Bacterial analysis of urine of pregnant and non-pregnant women having urinary tract infection (UTI), attending the General Out-Patient (GOP) clinic of the University College Hospital (UCH), Ibadan, Nigeria

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Abstract: This study was conducted in order to determine the prevalence of urinary tract infection among pregnant and non-pregnant women with a view of identifying the uropathogens responsible for infection and their antibiotic sensitivity patterns. Clean void mid-stream urine samples were collected in sterile universal bottles from fifty pregnant and fifty non-pregnant women within age range of 18 to 40 years attending the General Outpatient Clinic of the University College Hospital (UCH), Ibadan, Nigeria. The average ages of pregnant and non-pregnant women were thirty-four years and twenty-seven years respectively. Approximately 70% of pregnant women in the study group were infected while 75% of the non-pregnant women were also infected. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and *Enterococcus faecalis* were isolated in both groups. However, *Escherichia coli* was predominant in the groups of women. Of the antibiotics used in the study, amikacin followed by nitrofurantoin were most effective. Cefuroxine was least effective.

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Introduction

Urinary tract infections connote the presence and growth of microorganisms in the urinary tract with progression to the kidneys, ureters, bladders, urethra, and the prostate glands (Holt et al., 1994). Complications of infection including acute cystitis and acute pyelonephritis (Karen et al., 1994) may result in anatomical and structural abnormalities that impair the ability of the tract to clear out urine and therefore bacteria (Conway et al., 2007).The urine and related organs and structures of the urinary tract are normally meant to be sterile with absence of microorganisms (Krieg and Sneath, 1994). In the year 2007 alone, an estimate of approximately one billion dollars was reported invested on treatment of urinary tract infection (cystitis and pyelonephritis inclusive) in the United States of America (Mackie et al., 2007). The kidneys, ureters, bladder and urethra play different roles in the removal of body waste (Akinyemi, 2005).

Urinary tract infections typically occur when bacteria enter the tract through the urethra and begin to multiply in the bladder. The design of this study comprised the determination of infection of the tract amongst pregnant and non-pregnant women attending the General Out Patient (GOP) clinic of the University College Hospital (UCH), Ibadan, Nigeria with a view of identifying the uropathogens responsible for infection and their antibiotic sensitivity patterns.

Materials and Methods

2.1 Study Setting

Study was conducted at the Microbiology Laboratory, Department of Medical Microbiology, University College Hospital, Ibadan, Oyo State, Nigeria.

2.2 Study Population

The study population was drawn from patients attending the General out Patient (GOP) clinic of the Teaching Hospital. One hundred (100) samples of the urine that were randomly selected from the laboratory were used. Those patients that were already on antibiotics therapy were excluded from the study.

2.3 Collection of Samples

Mid-stream urine samples were collected using sterile, wide-mouthed plastic bottles with screw cap tops. They were submitted by the patient to the laboratory. On the urine sample bottle were indicated name, age, sex and time of collection. The samples were analyzed bacteriologically using the methods of Stamm et al. (2009).

2.4 Culture

A calibrated sterile platinum wire loop, 4.0mm in diameter, designed to deliver 0.01ml was used for the plating. A loopful of the well mixed urine sample was inoculated onto duplicated culture plates of blood and CLED agar. After incubation, all plates were examined macroscopically and microscopically for bacterial growth. The bacterial colonies were counted and multiplied by 100 to know the significance of the bacterial colonies present. This also gives an estimate

of the number of the bacteria present per millilitre urine (Stamm and Hooton, 1993).

Culture media used were blood agar, cysten lactose electrolyte deficient (CLED) agar, Mueller-Hinton agar with peptone water.

2.5 Analysis of Samples

Each of the urine samples was mixed and aliquot centrifuged at 3000rmp for 5 min. The deposits were examined under the microscope using x10 and x40 objectives. Any sample which had more than 10 white blood cells/mm^3 was regarded as pyuric (Cheesdrough, 2000). About 2 drops of each of the centrifuged urine samples was placed on a microscope glass slide and air-dried. They were thereafter subjected to Gram staining before examining under the microscope. Bacterial isolates were identified generally using a battery of tests (Cheesbrough, 2000).

2.6 Antimicrobial Sensitivity Testing

Antimicrobial sensitivity was carried out using agar disc diffusion technique (CLSI, 2006) with commercial antibiotics containing discs (Dyanamcro Labs, PVT, LTD, India). Zones of growth of inhibition were then measured to the nearest millimeter using a transparent ruler and recorded. Interpretation of results was done with zones of inhibition $\geq$ 18mm considered sensitive, 13-17mm considered intermediate and < 13mm considered resistant. Isolates were classified as either resistant or intermediate sensitive based on the definition of the Clinical and Laboratory Standard Institute (CLSI, 2006). An isolate was considered multi-drug resistant if it was resistant to at least three of the antibiotics (Cheesbrough, 2000).

2.7 Biochemical Tests for Identification

Biochemical tests carried out on the organisms isolated were catalase, coagulase, indole, citrate, urease and oxidase tests. Motility test was also carried out.

2.8 Preparation of the McFarland Standard

This was done as previously described by Andrew (2006), where 0.5ml of 0.048M $BaCl_2$ (1:17% w/v $Bacl_2.2H_20$) was added to 99.5ml of 0.18M H_2SO_4 (1%v/v) with constant stirring. The standard was then distributed into screw cap tubes of the same size and with the same volume as those used in growing broth cultures. The tubes were sealed tightly to prevent loss by evaporation. The tubes were protected from light and placed at room temperature. The turbidity standard solution was poured into a tube identical to the one used for the broth sample. It was also stored in the dark at room temperature for six months, properly sealed to prevent evaporation. The main purpose was to measure 0.5 turbidity of the inoculated broth before sensitivity was carried out for moderate growth on the plates (Andrew, 2006).

3. Results

Out of 100 women who had urinary complaints and used for the study, 50 (50%) were pregnant and were between 28 and 40 years of age. The other 50 (50%) were non-pregnant women and were between 18 and 35 years. Furthermore, 15 out of the pregnant women and 10 of the non-pregnant women had no infection. There were no bacterial growths in their urine cultures after 48 hours of incubation. Five out of the pregnant women and 3 out of the non-pregnant women had insignificant growth with cultures of their urine samples having bacterial colonies below 10^5 per ml of urine. On the other hand those samples that had 10^5 and above of bacterial colonies per ml of urine had significant growth. A total of seventy two women had pathogens in their urine consisting of thirty five pregnant women and thirty seven non-pregnant women. The frequency of occurrence of bacteria isolated was *Escherichia coli* (27), *Pseudomonas aeruginosa* (6) and *Klebsiella pneumonia* (10). However, *Staphylococcus aureus* (14), *Staphylococcus saprophyticus* (10) and *Enterococcus faecalis* (5) showed the least frequency of occurrence.

Table 1 shows the statistics of the patients used for the study. Table 2 shows the collation of frequency of occurrence of bacteria isolated from urine samples. Table 3 shows the biochemical tests used to identify the organisms isolated in this study. Table 4 shows the frequency of occurrence of the bacteria isolated from the samples of pregnant women. Table 5 shows the frequency of occurrence of the bacteria isolated from the samples of non-pregnant women. Table 6 shows characteristics of bacteria isolated from urine. Table 7 shows the range of zones of inhibition produced by antibiotics on isolates.

It was also observed that the number of isolated *Staphylococcus aureus* was slightly higher in pregnant women. *Escherichia coli* was higher in non-pregnant women. In the pattern observed from the antibiotics sensitivity test, amikacin followed by nitrofurantoin (Table 4) was the most potent of all the antibiotics indicated by the frequency of clear zones of inhibition on both Gram negative and Gram positive bacteria. Ceftazidine was poorly effective, shown as having the highest frequency of resistance (Table 4).

Table 1: Statistics of the patients used during the study

	Number of patients	Average age of patients	Those infected
Pregnant women	50	34	35
Non-pregnant women	50	27	37
Total	100		72

Table 2: Frequency of occurrence of bacteria isolated from urine samples

Organism	Pregnant women	Non-pregnant women	Total
Escherichia coli	12	15	27
Klebsiella pneumonia	7	7	14
Staphylococcus aureus	5	5	10
Pseudomonas aeruginosa	5	5	10
Staphylococcus saprophyticus	3	3	6
Enterococcus faecalis	3	2	5
Total	35	37	72

Table 3: Biochemical tests used to identify the organisms isolated

Citrate	Urease	Oxidase	Catalase	Coagulase	Indole	Motility	Gram reaction	Organism
-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Escherichia coli*
+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	*Klebsiella pneumoniae.*
-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	*Staphylococcus aureus*
-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	*Pseudomonas aeruginosa*
-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	*Staphylococcus saprophyticus*
-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	*Enterococcus faecalis*

Table 4: Frequency of occurrence of bacteria isolated from the urine samples of pregnant women

Isolate	Gram reaction	Frequency of occurrence
Staphylococcus aureus	Gram positive cocci in clusters	7
Staphylococcus saprophyticus	Gram positive cocci in clusters	5
Klebsiella pneumoniae.	Gram negative rods	5
Pseudomonas aeruginosa	Gram negative rods	3
Escherichia coli	Gram negative rods	12
Enterococcus faecalis	Gram positive cocci in chains	3

Table 5: Frequency of occurrence of bacteria isolated from the urine samples of non-pregnant women

Isolate	Gram reaction	Frequency of occurrence
Staphylococcus aureus	Gram positive cocci in clusters	7
Staphylococcus saprophyticus	Gram positive cocci in clusters	5
Klebsiella pneumonia	Gram negative rods	5
Pseudomonas aeruginosa	Gram negative rods	3
Escherichia coli	Gram negative rods	15
Enterococcus faecalis	Gram positive cocci in chains	2

Table 6: Characteristics of bacteria isolated

Bacteria isolated	Colonial characteristics	Lactose fermentation	Gram reaction
Escherichia coli	They were raised, dry and there were discrete colonies along line of streaking	Lactose fermenters on CLED	They were Gram negative rods
Pseudomonas aeruginosa	They showed a greenish pigmentation on Muller Hinton agar (sensitivity), the colonies were raised, dry but the colonies were not discrete	Non-lactose fermenters on CLED	They were Gram negative rods
Klebsiella pneumonia	They were raised, mucoid but no discrete colonies along the line of streaking	Lactose fermenters on CLED	They were Gram negative rods
Enterococcus faecalis	The colonies appeared raised, dry their colonies were smaller compared to the other bacteria that were isolated.	Lactose fermenters on CLED	They were Gram positive cocci in chains.
Staphylococcus aureus	The colonies appeared raised, dry there were descrete colonies along the line of streaking	Lactose fermenters on CLED	They were Gram positive cocci in chains.
Staphylococcus saprophyticus	The colonies appeared raised, dry there were descrete colonies along the line of streaking	Lactose fermenters on CLED	They were Gram positive cocci in chains.

This table shows that the isolates that fermented lactose (ability of the organisms to make use of the lactose in the medium to produce acid) were more than those that did not ferment lactose on CLED.
Note: CLED: Cysteine Lactose Electrolyte Deficient.

Table 7: Average range of zones of inhibition of organisms by the antibiotics.

Antibiotics	Zones of inhibitions (mm) of the bacteria isolated				
	Staphylococcus aureus	*Staphylococcus saprophyticus*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Escherichia coli*
Amikacin (30 µg)	38	33	21	29	32
Nitrofurantoin (10 µg)	29	30	29	25	21
Cefuroxine (10 µg)	09	07	0	0	0
Levofloxacin (10 µg)	13	15	14	15	18
Ciprofloxacin (10 µg)	18	15	16	17	11
Erythromycin (10 µg)	24	11	19	18	11
Pefloxacin (30 µg)	30	24	11	13	09
Ceftazidine (10 µg)	27	20	07	11	10

From Table 7, amikacin, closely followed by nitrofurantoin had the highest zones of inhibition. Amikacin with the highest frequency of clear zones of inhibition had ranges of zones of 21-38mm. The least zone of inhibition is shown as cefuroxine which has the lowest frequency of clear zones of inhibition (0-09mm).

4. Discussion

This investigation shows that the incidence of urinary tract infections in the pregnancy was less than that in the non-pregnant women (Tables 4 and 5). This may be due to the fact that sexual activity is higher in non-pregnant women than in pregnant women. Probably, pregnant women attending anti-natal clinic receive more information on infection and are able to abide to rules guarding prevention of infection during pregnancy. Stein and Funstruck (2002) reported a substantial risk of infection of 30-40% among pregnant women especially during the first trimester and a risk of 40-65% among non-pregnant women in their early stage of life. This result disagrees with the report of Jellheden, (2000) who claimed a prevalence rate of 15-35% in pregnant women and 10-20% in non-pregnant woman that were less than 50 years of age. The results from this study, as observed in Tables 3 and 4, also compared favourably with a 58% incidence in pregnant women and 79% incidence in non-pregnant women reported by Onifade (2005), in a similar study among pregnant and non-pregnant women in Nigeria. However, Elder (2001) reported a higher incidence of 66% in pregnant women while Omonigho, (2001) and Ebie, (2001), reported a lower incidence of 26.7, 22.3, and 35.3% respectively. These high incidences of urinary tract infections may be due to hormonal effect observed in pregnancy which reduces the tone of the ureteric musculature aided perhaps by mechanical pressure from the uterus leading to urinary stasis, thus encouraging bacterial proliferation in urine which is an excellent culture medium (Obiogbolu, 2009). Similarly, the incidence of urinary tract infection among pregnant women in Nigeria has been reported as 23.9% (Omonigho, 2001). These studies suggest that the higher standards of living in the industrialized world may contribute to the lower incidence rates of urinary tract infection there.

The high incidence of *Escherichia coli* signify the fact that that *Escherichia coli* is a commensal of the bowel and that such infection in women is mostly by faecal contaminantion from the anus to the urogenital tract which houses the entrance of female urinary tract whose proximity to the anus is very close (Obiogbolu, 2004). Other pathogens isolated in order of prevalence include *Klebsiella. spp*. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The isolated pathogens in this study were coliforms which are index organisms of faecal contamination. They are implicated in safety, good hygiene and sanitary quality. This confoms to the report of Anyamene et al. (2002) that the dominant etiologic agent accounting for more than 85% of causes of UTIs are the gram-negative bacilli which are normal flora of the intestinal tract.

4.1 Conclusion

Significant bacteriuria in pregnancy is common and a serious cause of maternal and prenatal morbidity and mortality. Clinical presentations include asymptomatic bacteriuria. Acute cystitis and pyelonephritis. All are amenable to investigation and treatment with substantially improving outcome. Pregnant women should be screened for asymptomatic bacteriuria by urine culture and treated with appropriated antimicrobials. Acute cystis and pyelonephritis demand full assessment and treatment with early involvement of other specialists in severe or systemic infection.

All women should be reviewed to confirm post-treatment urine sterility. Empirical antimicrobial treatment will occasionally be required but any decision to treat should be reevaluated once culture and sensitivity reports of the available. When choosing an antimicrobial, the pharmacokinetics and bioavailability of the individual drugs in pregnancy must be considered along with the resistance profiles of microorganisms in the local antenatal population. It is also vital to use treatments with an established safety profile and most importantly, without any genetic risk.

4.2 Prevention

There are several simple, do-it-yourself techniques that can be employed to avoid urinary tract infection (Onifade, 2005). Some may work some of the time only in some women. But because they have no side effects, they certainly are worth trying to prevent the often painful and bothersome symptoms the infection can bring. The following step is a preventive measure recommended for women either pregnant or not:

Drinking plenty of fluid – the equivalent of six to eight 8 glasses every day to flush bacteria out of the urinary system. Water is the ideal fluid because it is readily available, inexpensive and non-caloric, but other beverages also count toward your fluid intake. These include juices, milk and herbal teas. Even alcoholic beverages such as coffee and colas help replenish body fluids but do not rely heavily on them because they have diuretic properties. Additionally, alcohol and caffeine, as well as spicy foods, are among the substances that may irritate the bladder and, thus, should be avoided.

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