E3 Journal of Medical Research Vol. 1(6). pp. 074-083, July, 2012
Available online @ http://www.e3journals.org
ISSN 2276-9900

Full length research paper

Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana

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Accepted June 5, 2012

Urinary tract infections culminating from poor diagnosis during pregnancy puts pregnant women at high risk of serious complications. This study investigated the incidence of urinary tract infections among pregnant women attending antenatal clinics in the Cape Coast Metropolis of the Central Region of Ghana. Physical, chemical, microscopic, and microbial analysis were performed on urine samples obtained from 200 pregnant women aged 15 - 45 years attending the University of Cape Coast Hospital, Cape Coast Metropolitan Hospital and Ewim Urban Health Centre. The prevalence of urinary tract infections in the three trimesters was determined together with sensitivity testing of the bacteria isolates to antimicrobial drugs. Overall prevalence stood at 56.5 %, although comparatively high in pregnant women in the second trimester (50.4 %). *Escherichia coli* were the most implicated organism (48.7 %). Pregnant women aged between 15 – 32 years were the most affected and gentamycin was the most effective antimicrobial against the bacteria isolates. Results indicated that the incidence of urinary tract infections was high among pregnant women in the study area; therefore, urine microbial screening should be included in the routine antenatal checkups for pregnant women to detect the asymptomatic infections to reduce its risk to pregnancies.

Key Words: Cape Coast; *Escherichia coli*; Microbial load; Gentamycin

INTRODUCTION

Urinary Tract Infections (UTIs) describe the microbial invasion and subsequent multiplication on a part or the entire urinary tract. Although urine is fluid with a variety of molecules and salts some of which are waste products, it does not usually have bacteria as a normal component, therefore when a bacterium by any means get into the bladder and subsequently multiply, it may cause UTI (Patterson and Andriole, 1997).UTIs are among the most common bacterial infections in humans, both at the community and Hospital settings and have been reported in all age groups in both sexes (Hooton, et al., 1995).

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UTI has become the most common hospital-acquired infection, accounting for as many as 35 % of nosocomial infections, and is the second most common cause of bacteremia in hospitalized patients (Weinstein et al., 1997; Kolawole et al., 2009). UTI which usually presents as asymptomatic bacteriuria could be described based on the part of the urinary tract it affects. The upper urinary tract infection (pyelonephritis) is the most common urinary tract complication in women, occurring in approximately 12 % of all pregnancies (Delzell et al., 2000); lower urinary tract infection (cystitis) which is infections of the bladder is less common (Stamm, 1998).

The most common bacteria implicated in the pathogenesis of UTI and its complications include *Escherichia* coli (Stein G, Funfstuck R, 2000; Ebie et al., 2001; Bloomberg et al., 2005; Obiogbolu et al., 2009), Klebsiella spp (Omonigho et al., 2001), and *Staphylococcus aureus* (Ugbogu et al., 2010). In pregnant women, UTI is characterized by fever, flank pain, and tenderness in addition to significant bacteriuria. Other symptoms may include nausea, vomiting, frequent urination, urgency, dysuria, premature birth, and low birth–weight (Ronald, 1987; McGregor et al., 1990; Schultz et al., 1991). Untreated UTI had also been reported to increase the risk of pyelonephritis (Mittendorf et al., 1992; Gratacos et al., 1994), transient renal failure, acute respiratory distress syndrome, sepsis, shock, and hematological abnormalities (Obirikorang et al., 2012).

Pregnancy causes numerous changes in the physiology of a woman's body. Hormonal and physical changes increase urine stasis and the climbing up of urine from the bladder into the ureters. These changes, along with an already short urethra (approximately 3 - 4 cm in females) increase the frequency of UTI in pregnant women (Johnson and Kim, 2012). In general, pregnant women are considered immunocompromised UTI hosts because of the physiological changes associated with pregnancy (Ceisla, 2007). These changes increase the risk of infections that could be either symptomatic or asymptomatic.

In the first quarter of 2010, 36 % of pregnant women who embarked on antenatal visits to the Cape Coast Metropolitan Hospital (CCMH) recorded proteinuria (presence of proteins in urine). In that same quarter of the year, the Central Regional Hospital (CRH) also in the Cape Coast Metropolis recorded a positive bacterial growth in 28 % of the total urine samples cultured, with greater percentage coming from pregnant women (CRH Laboratory Records, 2010, unpublished). These observations raise serious concerns about the prevalence of UTIs among female attending in the hospitals and the Cape Coast Metropolis as a whole. This study sought to investigate the incidence of UTI, the common bacteria species implicated in UTI, whether incidence of UTI is trimester related and the antimicrobial drugs effective against the possible bacteria isolates, to inform the Ghana Health Service on policies to implement in routine antenatal checks.

MATERIALS AND METHODS

Study area

The study was conducted in the Metropolitan District of Cape Coast (Area: 9,826 km²), the Capital of the Central Region of Ghana from January to March, 2012. The Central Region is a hub of education in Ghana (harboring some of the best schools in the country). Some of the most beautiful beaches, national parks and small cities in West Africa make Cape Coast a major center for tourism within Ghana. University of Cape Coast Hospital, Cape Coast Metropolitan Hospital, and Ewim Urban Health Centre are the three major health care facilities in the District that service tourist, workers and students in the various educational institutions, and the fishing and farming communities from the surrounding towns and villages.

Study population

A total of 200 pregnant women from the three Hospitals were randomly selected for the study. The convenience sampling method was used and this permitted only the willing and available pregnant women who at the time of the study satisfied the inclusion and exclusion criteria to be recruited into the study.

Ethical considerations

Ethical clearance was sought for and granted by Ethics Committee of the Hospitals involved. Permission was also obtained from Administrators of the Hospital before the study was commenced. In addition, all the pregnant women recruited into the study agreed to a written informed consent after thorough explanation of the rationale of the study.

Collection of urine samples

After demographic information (e.g. age, age of pregnancy, term of pregnancy, and anti - biotic use for the past week) were obtained from their folders in the antenatal clinic, the pregnant women were then adequately educated on how to take mid-stream urine sample of their first urine (on the day of submission) into sterile capped, dry, wide-necked, leak - proof, and labeled sample tubes which were given to each of them to take home.

Urinalysis

Physical examination

The physical characteristics of the urine samples such as color, and flow were recorded immediately after collection from the pregnant women.

Urine chemistry

The urine samples were subjected to a semi-quantitative test using Dirui H 10 urine test strips (Dirui Industrial Company, China) to characterize the presence and levels of chemical entities such as proteins, nitrite, glucose, and ketones. This was done by dipping a test strip into each urine sample and comparing the observed color changes on the strip to a reference color chart provided on the package of the test strip.

Microscopy

A portion of each urine sample was poured into test tubes and span at 3000 rpm at 4^{o}C for three minutes using a Nuvu NF 200 centrifuge (Nuve SanayiTaalzemeleriImalat A.S., Turkey). The clear portion of the urine was discarded and the deposits remixed by tapping the bottom of the tube. A drop of the deposit was transferred unto well cleaned and dry glass slide and covered with a cover slip. The slide was then examined under a Olympus CH binocular microscope (Olympus Optical Company Limited, Japan) using both low and high power objective lens (10X and 40X) with the condenser iris closed sufficiently to give good contrast for identification of pus cells, Schistosoma ova, crystals, and other unusual components.

Urine culture

A loopful (0.01 ml) of urine sample was taken from each case using a standard loop calibrator and inoculated on a cysteine lactose electrolyte - deficient agar (CLED) and finally incubated aerobically at 37^{o}C overnight in an IPF 400 Precision incubator (Memmert, Germany).

Identification and counting of bacteria isolates

The different bacteria colonies were identified on the basis of their colony morphology (color, growth size and growth pattern). Further identification was done using standard biochemical tests including Citrate, Urease, Indole, Catalase and Coagulase tests as described by Cheesbrough (2006). Bacterial counts were determined by the product of colony count (colony on CLED) and loop volume. Significant UTI (significant bacteria growth) was determined by a bacterial count greater than 1 x 105/ml, while a bacterial count less than 1 x 102/ml was regarded insignificant UTI (insignificant growth).

Antimicrobial susceptibility test (AST)

The susceptibility of the isolates to selected antimicrobial agents was determined by the disc diffusion method and the Kirby Bauer method (Bauer et al., 1966) using antibiotic-impregnated paper discs (Medical wire and Equipment Co. Ltd., PotleyCorsham, England) containing the following antibiotics: amikacin (AMK, 30 µg), gentamicin (GEN, 10 µg), tetracycline (TET, 30 µg), erythromycin (ERY, 15 µg), chloramphenicol (CHL, 10 µg), cotrimoxazole (COT, 25 µg), cefuroxamine (CXM, 30 µg), cloxacillin (CXC, 5 µg), ampicillin (AMP, 10 µg), penicillin (PEN, 10 µg) and cefotaxime (CTX, 30 µg). Pure colonies of fresh isolates were emulsified in peptone water using a straight sterile wire, and the turbidity was adjusted to the equivalent of 0.5 MacFarland standards. A sterile cotton swab was then used to obtain a portion of the emulsified suspension to make a three – dimensional streak on a Mueller Hinton agar plate. An appropriate antibiotic disc based on the organism's Gram reaction was then placed on the plated agar lawn within 15 minutes of seeding and then incubated at 37^{0}C overnight (20 – 24 h). The diameter of the zone of inhibition was determined using a ruler and compared with a standard chart to determine susceptibility graded as sensitive or resistant. *Escherichia coli* (NCTC 10418) a Gram–negative organism and *Staphylococcus* aureus (NCTC 6571) a Gram–positive organism, were used as controls.

STATISTICAL ANALYSIS

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. One -way ANOVA followed by Dunnett's post hoc test was used to determine the significance of the differences in UTI incidence among the trimesters, month of pregnancy and age groups. $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

A total of two hundred urine samples were collected from pregnant women attending the three hospitals involved in the study and after physical, chemical and microscopic analysis, followed by urine culture using CLED agar as described by Cheesbrough (2006) the observations made included:

Physical appearance

Normal urine color ranges from pale yellow to deep amber and this is as a result of a the pigment called urochrome, however disease states can cause the color of urine to change from the normal color extremes (pale yellow – deep amber). The urine colors observed in this particular study ranged from pale yellow (66 %) to red (4 %) (Table1).

Urine chemistry

Nitrite was observed markedly in the samples from which Gram-negative bacteria were isolated. Traces of protein

Table 1: Urine colors recorded of the 200 urine sample obtained from the pregnant women

Urine color	Number (%)
pale yellow	132^ (66)
Colorless	21* (10.5)
Milky	5* (2.5)
Amber	37^ (18.5)
Red	4* (2)
Orange	0

^ - Normal urine appearance, * - Deviation from normal urine appearance

Table 2: Microscopic examination of urine samples

Deposits	Percentage (%)
Yeast cells	5* (10)
Trachomonasvaginalis	1.5* (3)
Schistosomahaematobium	0.5* (1)
Calcium oxalate crystals	1.5* (3)
Red blood cells	0.5* (1)
Pus cells	34* (68)

Numbers in parenthesis represents the number of samples that tested positive for each deposit.

were recorded in 51 (25.5 %) samples, whereas 45 (22.5 %) samples tested positive for glucose, from which 70 % contained yeast cells. Thirty six (18 %) samples tested positive for ketones.

Microscopy

Microscopic examination of the 200 samples revealed high percentage of pus cells and low percentage of red blood cells and Schistosoma haematobium ova (Table 2).

Urine culture

Out of the 200 samples, 113 (56.5 %) samples showed significant bacterial growth (bacteria count > 1 x 105/ml), determined by morphology and colony count greater than 105/ml, whiles 87 (43.5%) samples showed no bacterial growth (bacteria count < 1 x 102/ml). It was observed that pregnant women within the ages of 15–32 years recorded high UTI prevalence; whereas those above 33 years recorded lowest prevalence (Table 3).

Table 3: Incidence of UTI in relation to age distribution of pregnant women

Age group (years)	Number tested (%)	Number positive (%)	Number negative (%)
15 – 20	55a (27.5)	28b (24.8)	27c (31.0)
21 – 26	57a(28.5)	34b (30.1)	23c (26.4)
27 – 32	58a (29)	38b (33.6)	20c (23.0)
33 – 38	22a (11)	9b (8.0)	13c (14.9)
39 – 44	8a (4)	4b (3.5)	4c (4.6)
Total	200	113	87

a (represents the number of samples tested in each age group),
b (represents number of samples that showed significant bacterial growth)
c (samples that had no bacterial growth).

Table 4: Frequency of isolation of bacteria in pregnant women

Isolates	Percentage (%)
*Escherichia coli (E. coli)**	48.7 (55)
*Klebsiella pneumoniae.**	23.9 (27)
*Proteus mirabillis.**	7.1 (8)
*Staphylococcus aureus***	16.7 (19)
*Pseudomonas aeruginosa**	1.8 (2)
*Enterococcus faecalis**	1.8 (2)

* (Gram - negative bacteria), ** (Gram – positive bacteria), numbers in parenthesis represents the number of samples that tested positive for each bacterium).

The highest UTI incidence was recorded in the fourth month of pregnancy while significant incidence was also recorded in the third, fifth and sixth months, with the first month having the lowest incidence (Figure 1). Comparison of the incidence of UTI among the three trimesters shows a higher incidence ($P \leq 0.05$) in the second trimester, with the first trimester recording the lowest (Figure 2).

Isolation and identification of bacteria

A total of six (6) bacteria species were isolated and identified, out of which five (5) were Gram–negative bacteria and one (1) was Gram - positive bacterium (Table 4). The distribution of bacteria species (etiologic agents) over the three trimesters in a decreasing order followed the pattern *E. coli* >*Klebsiella pneumonia* > *S. aureus* > *Proteus mirabilis* > *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Table 5).

Table 5: Distribution of isolates by trimesters

Trimester	Isolates						Total
	E. coli	*Klebsiella pneumonie*	*Proteus mirabillis*	*S. aureus*	*Pseudomonas aeruginosa*	*Enterococcs faecalis*	
1	13	4	4	4	0	1	26
2	27	14	1	12	2	1	57
3	15	9	3	3	0	0	30
Total	55	27	8	19	2	2	113

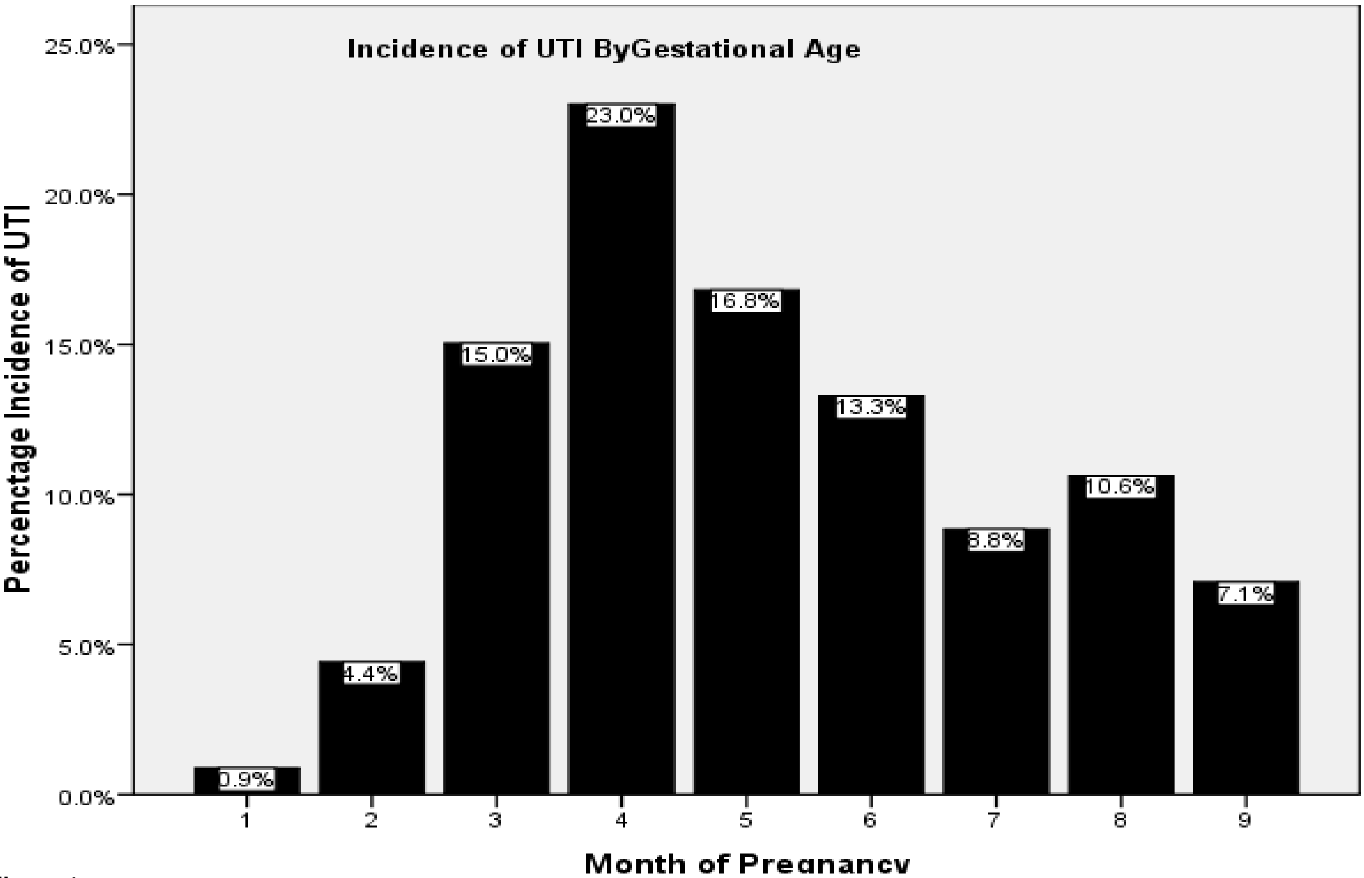

Figure 1: The incidence of UTI over the gestational period (9 months), each bar represents the percentage incidence of UTI in a month, determined by the number of pregnant women in a given month with positive urine microbial culture (microbial count > 10^5/ml).

Antimicrobial susceptibility testing (AST)

The bacteria species showed varying susceptibility patterns to five of the antimicrobial drugs, for instance in decreasing order the pattern stood at Gen > Tet > AMK > Amp > Ery (Table 6 and Figure 3). Both Gram - negative and Gram - positive bacteria however exhibited no susceptibility mostly to Amikacin and Penicillin respectively (Table 7).

DISCUSSION

The study reports on the use of microbiological and physicochemical analysis as a basis for investigating asymptomatic urinary tract infection among pregnant women within the ages of 15 - 45 years. Although a high percentage (66 %) of the pregnant women presented urine samples with normal color and odour, the overall incidence of UTI was high, suggesting a negative

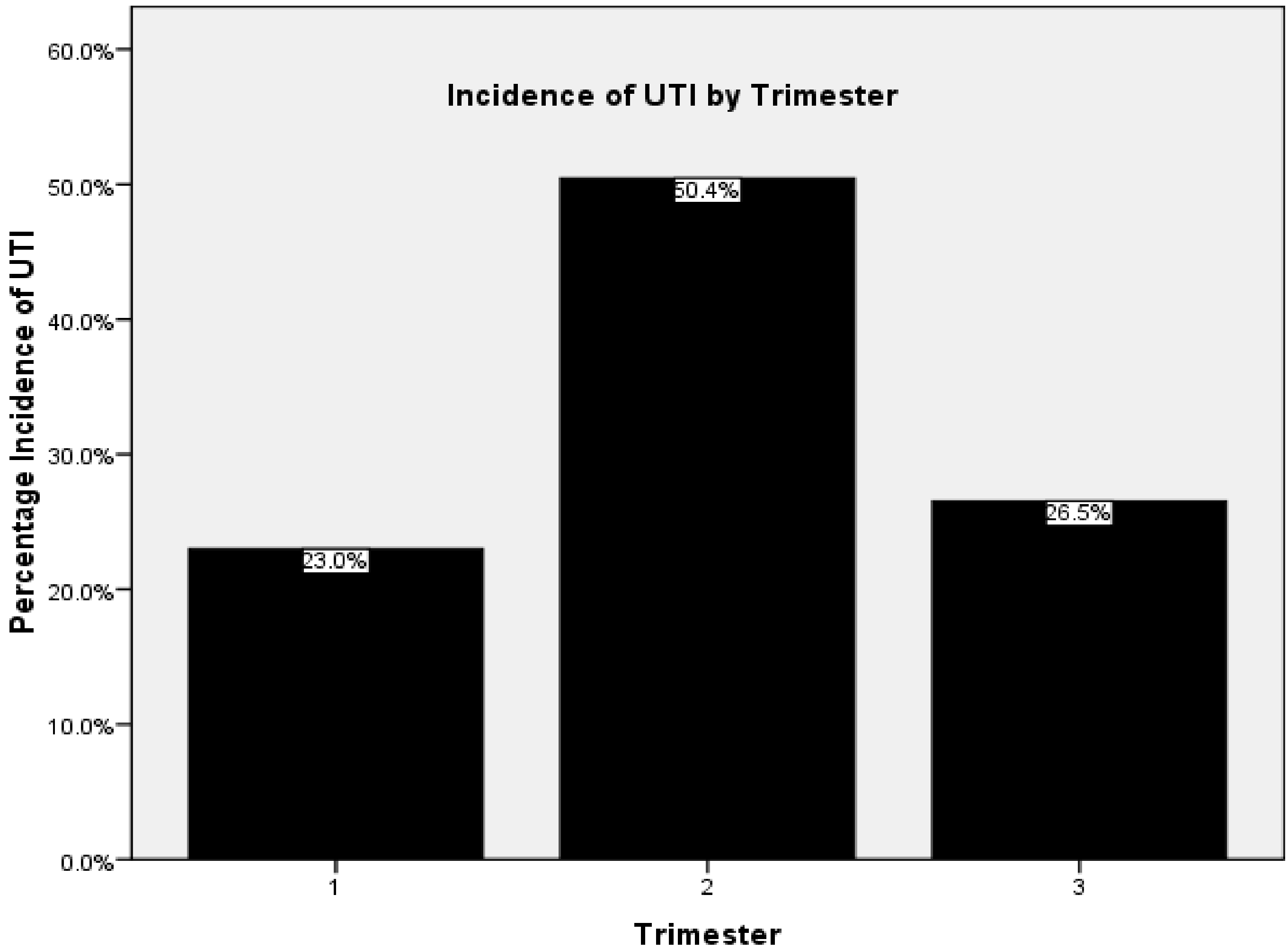

Figure 2: The percentage incidence of UTI over the three trimesters of a normal full term pregnancy. Each bar describes the percentage incidence of UTI for the first (1), second (2) and third (3) trimesters of pregnancy.

Table 6: Susceptibility patterns of bacteria to antimicrobial drugs

Isolates	**Antimicrobial Drugs**					**Total**
	Gen	**Tet**	**Amk**	**Amp**	**Ery**	**Total**
*E. coli**	28	27	0	0	0	55
*Klebsiellapneumoniae**	17	0	10	0	0	27
*Proteus mirabillis**	2	0	0	6	0	8
S. aureus	13	5	0	0	1	19
*Pseudomonas aeruginosa**	2	0	0	0	0	2
*Enterococcus faecalis**	2	0	0	0	0	2
Total	64	32	10	6	1	113

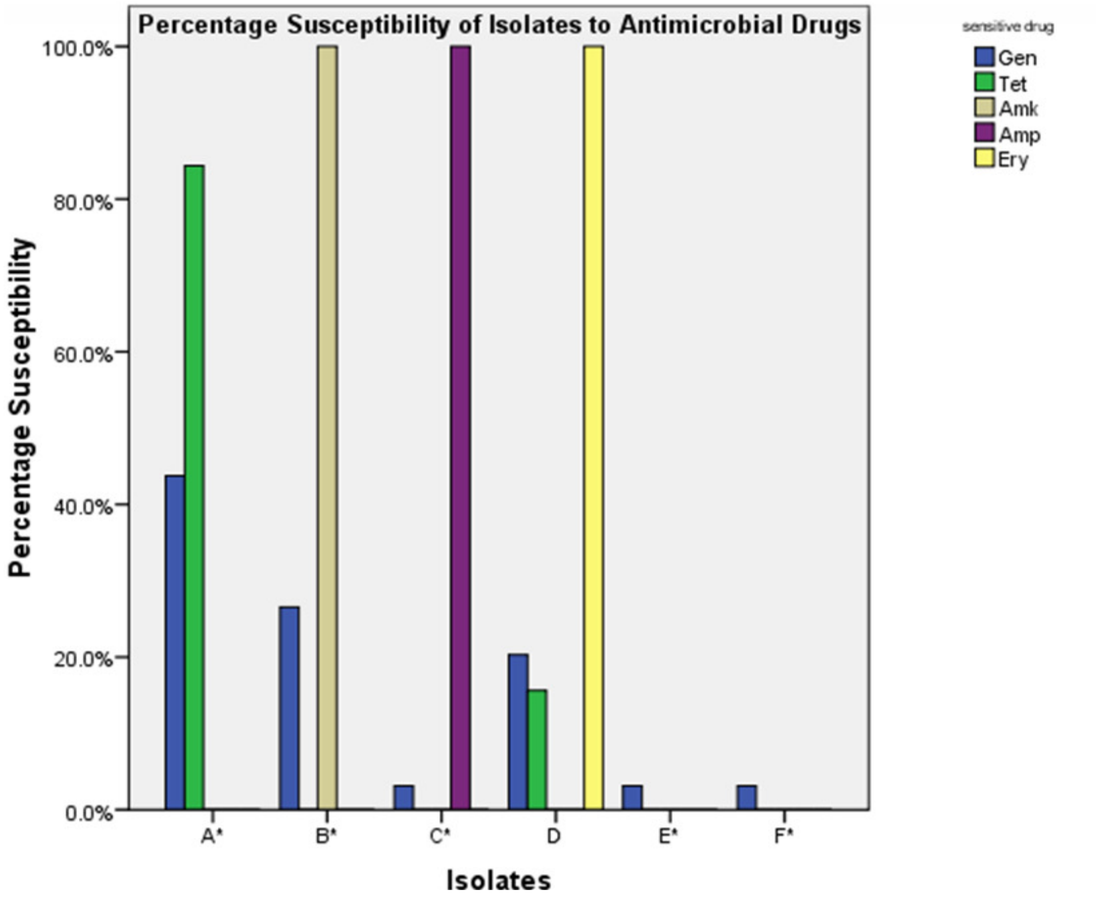

Figure 3: The bacteria that showed no susceptibility to the various antimicrobial drugs shown in table 5 as (0) are again shown here in the figure above by zero percentage susceptibility bars (0.0 %). For instance, *E. coli* was not susceptible to Amikacin, Ampicillin and Erythromycin, hence their virtual absence against *E. coli*. The effect of Amikacin was observed only in *Klebsiellapneumoniae*, hence the full percentage susceptibility bar: A (*E. coli*), B (*Klebsiella pneumonia*), C (*Proteus mirabilis*), D (*S. Aureus)*, E (*Pseudomonas aeruginosa*), and F (*Enterococcus faecalis*), * (Gram - negative bacteria).

correlation between urine color and odour, and UTI. Proteinuria as observed in some of the samples studied could be a non – specific biomarker for UTI. Nitrites were always detected in samples from which Gram – negative bacteria were isolated. Gram-negative organisms like *E. coli*, *K. pneumonia*, and *Proteus spp* are able to reduce nitrate present in urine to nitrite, hence could account for the nitrite present. We hereby agree with Cheesbrough (2006) who had linked bacteria invasion to the presence of protein and nitrite in urine. The present UTI incidence of 56.5 % represents one of the highest so far compared to earlier reports such as 3.3 % (Moghadas and Irajian, 2009), 3.7 % (Mobasheri et al., 2002), 6.1 % (Hazhir, 2007), 7.3 % (Turpin et al., 2007), 8.4 % (Hernandez et al., 2007), 9.5 % (Obirikorang et al., 2012), 9.8 % (Tadesse, 2007), 22.2 % (Famurewa, 1992; Omonigho et al., 2001), 23.9 % (Olusanya et al., 1993), and 35.5 % (Ebie et al., 2001). However, the result of the present study was comparable with a 58 % incidence of UTI reported by Onifade et al., (2005). This rather high incidence could be attributed to low economic status and low level of personal hygiene among the pregnant women. The variation in UTI incidence from one geographical location to another could be attributed to differences in UTI perception, mode of screening, and compounding risk factors such as age, parity (Obirikorang et al., 2012), pregnancy (Delzell et al., 2000) and in our view host behavioral factors.

In affirmation of earlier reports, UTI incidence was quiet high in the 21 – 32 age groups. Naturally, both men and women in this age group are more sexually active. The anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse which could result in increased tendency of bacteria being massaged up the urethra into the bladder.

UTI incidence was comparatively high in the second

Table 7: Susceptibility pattern of Gram - negative bacteria to antimicrobial drugs

Isolates	Antimicrobial Drugs						Total
	Gen	Amk	Cot	Amp	Pen	Flx	
*E. coli**	1	37	9	8	0	0	55
*Klebsiellapneumoniae**	0	0	10	17	0	0	27
*Proteus mirabilis**	0	7	1	0	0	0	8
S. aureus	0	0	0	2	14	3	19
*Pseudomonas aeruginosa**	0	0	0	0	2	0	2
*Enterococcus faecalis**	0	2	0	0	0	0	2
Total	1	46	20	27	16	3	113

* (Gram - negative bacteria), 0 (bacteria showed no susceptibility to that antimicrobial drug)

and third trimesters and 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 favorable for microbial invasion. Though incidence of UTI 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 adds to Vazquez and Villar (2000) who had reported that 10 - 30 % of women with bacteriuria in the first trimester develop upper UTI in the second and third trimester; this possibly could mean that the incidence of UTI is trimester - related reflecting perhaps the observation that incidence increases with aging pregnancies.

The present observation like other works showed that the most common bacteria isolated from the urinary tract in a decreasing order are *E. coli* (48.7 %), *K. pneumoniae* (23.9 %) and *S. aureus* (16.7 %). Other bacteria isolated included *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* agreeing with previous observations that UTI etiology is fast changing with the recruitment of many diverse bacteria species. This finding is similar to other reports that Gram - negative bacteria, especially *E. coli* is the most pathogen isolated in patients with UTI (Delzell 2000; Ebie et al., 2001; Onifade et al., 2005; Aiyegoro et al., 2007). Gram-negative bacteria which are the dominant etiologic agents account for more than 85 % of cases of UTI and are normal flora of the intestinal tract especially the rectum which is in close proximity to the urethral orifice (Anyamene et al., 2002; Obiogbolu et al., 2009).

The dominance of Gram–negative bacteria in UTI could be attributed to an increase in levels of amino acids and lactose during pregnancy which particularly encourages *E. coli* growth. It could also be due to infection by faecal contamination due to poor hygiene (Obiogbolu, et al., 2009).

The dominance of *E. coli* over *Klebsiella pneumonia* in this study is however in contrast to the findings of Omonigho et al., (2001) who found *K. pneumonia* to be more prevalent than *E. coli* in UTI and this may be attributed to differences in host susceptibility to these two pathogens as a result of both biological and environmental factors that encourage biodiversity in host, pathogens, vectors and social factors such as people's efforts in controlling disease. The 23.9 % prevalence recorded for *K. pneumonia* however could be that they are now gaining clinical prominence as etiologic agents of UTI. According to Murray et al. (1998), *S. aureus* is believed to cause cystitis in many young sexually active females, it was similarly found in the present study. This could be indicating that it is also gaining clinical prominence in the etiology of UTI during pregnancy.

Finally, both the Gram-negative and Gram-positive bacteria showed greater susceptibility to Gentamycin. This observation compares favorably with the observations made by Kenechukwu et al. (2005) that Gram-negative bacteria have the highest sensitivity to Gentamycin after Nitrofurantoin. Gentamycin is an aminoglycoside antibiotic that works by binding to the subunit of the bacterial ribosome, interrupting protein synthesis thereby preventing bacteria from performing vital functions needed for survival. Per the present finding gentamicin can be effective in treating bacterial infections especially those by Gram - negative bacteria as has also been reported (Miglioli et al., 1999). Gram-negative bacteria showed greater resistance to the effect of Amikacin, whereas Gram - positive bacteria exhibited minimal resistance to Penicillin.

CONCLUSION

An asymptomatic UTI prevalence rate of 56.5 % observed in the present study reveals the clinical and epidemiological significance that UTI has attained in the study area. This calls for the need to include compulsory UTI diagnosis, specifically by urine culture for pregnant women as part of their routine antenatal visits for early detection and treatment. There is the urgent need for education and creation of awareness on the importance of personal hygiene, particularly during pregnancy. Finally, key stakeholders in the health ministry, for instance the Ministry of Health and the Ghana Health Service must come out with measures both in the short and long terms to address UTI and its complications in pregnancy, especially at a time when the Nation struggles to meet the Millennium Development Goals pertaining to Maternal Health.

ACKNOWLEDGEMENT

We are very grateful to the following: all the pregnant women who out of their own will made themselves available for the study, the Administrators and Ethics Committee members of the three hospitals used in the study, and the laboratory staff of the three Hospitals. To all we say thank you.

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