

Characterization And Susceptibility Pattern of Gram Negative Bacteria Isolated From Urinary Tract of Pregnant Woman Attending Antenatal Clinic In Makurdi Metropolis

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ABSTRACT

The study investigated the susceptibility pattern of gram negative bacteria isolated from pregnant women attending antenatal at BishopMurray hospital, Makurdi Benue State Nigeria. A total of 40 clean catch midstream urine samples were collected from the hospital laboratory in sterile bottles and taken to the school laboratory. Inoculation of the samples to the media was done using the streak method. Twenty eight (28) samples contained gram negative isolates identified by colony morphology, microscopy and biochemical test which included: Escherichia coli 12(30%), Pseudomonas aeruginosa 9(22.2%), and Klebsiella pneumoniae 7(17.5%). The susceptibility of the isolated bacterial strains were tested against 10 different antibiotics using the disc diffusion method and their activity was evaluated by observing zones of inhibition, measured and recorded. The results showed that E. coli, Pseudomonas aeruginosa and Klebsiella pneumoniae were highly susceptible to ciprofloxacin (with zone inhibition 26.50 mm, 24.50 mm, and 25.00 mm respectively), Augmentin(21.3 %) Tarivid (20.6 %), Streptomycin (14.3 %), Gentamycin(18.0 %), Perfloracin (10.0 % with Pseudomonas completely resistant), Septrin(15.3 %), Amoxicillin (12.9 % with E. coli completely resistant) Sparfloxacin (11.0 % with Klebsiella resistant). The isolates were resistant to chloramphenicol with no zone of inhibition.

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Introduction

The urinary tract consists of organs that amass and store urine and discharge from the body which includes bladder, kidneys, and urethra [1]. A urinary tract infection (UTI) is a situation in which one or more parts of the urinary system (the bladder, kidneys, ureters and urethra) are infected. UTIs are the mainly bacterial infections and can arise anytime in a disposed individual. Almost 95 % of reported cases of UTIs are caused by bacteria that usually grow at the notch of the urethra and move up to the bladder. To a great extent, less often bacteria extend to the kidney beginning from the bloodstream [2].

Urinary tract infection (UTI) certainly is a familiar clinical ailment encountered in conventional health establishments worldwide. It is by and large approximated that millions of people are affected annually, with a huge percentage of the infections being perceptible; many also evident with palpable proven sort while others demonstrate difficulties in addition [3].

Urinary tract infection (UTI) is branded by bacterial invasion and reproduction relating to the kidneys and urinary tract pathways. UTI has turned out to be a largely universal hospital-acquired infection, responsible for almost 35 % of nosocomial infections, as the second most widespread source of bacteraemia in hospitalized patients [4-6].

Urinary tract infections are the most regular bacterial infection in women, and it takes place four times most often in females compared to males [7,8]. They happen most habitually amid the age of 16 and 35 years, with 10 % of women contracting an infection yearly and 60

% having an infection at instances in their lives [2]. Other foremost factors which make females more susceptible to UTI are pregnancy and sexual intercourse.

During pregnancy, the physiological boost in plasma volume and decline in urine concentration accounts for the advance of glycosuria in up to 70 % women which finally leads to increased bacterial population in urine [9]. Abnormalities of urinary tract or stones, immuno-suppression, diabetes mellitus and previous history of UTI are liable to amplify the menace [10]. In addition, female urine reportedly has a more suitable pH and osmotic pressure for the multiplication of Escherichia coli compared to the urine of males. Other factors such as reprehensible tidiness of the perineum, utilisation of napkins and hygienic towel in concert with pregnancy and sexual intercourse put in to elevated occurrence of UTIs in diverse women [2,11].

The anatomical association of the female urethra to the vagina makes it responsible for the ordeal for the period of sexual intercourse with bacteria being massaged up the urethra into the bladder all through pregnancy or childbirth. The humid surroundings of the females' perineum guarantees microbial growth and predispose the female bladder to contamination. Increase in the concentration of amino acids and lactose during pregnancy are said to encourage the growth of E. coli in urine. The presence of an indwelling catheter increases the likelihood of bacteremia almost 40-fold. UTIs are one of the main reasons for hospitalization, and are also the rationale for substantial antibiotic exploit in the LTC setting [12].

Intermittent infections are common and can lead to irretrievable harm to the kidneys, resultant in renal hypertension and renal malfunction in rigorous cases [13]. Occurrence of infection in females increases unswervingly with sexual action and childbirth. In the women, 25-30% of women sandwiched between 20-40 years of age will acquire UTIs [14]. These infections account for about 8.3 million that seek medical attention yearly [15].

Urinary tract infections linger as the most frequent bacterial infection in human populace and it is largely the most regularly stirring nosocomial infections [16]. Its annual global occurrence is of almost 250 million [17]. Worldwide about 150 million people are diagnosed with UTI each year, costing the global economy in surplus of 6 billion US dollars [18]. They are usually classified as uncomplicated or complicated, depending on the factors that activate the infections primarily depending on whether the infection is taking place for the first time or is a replicate incident

The uncomplicated UTIs are owing to a bacterial infection, most especially *E. coli*. They affect women much more regularly than men for the reason that the urethra is shorter and nearer to the anus, which is an invariable basis of faecal bacteria. Complicated infections, which take place in men and women irrespective of age, are also caused by bacteria but they tilt to be more brutal, more complex to take care of, and persistent. They are frequently the creation of some anatomical or structural defect that impair the capacity of the urinary tract to clear away urine and consequently bacteria, catheter use in the hospital or unrelieved indwelling catheter in the outpatient locale, bladder and kidney dysfunction, or kidney transplant (especially in the first 3 months after transplant). Recurrences can take place in patients with convoluted UTI if the underlying structural or anatomical abnormalities are not corrected.

Urinary tract infection is caused by bacteria which could be symptomatic or asymptomatic. Subordinate urinary tract infection is referred to as bladder infection. The most universal symptoms are a burning sensation during urination and having an push to urinate repeatedly without vaginal release and a major hurt [2]. These symptoms may differ from placid to harsh and in healthy women preceding an archetypal of six days [7]. Symptoms of an upper urinary tract infection or pyelonephritis, such as border pain fever or queasiness and vomiting in addition to the classic symptoms of a lower tract infections, bloody urine or visible pus in the urine are being documented [8].

Three common clinical manifestations of UTIs in pregnancy are: asymptomatic bacteriuria, acute cystitis and acute pyelonephritis [19]. UTI in pregnant women is also characterised by fever, flank pain and tenderness in addition to significant bacteriuria. Other symptoms may include nausea, vomiting, common urination, exigency, dysuria, untimely birth and low birth weight [20]. Bloody urine is reported in as many as 10% of cases of UTI in otherwise healthy women; this condition is called hemorrhagic cystitis. Fevers, chills and malaise may be noted in patients with cystitis, though these findings are related more frequently with upper UTI. The desire to urinate frequently, which may persist instantly after the bladder, is emptied, a painful burning sensation when urinating. (If this is the only symptom, then the infection is most likely urethritis, an infection restricted to the urethra), uneasiness or pressure in the lower abdomen. The abdomen can feel swollen. The urine frequently has a burly smell, looks dull, or contains blood. This is a signal of pyuria, or an elevated white blood cell count in urine, and is a very dependable pointer of urinary tract infections [19].

If the urine is composed majorly with bacteria but devoid of symptoms, the circumstance is referred to as asymptomatic bacteriuria. According to Nicolle, the regularity and usual history of asymptomatic bacteriuria show a discrepancy from dissimilar populations. Bacteria isolated from the urine of patients with asymptomatic bacteriuria typically start off as colonizing flora of the gut, vaginal or periurethra locale [2].

Asymptomatic bacteriuria occurs subsequently by ascension of bacteria from the urethra into the bladder occasionally with successive ascensions to the kidneys. Organisms will then persevere in the urinary tract without showing a host reaction adequately to generate symptoms or cause nuisance [2]. It's been acknowledged for a number of times that asymptomatic bacteriuria is frequent during pregnancy thus women are at increased risk of UTIs. Nicholson reported that except for a dumpy period instantaneously after delivery (infant period), females far surpass males in the occurrence of asymptomatic bacteriuria [21].

E. coli is by far the commonest source of uncomplicated community-acquired UTIs in both outpatient and inpatient settings. The rampant organisms habitually isolated from UTIs patients are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Proteus spp* and *Enterobacter spp*. The pervasiveness and scale of incident of one or two of the organisms in surplus of others are dependent on the environment. Gram negative bacteria have being conventional mainly in UTIs cases and reported by a number of authors with *E. coli* and *Klebsiella spp* as the foremost bacteria [21].

Pathogenesis of UTIs in women begins with colonization of the vaginal introitus by uropathogens that have faecal origin, followed by ascension through the urethra into the bladder. Pyelonephritis develops as bacteria pathogens come up to the kidneys passing through the ureters. The host and microbial factors that inspire the order from bladder to kidney infection necessitate further enquiry. Pyelonephritis can also be caused by seeding of the kidneys from bacteremia. It is probable that some cases of pyelonephritis are linked through seeding of the kidneys from bacteria in to the lymphatics [22]. UTI represents one of the largely infectious pathologies, disturbing pregnant women reportedly among 20 % of pregnant women and it is generally the most regular source of admission in obstetrical wards [23]. Abortion, preterm labour, maternal anemia, small birth size, thrombosis, hypertension, phlebitis and chronic pyelonephritis are connected to urinary tract infection throughout pregnancy [24,25].

The core cure of Urinary tract infections is antibiotics; nevertheless the growing menace of antibiotic resistance has presented a challenge in administration of effective antibiotics to patients with recurring and complicated UTI. Bacteria resistance to antibiotic is due to regular consumption or ingestion of antibiotics. It is important however to perform antibiotic sensitivity test earlier to treatment so as to make certain the efficacy of the prescribed antibiotic [26].

The aim of the study is to: characterize the susceptibility pattern of gram negative bacteria isolated from urinary tract of pregnant women. Considering the risk induced by UTIs in pregnant women, which is responsible for several complications during pregnancy, its diagnosis and treatment, is essential to maintain the health of the mother and baby. As such, this study when carried out will help examine the susceptibility pattern of gram negative bacteria causing UTIs, determine the exact antibiotic prior to infection and also create general awareness to the public on importance of carrying out test in cases of suspected UTIs.

Materials and Methods

Study Area

This prospective observational study was carried out among pregnant women attending antenatal in Bishop Murray hospital Makurdi which is the state capital of Benue state in Nigeria, Makurdi is located in the north central zone of Nigeria with Tiv, Idoma and Igede being the major ethnic groups.

Sample size and Sample Collection A total of forty (40) urine samples were collected from pregnant women excluding those who were on antibiotic treatment. The urine samples were collected in disposable wide mouthed sterile capped containers after proper clean up of the genitalia, the pregnant women were properly educated on how to collect clean catch midstream urine as described by Obirikwurang et al [27].

Media preparation and analysis of samples. A measured amount of nutrient agar, Mac-Conkey agar and Eosin Methylene Blue Agar (EMBA) were prepared according to the Manufacturers instruction and used in culturing of bacteria. The urine samples were first cultured on nutrient agar by streaking four times on the media and incubated at 37 °C for 24 hours. After incubation the inoculated culture plates were observed for bacterial growth, colony morphology (colour, shape edge, elevation, surface). Single colonies from the plates were sub cultured by streaking method on mac-conkey agar and Emb (Eosin methylene blue) and incubated at 37 °C for 24 hours for further identification [27].

Characterization and Identification of the isolates Growths on the culture media were identified using the colony descriptions of the isolates, morphological characteristics, gram stain and biochemical reactions of the isolates [27]. The Biochemical test; Oxidase test, Indole Test, Citrate Test and Voges-Proskauer (Vp) Test were done to identify gram positive and gram negative 11 bacteria. All bacteriological isolates were examined and confirmed according to Baron and Bergeys manual of determinative bacteriology [28].

Antibiotic Susceptibility Test The disc diffusion method was used to carry out the antibiotic susceptibility testing. The test organism was seeded on Mueller Hinton agar using streak method. A sterile forcep was used to place the disc containing small wafers of antibiotics on the surface of the medium, the set up was incubated aerobically at 37 °C for 24 hours. The inhibition zone diameters were measured using the meter rule and recorded. A clear ring or zone of inhibition indicates bacteria sensitivity [27].

Results Out of the 40 urine samples examined in this study, 28 samples were confirmed to be gram negative bacteria which are E. coli, Pseudomonas, aeruginosa and Klebsiella pneumonia. Table 1 Shows the frequency distribution of gram negative bacteria isolated from the urine of pregnant women. E. coli was the most predominant organism with percentage of 12(30 %) followed by Pseudomonas aeruginosa 9(22.2 %) and Klebsiella 7(17.5 %).

Table 2 Shows the susceptibility pattern of the gram negative bacteria isolated to various antibiotics. E. coli was more susceptible to ciprofloxacin with a zone of inhibition of 26.00 mm and was least inhibited by amoxicillin and Chloramphenicol with no zone of inhibition at all.

Table 1: Frequency distribution of gram negative isolates

Bacteria spp	Frequency of occurrence	Percentage
E. coli	12	30%
Pseudomonas	9	22.2%
Klebsiella	7	17.5%
Total	28	

Table 2: Susceptibility pattern of gram negative isolates against tested antibiotics

Antibiotics	E. coli	Pseudomonas	Klebsiella
AU	24.50	19.50	20.00
OFX	16.00	22.50	23.50
S	11.00	15.00	17.00
CN	21.00	22.00	11.00
PEF	17.00	15.00	17.00
SXT	14.00	14.50	17.50
AM	0.00	20.50	18.20
CH	0.50	0.500	0.00
SP	17.50	15.00	0.00
CPX	26.50	24.50	25.00

AU = Augmentin, OFX = Tarivid, S = Streptomycin CN = Gentamycin PEF = Pefloxacin, SXT = Septrin, AM = Amoxicillin, CH = Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin Pseudomonas was more susceptible to ciprofloxacin with zone of inhibition 24.50 mm and least susceptible to Chloramphenicol & pefloxacin with no zone of inhibition. Klebsiella was more susceptible to ciprofloxacin with zone of inhibition of 25.50 mm.

- Augmentin was more effective against E. coli and least effective against Pseudomonas.
- Tarivid was more effective against Klebsiella and least against E. coli.
- Streptomycin was more effective against pseudomonas and least with klebsiella.
- Gentamycin was more effective against pseudomonas and least against Klebsiella.
- Pefloxacin was more effective against E. coli and no effect at all on pseudomonas
- Septrin is more effective against klebsiella and least against Pseudomonas.
- Amoxicillin is more effective against Pseudomonas and no effect at all on E. coli
- Chloramphenicol had no effect on the isolated bacteria.
- Sparfloxacin was more effective against E. coli and no effect on Klebsiella
- Ciprofloxacin had more effect on E. coli and least on Pseudomonas.

Table 3 Shows the mean effect of Ten antibiotics tested against the isolated gram negative bacteria.

Antibiotic conc. In Ug		Mean	± Standard deviation
AU	25	21.33 a	2.75
OFX	30	20.67 b	4.07
S	30	14.33 c	3.05
CN	10	18.00 d	6.08
PEF	10	10.00 e	8.89
SXT	30	15.33 f	1.89
AM	130	12.90 a	11.23
CH	30	0.00 u	0.00
SP	10	11.00 l	9.57
CPX	10	25.33 j	1.04

Discussion

The results of this study revealed that *E. coli* 12 (30%) was the most predominant organism causing UTI among pregnant women followed by *Pseudomonas aeruginosa* 9 (22.2%) and *Klebsiella pneumonia* 7 (17.5%) being the least isolated. The dominance of *E. coli* over *Klebsiella pneumonia* in this study is however in contrast with the findings of Omonigho et al., who found *K. pneumonia* to be more prevalent compared to *E. coli* in UTI. Aiyegoro et al., also reported in their study high incidence of *E. coli* in UTIs [29]. Previous research report of Getachew et al. which screened midstream urine samples from pregnant women implicated *E.*

E. coli as the most predominant bacteria. Other research findings in similar studies from other parts of Nigeria and different parts of the world have also reported *E. coli* as the most frequently occurring bacteria in UTI from pregnant women which corroborates our findings reported high incidence of UTI from Maiduguri, Nigeria in both pregnant and non pregnant women with *E. coli* as the most common agent.

The dominance of gram-negative bacteria in UTI could be attributed to an increase in the levels of amino acids and lactose during pregnancy which particularly encourages the growth of *E. coli*. It could also be due to infection by means of faecal contamination owing to poor hygiene [11]. Urinary tract infections are widely spread infections that could be seen in hospitals and the second most common infection seen in the broad-spectrum population [30].

UTI occurrence is irrespective of age and gender. It is nevertheless more recurrent in women, and pregnancy aggravates the hazard of UTI in women due to a number of versatile observations all through the pregnancy, amplified progesterone and oestrogen levels normally leads to a decrease in urethral and bladder volume. Increased plasma volume at some stage in pregnancy has led to a decrease in urine concentration and improved bladder volume. The recipe of these factors normally leads to urinary stasis [31]. Umeh et al. reported that bacteriuria infection in women from Makurdi, Benue State was 5 times higher in women compared to men.

Resistance of pathogenic microorganisms isolated from urine to antibiotics is reportedly on the increase globally. The result of the antibiotic susceptibility tests in the present study showed that the isolated gram negative bacteria showed the highest susceptibility to ciprofloxacin. This corroborates the report of other studies that ciprofloxacin, a member of the fluoroquinolones is effective against a wide range of organisms [32]. Augmentin inhibited the three isolates

with the following zones, *E. coli* (24.50mm) *Pseudomonas aeruginosa* (19.50mm) and *Klebsiella pneumonia* (20.00 mm). The isolates were also susceptible to tetracycline, streptomycin, septrin and gentamycin; an aminoglycoside antibiotic that worked by fastening to a subunit of the bacteria ribosome that interrupts with protein production thus preventing bacteria from carrying out fundamental functions necessary for survival. Gentamicin is used for severe UTIs but it possibly will entail severe side effects such as damage to hearing, sense of stability and kidneys. The uppermost efficiency of gentamicin in the cure of UTIs has been reported by Al-Sweih et al., [33].

Previous research results showed varying degrees of sensitivity of bacteria isolates; *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* etc to antibiotics such as ciprofloxacin, ofloxacin, augmentin, amoxicillin, erythromycin and gentamicin among others. MDR was observed in 100 % of bacteria isolates which included *E.*

coli, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* from pregnant women in a related study. All the isolates in the present study however resisted chloramphenicol, (0.00); *Pseudomonas aeruginosa* was resistant to perfloracin, *E. coli* was resistant to amoxicillin, and *Klebsiella pneumonia* was resistant to sparfloracin.

The present study corroborates previous research reports on the rising cases of UTI in pregnant women and increasing resistance of uropathogens to a wide range of antibiotics. We therefore, advocate for antibiotic susceptibility test prior to treatment of positive cases, periodic surveillance of established and incident cases of multidrug resistance and the search for alternative drugs.

Ethical Considerations

We sought and obtained ethical clearance from Benue State Hospital Management Board and the Hospital ethical committee before the study commenced. The Patients consent were also obtained after due explanations on the basis of the study [34,35].

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