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Isolation and antibiotic sensitivity of *Escherichia coli* from pregnant and non-pregnant women attending the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria

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ABSTRACT: This study was carried out to isolate and determine the antimicrobial susceptibility patterns of *Escherichia coli* from pregnant and non-pregnant women with urinary tract infection attending University of Maiduguri Teaching Hospital (UMTH) Maiduguri, Nigeria from X- 2008.Clean voided mid-stream urine samples were collected in sterile universal bottles while high vaginal swab samples were obtained from patients with the use of sterile swab stick. A total of 260 samples were collected comprising 65urine samples each from pregnant and non-pregnant women and 65 high vaginal swabs each from pregnant and non-pregnant women.

The results showed that out of the sixty-five (65) urine samples collected from pregnant women attending the health institutions, 48 (73.9%) were positive for various species of bacteria which includes *Escherichia coli* 26 (40.0%), *Staphylococcus aureus* 10 (15.4%), *Proteus species* (15.4%) and *Klebsiella species* 2 (3.1%). Out of the 65 high vaginal swab samples collected from the pregnant women, 16 (24.6%) were positive for various species of bacteria among which are *Escherichia coli* 7 (10.8%), *Staphylococcus aureus* 6 (9.2%), *Proteus species* 3 (4.6%).

Out of the sixty-five (65) urine samples collected from non-pregnant women attending the health institutions, 33(50.8%) were positive for various species of bacteria which includes *Escherichia coli* 15 (23.1%), *Staphylococcus aureus* 10 (15.4%) and *Proteus species* 8 (12.3%). Out of the 65 high vaginal samples collected from non-pregnant women attending the health institutions 17 (26.1%) were positive for the following species of bacteria, *Escherichia coli* 6 (9.2%), *Staphylococcus aureus* 5 (7.7%) and *Proteus species* 6 (9.2%).

The result of antibiotic sensitivity test on *E.coli* isolates showed that they are highly sensitive to the quinolone group of antibiotics such as Ciprofloxacin, Ceporex and Perflacin. The organism is intermediate sensitive to Streptomycin, Tarivid and Gentamycin but resistant to Augmentin, Nalidixic acid, Septrin and Ampicillin.

Introduction

In the female human subject, the urinary tract has an important relationship with the reproductive organs because of its proximity. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state; the enlarging uterus affects all the tissues of the urinary tract at various times (1). The urethra is shorter in females than in males and is more readily transversed by microorganisms. This is why urinary tract infections are more common in females (2).

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The vaginal microflora is normally made up of 5 to 15 different bacterial species, including aerobes and anaerobes, and has the potential to inflict different clinical syndromes (e.g. bacterial vaginosis) and diseases (e.g.pelvic inflammatory disease). Gram-negative facultative anaerobic *Escherichia coli* are one of the common organisms in the microflora of pregnant as well as non-pregnant women (3).

UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients. UTI accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria (in particular, *Escherichia coli*) remained the most frequent cause of UTI, although the distribution of pathogens that cause UTI is changing. There are several factors and abnormalities of UTI that interfere with its natural resistance to infections and these factors include sex, age and disease, hospitalization and obstruction of the urine flow (4). Females are however believed to be more affected than males except at the extremes of life (5), this is as a result of shorter and wider urethra. The anatomical relationship of the female's urethra and the vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy/ child birth (6, 7). The highest incidence of urinary tract infection occurs in the child bearing age and this has been linked directly to sexual activity and aging (8). UTI are responsible for considerable morbidity and when associated with urinary obstruction or renal papillary damage, can lead to serious kidney damage.

UTIs are usually treated with antibiotics including nalidixic acid, nitrofurantoin, ofloxacin, perfloxacin, ciprofloxacin, gentamycin, etc. In the last couple of years, there has been a lot of focus in scientific literature on inappropriate use of antimicrobial agents resulting in the spread of bacterial resistance (9, 10). The widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents (11). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance to that agent. The emergence of antimicrobial resistance in the management of urinary tract infections is an important public health issue. While many antibiotics including penicillin, macrolides and tetracyclines were very useful in the treatment of urinary tract infections in the past, the rates of bacterial resistance to antimicrobial agents has significantly increased and are increasing in many countries in recent times (12). Despite the well-publicized concerns about the problems of inappropriate use of antimicrobial agents, or use of broad spectrum antibiotics (when narrow spectrum drugs would be effective), increasing resistance of bacteria causing urinary tract infections to antimicrobial agents remains a serious problem. With the high prevalence of fake and substandard drugs in Nigeria (13), the rate of bacterial resistance to antimicrobial agents in the management of UTIs is likely to be much higher compared to many other countries.

The aims and objectives of this study are to isolate and identify microorganisms implicated in urinary tract infection as well as determining the susceptibility pattern of the isolates. The result of this study is expected to provide very useful information which would assist physicians in drug prescribing methods towards a better management of urinary tract infections.

Materials and Methods

Study population

Clean voided mid-stream urine and high vaginal swab samples were collected from pregnant and non-pregnant women attending the University of Maiduguri Teaching Hospital, Nigeria. Some categories of patients were excluded from this study and these are patients on any antibiotic therapy one week before the samples were collected. For collection of urine samples the patients were advised not to take large quantities of fluids an hour before sample collection.

Collection of samples

High vaginal swab samples were obtained from patients with the use of sterile speculum and a sterile swab stick. The speculum was inserted into the vagina and was used to open it until the cervix of the patient become visible; the swab stick was then used to collect the sample at the site below the cervix by gently rotating it around the site.

Clean voided mid-stream urine specimens were obtained from patients in sterile universal bottles containing boric acid (0.2 mg) to arrest multiplication of bacteria in urine. All the patients were instructed on how to collect the sample aseptically and taken to the laboratory immediately for culture.

Isolation and identification of bacteria

Ten-fold serial dilutions were made by transferring 1.0 ml of the urine sample into 9.0 ml of sterile physiological saline. One ml was then dispensed into molten nutrient agar in Petri dishes and rotated gently to mix and be uniformly distributed. The contents were allowed to set and the plates were then incubated at 37^{o} C for 24 h. Bacterial colonies appearing on the plates after the incubation period were enumerated to determine urine samples with significant amount bacteriuria. A loopful of each urine sample was also streaked on MacConkey agar, Eosin Methylene Blue (EMB) and Blood agar plate for the isolation of the bacteria present in the urine. After incubation, plates with growth were selected and the colonies isolated using sterile inoculating wire loop and subsequently sub cultured on nutrient agar slants for further biochemical tests.
The samples from high vaginal swab were equally streaked on MacConkey, EMB and Blood agar and incubated at 37^{o}C for 24 hours. Identification of bacterial was made on the basis of gram reactions, colonial appearances of the isolates and the biochemical characteristics.

Antimicrobial susceptibility testing

The patterns of all the bacteria isolates to the following antibiotic agents, Tarivid 10mcg, Ceporex 10mcg, Gentamicin 10mcg, Augmentin 30mcg, Nalidixic acid 30mg, Ciprofloxacin 10mcg, Streptomycin 30mcg, Perflacin 10mcg, Septrin 30mcg and Ampicillin 30mcg were determined by using the agar-disc diffusion method as described by Bauer *et al* (14). The inoculated plates containing the antibiotics were incubated at 37^{o}C for 24 h after which the diameter of zone of inhibition around each antibiotic disc were then measured to the nearest millimeter and interpreted according to the current CLSI standard (15).

Results

The results showed that out of the sixty-five (65) urine samples collected from pregnant women attending the health institutions, 48 (73.9%) were positive for various species of bacteria which includes *Escherichia coli* 26 (40.0%), *Staphylococcus aureus* 10 (15.4%), *Proteus species* (15.4%) and *Klebsiella species* 2 (3.1%). Out of the 65 high vaginal swab samples collected from the pregnant women, 16 (24.6%) were positive for various species of bacteria among which are *Escherichia coli* 7 (10.8%), *Staphylococcus aureus* 6 (9.2%), *Proteus species* 3 (4.6%).

Out of the sixty-five (65) urine samples collected from non-pregnant women attending the health institutions, 33(50.8%) were positive for various species of bacteria which includes *Escherichia coli* 15 (23.1%), *Staphylococcus aureus* 10 (15.4%) and *Proteus species* 8 (12.3%). Out of the 65 high vaginal samples collected from non-pregnant women attending the health institutions 17 (26.1%) were positive for the following species of bacteria, *Escherichia coli* 6 (9.2%), *Staphylococcus aureus* 5 (7.7%) and *Proteus species* 6 (9.2%).

The result of antibiotic sensitivity test on *E.coli* isolates showed that they are highly sensitive to the quinolone group of antibiotics such as Ciprofloxacin, Ceporex and Perflacin. The organism is intermediate sensitive to Streptomycin, Tarivid and Gentamycin but resistant to Augmentin, Nalidixic acid, Septrin and Ampicillin.

Table 1: Prevalence of microorganisms isolated from pregnant women.

Sample source	No of samples	Bacteria isolated	No of positive isolates	Percentage isolated
Urine	65	*Escherichia coli*	26	40.0
		S. aureus	10	15.4
		Proteus species	10	15.4
		Klebsiella species	2	3.1
	Total		48	73.9
High vaginal swab	65	*Escherichia coli*	7	10.8
		S. aureus	6	9.2
		Proteus species	3	4.6
	Total		16	24.6

Table 2: Prevalence of microorganisms isolated from non-pregnant women.

Sample source	No of samples	Bacteria isolated	No of positive isolates	Percentage isolated
Urine	65	*Escherichia coli*	15	23.1
		S. aureus	10	15.4
		Proteus species	8	12.3
	Total		33	50.8
High vaginal swab	65	*Escherichia coli*	6	9.2
		S. aureus	5	7.7
		Proteus species	6	9.2
	Total		17	26.1

Discussion

The outcome of our study revealed that *E. coli* constitutes one of the predominant organisms incriminated as the major causes of urinary tract infection, because out of the 114 positive isolates, *E.coli* constituted 54 (47.4%) out of the organisms isolated from the pregnant and non-pregnant women, this was followed by *Staphylococcus aureus* 31(27.2%), *Proteus* 27 (23.7%) and *Klebsiella* 2 (1.7%) (only from the urine of pregnant women). Similar finding was reported by Inabo and Obanibi (16) in Kaduna where *E. coli*, *S. aureus* and *Klebsiell*a were the predominant bacteria isolated from patients suffering from urinary tract infection. Out of the 260 samples obtained from both pregnant and non-pregnant women (urine and high vaginal swab), gram negative bacteria constitutes 83(31.9%) as compared to the gram positive bacteria which constitutes only 31 (13.5%) of the total isolates. During sample collection, all the pregnant women either had a history of urinary tract infection or were having urinary tract infection while few among the non pregnant women had a history of urinary tract infection mostly during previous pregnancies, this observation may likely be due to *E. coli* preference for urinary stasis, a condition which is common during pregnancy. This finding is similar to other reports which indicated that a gram negative bacterium, particularly *E. coli,* is the commonest pathogen in patients with UTI (17, 18, 19).

The incidence of urinary tract infections is far more frequent in women than in men by reason of their fundamental physiological differences. The results showed that the organisms isolated from urine samples of the pregnant women were *E. coli, Staphylococcus aureus, Proteus spp.* and *Klebsiella spp.* The apparent distribution of these organisms in the pregnant women studied is a cause for concern, because they are prone to several complications during labour as well as a risk to the fetus. Some of these pathogens have been observed to bring

about miscarriages, prevent future conception and may cause blindness in the newborn. They are also known to gradually weaken the immune system of the mother resulting to secondary infection (20).

Similar studies in Benue and Ekiti state in Nigeria also indicated that members of the family *Enterobacteriaceae* are the predominant organisms implicated in urinary tract infections (21, 22). The predominant bacteria isolated were *Escherichia coli, Staphylococcus aureus and Klebsiella species. Proteus spp.* was incriminated in urinary tract infection, although infection may be endogenous but there is a possibility of infection being introduced from exogenous sources such as diagnostic or therapeutic instrumentation. The urease activity of *Proteus spp*. constitutes an important factor which determines its pathogenicity in the urinary tract. The organism rapidly forms ammonia from the urea in the urine, the kidney tissues become saturated with ammonia, which enhances infection. The alkalinity may cause deposition of phosphate stones which promote the retention of urine (23).

It was observed that high number of *E.coli* was isolated from urine samples of both pregnant and non-pregnant women as compared to the high vaginal swabs of the same patients, it was equally observed that a higher number was recorded from the urine samples of pregnant women when compared to the non-pregnant women, hence a high level of personal hygiene is expected from pregnant women as they are more exposed to urinary tract infections. In recent time, drug resistance has become a global problem which needs to be tackle globally, this is because bacteria are developing resistance to the antimicrobial agents that are commonly used in the treatment of UTI in the past.

The result from this work showed that *E.coli* was highly susceptible to the quinolone groups of antibiotics which includes Ciprofloxacin, Ceporex and Pefloxacin, the organism is intermediate sensitive to Streptomycin, Tarivid and Gentamycin but resistant to Augmentin, Nalidixic acid, Septrin and Ampicillin. . The drug of choice for the treatment of UTI over the years have always been includes septrin (co-trimoxazole), ciprofloxacin, ofloxacin, nitrofurantoin, gentamicin, ampicillin (24),and tetracycline, amoxicillin, and nalidixic acid (25), but in recent time the organisms have developed resistance to some of these antimicrobial agents and this is a cause for concern.

The most effective antibiotics in this study were the quinolones (ofloxacin, ciprofloxacin, and pefloxacin), these antibiotics are relatively expensive compared to the common antibiotics frequently used and this might have restricted their procurement and indiscriminate use by the populace, thereby making it effective against the organisms. Ampicillin, Nalidixic acid and Co-trimoxazole (septrin) which are commonly used antibiotics were not effective against majority of the organisms isolated in this study. It is recommended that antibiotic therapy should be use only after a thorough culture and antibiotic sensitivity tests have been carried out to avoid the emergence of drug resistance among bacteria.

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