2018).

Presence of nitrate fermenting bacteria in the pee of third trimester pregnant ladies

The discoveries of the current investigation showed that solitary 3.15% of the ladies in their third trimester had a development of nitrate fermenters in their pee. This is reliable with discoveries of comparable investigations by different creators announced a 2.1% - 4.2% pervasiveness of nitrate maturing microbes among ladies in their third trimester (Gilbert *et al.*, 2013; Elzayat *et al.*, 2017). About half of the ladies in their third trimester were seen to have a substantial development of *E. coli* upon assessment. The general pervasiveness of UTI in third trimester is seen to be moderately lower than 5%, particularly among ladies going to pre-birth care (Thakre *et al.*, 2012; Ochada *et al.*, 2015). The treatment and consideration got during pre-birth care is related with a diminished pace of UTI contamination among ladies in their third and last trimester before conveyance. Because numerous anti-toxins may cause birth issues (anencephaly, heart deformities, and congenital fissure) when utilized during the first trimester, they are just utilized as a first-line treatment for UTIs that emerge during the second and third trimesters (Ghouri *et al.*, 2018). Consequently, there is a low prevalence of UTI or nitrite fermenters among the women when treated properly. Similarly, an aggressive immune system returns near delivery where inflammation helps with the labour response (Tula and Iyoha, 2014; Oladeinde *et al.*, 2015).

Prevalence of nitrate fermenting bacteria among different trimester pregnant ladies

The predominance of nitrite fermenters was seen to be moderately higher among ladies in their first trimester (9.03%), firmly followed by ladies in their second trimester (8.89%) and ladies in their third trimester (3.15%). Measurable investigations showed the circulation of the nitrite fermenters in the various trimesters was not statistically significant ($p = 0.094$). This is predictable with the discoveries of other examination, which tracked down no huge varieties in the dissemination of UTI contamination among pregnant ladies in their first, second, or third trimesters (Gilbert *et al.*, 2013; Elzayat *et al.*, 2017). However, the current examination's discoveries repudiate past reports that tracked down a lot higher occurrence of nitrate aging microscopic organisms in ladies in their first trimester contrasted with those in their second and third trimesters (Oladeinde *et al.*, 2015; Nwachukwu *et al.*, 2018). According to Nwachukwu *et al.* (2018), the observed discrepancies could be attributable to similar hygiene habits among women or health-seeking behaviours that vary across the country. Scanty growth of nitrite fermenters was observed mostly among women in their third trimester (25.0%) compared to women in their second trimester (16.67%) and women in their first trimester (15.38%). The general prevalence of UTI in third trimester is observed to be relatively lower than 5%, especially among women attending prenatal care (Thakre *et al.*, 2012; Ochada *et al.*, 2015). The treatment and care received during prenatal care corresponds to a relatively lower UTI infection rate among women in their third and final trimester before delivery. Since specific anti-infection agents represent a possible danger for birth complications (anencephaly, heart defects, and congenital fissure) when taken during the first trimester, they are just viewed as a first-line treatment for UTIs happening during the second and third trimesters (Stanley and Kayode, 2014; Ghouri *et al.*, 2018). Notwithstanding, Moderate development of nitrate fermenters was noticed generally among ladies in their first trimester (38.46%) contrasted with ladies in their third trimester (25.0%) and ladies in their subsequent trimester had the most un-moderate *E. coli* growth (16.67%). The concentrate likewise tracked down that heavy *E. coli* development was generally normal in women in their subsequent trimester (66.67%), trailed by ladies in their third trimester (50.0%), and ladies in their first trimester of pregnancy (46.15%). Hormones are one of the causes. They cause changes in the urinary tract during pregnancy, making women more helpless to contaminations. Hormone changes can additionally cause vesicoureteral reflux, an illness where pee streams back up from the bladder to the kidneys.

CONCLUSION

Urinary tract infections brought about by deferred finding in pregnant women address a serious danger of pregnancy issues. The pervasiveness of UTIs and the bacteriological causal specialists among pregnant

ladies joining in pre-birth facilities at a Tertiary Care Hospital in Ile Ife, South-western Nigeria, is announced in this examination. The study found a 7% occurrence of nitrate fermenters in the pee of pregnant women in Port Harcourt. The frequency of nitrite fermenters in ladies was found to diminish from the first to third trimester (9.0 - 3.0%). An insufficient development of the nitrite fermenters was for the most part normal among women in their third trimester in contrast with second trimester and first trimester. The discoveries of the examination demonstrate that the principal trimester of pregnancy is a high-hazard window for the colonization of nitrite maturing uropathogens.

RECOMMENDATIONS

Based on the findings, the following recommendations were made:

1. Urine cultures are recommended early in pregnancy to detect ASB
2. Public campaigns ought to be encouraged to empower vagina-friendly cleanliness for pregnant women particularly women in their first trimester of pregnancy
3. Screening of asymptomatic bacteriuria is suggested in all pregnant women.

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