Prevalence of Urinary Tract Infections Among Pregnant Women Attending Antenatal Clinic In Some Selected Hospitals In Anambra State

'Okonkwo Nonye G., [2]Umeaku C. N.
[12]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli. 08036761216
chimeaku@yahoo, com ,

Abstract: Urinary Tract Infection is one of the most frequently seen medical complications in pregnancy. This study reports the prevalence of urinary tract infections among pregnant women attending antenatal clinics in some selected hospitals in Anambra State, Southeastern Nigeria. The aim of this work was to determine the prevalence of urinary tract infection among the pregnant women, the trimester peak of infection and the antibiotic sensitivity of the isolated organisms. Microbiological analysis was performed on the urine samples obtained from 500 pregnant women aged 21 - 40 years. Standard questionnaires was used to obtain socio-demographic data from the pregnant women and data obtained was analysed statistically to determine the relationship between the variables and their significance. Cystine lactose electrolyte deficient agar, MacConkey and blood Agar were used to isolate the organisms where every specimen that yielded pure heavy growth of bacterial pathogens of $>10^5$ cfu/mL of urine was considered significant. These bacterial isolates were identified based on cultural morphology, microscopic, biochemical and molecular characterization. The results obtained showed that 101 (20.2%) pregnant women were infected and age bracket 26-30 years had the highest incidence of UTIs with infection rate of 31.7%. Pregnant women in their second trimesters were most infected with infection rate of 49.5%. *Escherichia coli* was the commonest isolated organism 71(70.3%), followed by *Stapyloccocus aureus* 26(25.7%) and *Enterococcus feacalis* 4(3.96%), with 30%, 65% and 100% bacterial sensitivity to gentamycin respectively. Gentamycin therefore was the most effective antimicrobial drug against the bacterial isohtes. All the isolates assessed for *Escherichia coli, Enterococcus feacalis,* and *Staphylococcus aureus* genes wer£ positive with the amplification of 16sRNA gene of these organisms.

Keywords:

Introduction

Urinary tract infection and its associated complications are the cause of nearly 150 million deaths per year worldwide. The disease can be developed in 40% - 50% of women and 5% of men (Totsika *et al*., 2012). After anemia, UTIs are the second common complications in pregnant women, which if not properly controlled, can adversely affect the health of infant or the pregnant mother (Franklin *et al.*, 2000; Mittal, *et al.,* 2005). Various microorganisms are able to invade the urinary tract and can be involved in the pathogenesis of urinary tract infection (UTI) (Asadi *et al*., 2012; Rajaratnam *et al*., 2014; Gomi *et al*., 2015). As one of the most common recurrent acquired infections, urinary tract infection (UTI) has a conspicuous role in increasing the number of stillbirth deliveries (Masinde *et al.,* 2009; Litza and Brill, 2010; Al-Hadad, 2005).

Urinary tract infection (UTI) therefore is broadly the inflammatory response of urothelium to bacterial invasion, which is usually associated with bacteriuria and pyuria. Bacteriuria is the presence of bacteria in urine (Rajaratnam *et al*., 2014). UTI can also be said to be the invasion and multiplication of microorganisms that affect any part of the urinary tract (Awaness *et al.,* 2000). Urinary tract infection is a common clinical problem, which can involve the urethra, bladder, ureters, and kidney (Al-Dujiaily, 2000), affecting all age groups, but women are more susceptible than men. This is due to the short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Awaness *et al.,* 2000). Additionally, the physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosuria, which encourages bacterial growth in the urine. In simple terms, it can be referred to as a condition which women may encounter during the span of their life time and the prevalence is higher among women during pregnancy (Salvatore *et al.,* 2011). As the name indicates, the infected parts involve the urinary tract comprising of the upper and lower urinary tracts. The symptoms associated with the bladder and kidney infections are contrasting, which includes painful and frequent urination in case of cystitis as a result of bladder infection. Whereas conditions like high fever and flank pain are commonly experienced in case of kidney contagion which is referred to as phylonephritis (Ajide *et al*., 2016). The prevalence of the infection among children and elderly people is not clearly understood and is currently under study (Wood food and George, 2011). Bacteria are the prime perpetrator responsible for conferring the infection among humans but the role of certain fungi and viruses cannot be over looked. However, the incidence of UTI

*Corresponding author:
gracenonye.gn@gmail.com** * [1]**Okonkwo Nonye G.,

as a result of viral or fungal infection is considered to be rare (Amdekar *et al.*, 2011). Though the infection seems to be harmless in the initial stages, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances (Lane and Takhar, 2011). Research studies have defined urinary tract infection as the most common form of bacterial infection (Parveen *et al.*, 2011; Demilie *et al.*, 2012) Urinary tract infection can be a consequence of poor diagnosis and is regarded as the most common hospital acquired infection (Koffuor *et al.*, 2012; Kolawole *et al.*, 2009).

Urinary tract infection is an independent risk factor for renal carcinoma and bladder cancer, it is also a risk factor for premature delivery, foetal loss and kidney infections (Linhares *et al.*, 2013). Uropathogenic *Escherichia coli* isolates possess multiple virulence factors that promote colonization of bacteria and infection in the urinary tract such as fimbrae, adhesion, toxins, siderophores and capsular polysaccharide. Clinical experiences have shown a high rate of antibiotic resistance among uropathogens (Schaeffer, 2002).

Aim:

This study aims to determine the prevalence of urinary tract infection among pregnant women, the trimester of peak of infection and the antibiotic sensitivity pattern of the isolates.

Objectives:

Issuance of well-structured questionnaires.
Macrobiological and microbiological analysis.
Biochemical characterization of the isolates.
Molecular biologic analysis of the isolates.
Antibiotic susceptibility testing.

Materials and Methods

Study Area.

This study was conducted in Onitsha, Anambra State, and South-Eastern Nigeria. It is a metropolitan city, known for its river port. It is also an economic hub for commerce, industry, and education. Onitsha lies at a major east-west crossing point of the Niger River, on a latitude of 6° 10′ 0″ N and longitude of 6° 47′ 0″ E above sea level.

Study Population

A cross-sectional study of 500 pregnant women attending antenatal clinics from Our Lady of Lourdes Hospital, Holy Rosary and Maternity Hospital, Kanayo Specialist Hospital, Chinedu Hospital and Chisom Hospital all in Anambra State Nigeria were randomly selected for the study between the months of June to September, 2015. Women with history of urinary tract infection, diabetis mellitus, hypertension, renal diseases or sickle cell disease. Also women with previously treated urinary tract infections, those who are HIV positive, women who are currently on antibiotics or had taken antibiotics during the course of the index pregnancy were excluded in the study, socio-demographic data such as age, sex, occupation, level of education, religion, parity and gestational periods of pregnancy were collected from the pregnant women using standard questionnaires and kept confidential during the research. Only the willing and available pregnant women who at the time of the study satisfied the inclusion and exclusion criteria were recruited into the study.

Ethical Approval

Ethical approval letters were obtained from the administrators and chief medical directors of the hospitals and also individual consent obtained from each of the pregnant women included in the study who were assured of anonymity and confidentiality.

Sample Collection

Five Hundred (500) voided mid-stream urine samples were collected in sterile capped universal containers from non-catheterized pregnant women. The samples were labeled and transported in iced packed container to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University, Uli and were processed within 1 – 3hrs of sample collection.

Urine Culture

A loopful (0.01ml) of urine sample was taken from each sample using a standard loop and inoculated on Cysteine Lactose Electrolyte Deficient Agar (CLED), MacConkey agar and Blood agar, incubated aerobically at 37^0C 24 hours (Cheesbrough, 2002). Specimens that yielded heavy growth of bacterial pathogens of $>10^5$ colony forming unit per mL of urine were considered significant while specimens that produced $< 10^5$ colonies of urine were considered insignificant or due to contamination.

Microscopy

A portion of each urine sample was poured into a test tube and spun at 3000rpm for 10-15minutes using a centrifuge. The clear portion of the urine samples were discarded and the deposits remixed by tapping the bottom of the test tube. A drop of the deposit was transferred into well cleaned dry glass slide and covered with a cover slip. The slide was then examined under a microscope using both low and high pressure objective lens (10x and 40x), for pus cells, red blood cells, epithelial cells, casts, crystals, yeast-like cells. Pus cells >5/HPF were also considered significant for infection.

Identification of bacterial Isolates

The different bacterial colonies were identified on the basis of their colony morphology (colour, growth, size and pattern), Gram stain reaction was assayed for. The biochemical test which included: Indole, Catalase, Oxidaze, Citrate, Urease, Methyl red/

Voges Proskauer, Triple Sugar Iron Agar test and Coagulase test. (Cheesbrough, 2004). Colonies were counted and multiplied by loop volume. A bacterial count of 1 x 10^5 per ml was considered significant while a count less than 1 x 10^2 per ml was considered insignificant. Any urine specimen containing high colony counts with more than one species of bacteria was considered as being contaminated and hence, discarded.

Molecular biologic analysis

Polymerase chain reaction technique was used. DNA extraction was done using boiling method. Overnight broth cultures of the organisms were used for the DNA extraction. A portion of 1.5ml of the broth cultures were transferred to 2ml eppendorf tube. Centrifugation was done at 12,000rpm for 1 minute, after which the supernatant was decanted and vortexed for another 1 minute. The vortexed residues were heated using the heating block at 95°C for 10minutes. This was followed by final centrifugation at 12,000rpm for 1 minute. The supernatant (DNA) was collected and stored at –20°C until further use for PCR.

Statistical Analysis

All data where possible were analyzed using SPSS version 16.0. Analysis of variance (ANOVA) was used and the result presented as mean ± standard deviation. Results on pattern of microbial isolates were represented as percentage fraction of the total number of subjects.

Results

A total of 500 urine samples were collected from pregnant women attending the five hospitals involved in the study and after microscopic analysis, followed by urine culture, the observations made include. A total of Three (3) bacterial species were isolated and identified, two (2) were gram positive and one (1) gram negative.

Table 1: Frequency of isolation of bacterial species in preghant women

Isolates	No. of Isolates
Escherichia coli	71 (70.3%)
Staphylococcus aureus	26 (25.7%)
Enterococcus feacalis	4 (3.96%)

Table 2 shows that out of the 500 samples, 101 (20.2%) showed significant bacterial growth count greater than $1x10^5$cfu/ml determined by morphology and colony count, while 399 (79.8%) showed bacterial growth less than $1x10^2$cfu/ml. It was observed also that pregnant women within the ages of 26-30 years recorded high UTI prevalence whereas those above 36 years recorded low prevalence.

Table 2: Prevalence of UTI in relation to age distribution of pregnant women

Age group (years)	Number tested (%)	Number positive (%)	Number negative (%)
21-25	168 (33.6)	30(29.7)	138 (34.6)
26-30	182 (36.4)	32(31.7)	150(37.6)
31-35	120 (24.0)	22 (21.8)	98 (24.6)
36-40	30 (6.0)	17 (16.8)	13 (3.3)
Total	500	101	399

The prevalence of UTI in relation to their gestational age was relatively high in second trimester (49.5%) followed by third trimester (31.7%) and least was first trimester (18.8%).

Table 3: Prevalence of UTIs among the pregnant women in relation to gestational Age

Trimester	Isolates			
	E.coli	*S.aureus*	*E.feacalis*	TOTAL
1	14	5	0	19
2	36	13	1	50
3	21	8	3	32
TOTAL	71	26	4	101

Table 4: shows the prevalence of UTI's in relation to their educational status. The result showed that those women with primary education had the highest rate of infection (70.3%), secondary education (19.8%) and tertiary education (9.9%).

Table 4: Prevalence of UTIs among the pregnant women in relation to educational status

Educational status	Number tested	Number positive (%)
Tertiary	140	10 (9.9)
Secondary	150	20(19.8)
Primary	210	71(70.3)
Total	500	101

The prevalence rate of UTI's among the pregnant women in relation to their occupational status is shown on table 5. the highest infection rate was obtained among pregnant women who were traders (54. 5%).this was followed by housewives with infection rate of (30.7%) while the least prevalence occurred among civil servants with infection rate of (14.9%) (table 5).

Table 5: Prevalence of UTIs among the pregnant women in relation to occupational status

Occupational status	Number tested	Number positive (%)
Employed	122	15 (14.9)
Trader	290	55 (54.5)
Housewives	88	31 (30.7)
Total	500	101

Some bacterial isolates were susceptible to commercially available antibiotics as presented in Table 6.

Table 6: Susceptibility profile of bacteria to antimicrobial drugs

Isolates	APX	RD	AMX	CN	LEV	CPX	NB	E	S	CH	Total
Escherichia coli	0	10	10	21	0	14	0	16	0	0	71
Staphylococcus aureus	0	0	4	17	0	2	0	2	1	0	26
Enterococcus feacalis	0	0	0	4	0	0	0	0	0	0	4
Total	0	10	14	42	0	16	0	18	1	0	101

Key:
APX – Ampiclox, RD – Rifampicin, AMX – Amoxicillin, CN – Gentamycin, LEV – Levofloxacin, CPX – Ciprofloxacin, NB – Norfloxacin, CH – Chloramphenicol, E – Erythromycin, S- Streptomycin.

Discussion

In this study, the prevalence of urinary tract infection was (101)20.2% and the predominant pathogens were *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus feacalis* with prevalence rate of 70.3%, 24.8% and 4.0% respectively. This finding is in agreement with Omonigho *et al*., (2001), Olowu and Oyetunji (2003) and Akujobi *et al*., (2009).

This rather high prevalence rate reported in this study could be attributed to poor healthcare and public awareness program given to the pregnant women and poor personal and environmental hygiene they observe. The variation in prevalence of UTI from one geographical location to another could be attributed to differences in mode of screening, age, parity pregnancy (Obirikorang *et al*., 2012 and Dezell *et al*., 2000), according to my results of host behavioral factors. urinary tract infection was comparatively high among the pregnant women in the second and third trimesters; (49.5%) and (31.7%) as this was similar to the findings of Alex *et al*., (2012) and Akobi *et al*., (2014). This could be attributed to the anatomical and physiological changes experienced by pregnant women during these stages of pregnancy, for instance the uterus expands and also there are increased hormonal effects which together offset normal homeostatic balance making conditions favourable for microbial invasion. Though the incidence of urinary tract infection in the first trimester was low it could only be that it served as incubation period for most of the microbes, only for microbial invasion to be manifest in the second and third trimesters. This observation was in accordance to the results of Vazquez and Villar (2000), who had reported that 10–30 % of women with bacteriuria in the first trimester develop upper urinary tract infection in the second and third trimester; this possibly could mean that the incidence of urinary tract infection is trimester - related reflecting perhaps the observation that incidence increases with aging pregnancies.

The prevalence of urinary tract infections in relation to their educational status showed that those women with primary education had the highest rate of infection (33.8%), followed by secondary education (13.3%) and the least was tertiary education (7.1%), this could be attributed to the fact that the well educated women have stronger knowledge of personal hygiene. The prevalence rate of urinary tract infection among the pregnant women in relation to their occupational status showed the highest infection rate was obtained among pregnant women who were housewives(35.2%), this was followed by traders with infection rate of (19.0%) while the least prevalence occurred among those employed with infection rate of (12.2%).There is no significant difference between educational and occupational statues of the pregnant women. The prevalence of urinary

tract infection in this study is in conformity with the findings of Ezigbo *et al.* (2016) while in contrast with the findings of Masinde *et al.* (2009); Hamdan *et al.* (2011), which reports this as significant risk factors of urinary tract infection.

The prevalence of urinary tract infection among pregnant women in relation to parity has it that the highest infection rate was obtained among pregnant women with 0-1 parity, this was followed by those with >4 parity and the least was those with 2-3 parity. Multi parity has an increased risk factor of developing urinary tract infection among pregnant women. This has been previously reported by Hamadan *et al.* (2011); Emiru *et al.* (2013); Oladiende *et al.* (2015).

The most common bacteria isolated from the urinary tract infections among these pregnant women in this study was *Escherichia coli* (70.3%), *Staphylococcus aureus* (25.7%) and *Enterococcus feacalis* (3.96%) agreeing with the report by (Emiru *et al.*, 2013), stating that the commonest isolates were *Escherichia coli* (80%). This finding is also similar to other reports that gram negative bacteria, *Escherichia coli* is the most common pathogen isolated in patients with urinary tract infections (Ebie *et al.*, 2001; Onifade *et al.*, 2005; Aiyegoro *et al.*, 2007 and Delzell, 2008). This could be as a result of poor genital hygienic practices by the pregnant women who may find it difficult to clean their anus properly after defecating or clean their genital after passing urine. The prevalence of *Staphylococcus aureus* (25.7%) was also high in this study, this agrees with Amadi *et al.* (2007). This could be that it's gaining clinical prominence in the etiology of urinary tract infection during pregnancy. However, this finding contradicts that of Omonigho *et al.* (2001) who found *Klebsiella spp.* to be more prevalent than *Escherichia coli* in urinary tract infection.

Both gram negative and gram positive bacteria showed greater susceptibility to Gentamycin. This compares with the observations of Alex *et al.* (2012) and Kenechukwu *et al.* (2005). While Moderate activity against bacterial isolates was observed with Ciprofloxacin, Amoxicillin, Rifampicin, and Erythromycin, while there is very poor sensitivity of the isolated bacterial pathogens to Streptomycin, Chloramphenicol, Norfloxacin, Levofloxacin and Ampiclox. Prescription of antibiotics without laboratory guidance as well as over-the-counter sales of antibiotics without prescription is also a probable factor for increased bacterial resistance to antimicrobial agents.

Finally, all the isolates accessed for *Escherichia coli, Enterococcus feacalis,* and *Staphylococcus aureus* genes were positive with the amplification of 16sRNA gene of these organisms. This finding is also similar to the reports of Kenechukwu *et al.* (2005).

Conclusion

Urinary tract infection prevalence rate (20.2%) reported in this study reveals the clinical and epidemiological significance that urinary tract infection has attained in the study area. Therefore, this calls for education and creation of awareness on the importance of personal hygiene during pregnancy. Since urinary tract infection may be symptomatic and asymptomatic in most cases, it is therefore necessary that routine screening and proper laboratory diagnosis of urine culture for pregnant women be included in their routine antenatal visits.

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