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Prevalence of *Escherichia coli* in urinary tract infection among patients attending Umaru Sanda Ndayako General Hospital Bida, Niger State, Nigeria.

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Abstract
This study was carried out to investigate the prevalence of *Escherichia coli* in urinary tract infection among the patients attending Umaru Sanda Ndayako General Hospital, Bida. Samples were collected and diagnosed within the period of two months, from May- July, 2013. Growth on MacConkey agar was sub-cultured on eosin methylene blue for identification of colonial characteristics of *E. coli* and other associated microorganisms.One hundred (100) urine samples werescreened and 83 samples had significant bacteriuria. *Escherichia coli*, after standard laboratory urine culture using MacConkey agar and Eosine methylene blue coupled with biochemical tests such as Indole, Methyl red- Vogesproskauer test, and citrate utilization were used as confirmatory tests accounts for 36(40%).Out of the 36(43%) positive *E.coli* isolated 29(80%) were sensitive to ciprofloxacin, Trimethoprim, chloramphenicol and nitrofurantion. Apart from *E. coli*, other microorganism sensitive to these antibiotics include, *Enterobacter* sp, *Proteus mirabilis* and *Klebsiella* sp. 7(19.4%) of *E .coli* , 5(50%) of proteus mirabilis and 3(33%) *Klebsiella* sp were resistant to ceporex, Amoxicilline and Tetracycline. Forty-nine (49) (59%) positive cases of UTI were for female and male patient had 34(40%). During this research, middle age women from 21-30 (26.5%) and 31-40(18%) were found to possess the highest prevalence of UTI. The result of this study indicated that *E. coli* is the most prevalent organism responsible for UTI due to the possession of some virulent associated properties which enable them to adhere to the urinary tract. Appropriate measures should be taken to prevent further spread of this infection most especially in women and children.

Key words: Prevalence, *Escherichia coli*, Urinary, Infection

Introduction

Urinary Tract Infections (UTIs) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is perhaps the single most common bacterial infection of mankind (Morgan and McKenzie, 1993). Urinary tract includes the organs that collect and store urine and release it from the body which includes kidneys, ureters, bladder and urethra. *Escherichia coli* causes about 75 - 90% of uncomplicated UTIs (Karen *et al*., 2006), whereas *Staphylococcus saprophyticus* causes an estimated 5 - 15% of UTIs frequently in younger women (Michael *et al.*, 2007). *Enterococcus* and other Gram negative rods other than *E. coli* have also been implicated in

some cases (Benjamin *et al*., 2009). Significant bacteria is defined as the persistent isolation of > 10^5 colony forming units (cfu) of bacteria per ml of clean voided, mid-stream urine specimens plated within six hours of collection. In females, it is possible that slow growing microaerophiles, such as *Lactobacillussp, Corynebacteriumsp* and *Streptococcus mileri*may be involved in the pathogenesis of urinary tract infections. Symptoms are usually precipitated by sexual intercourse (Michael *et al*., 2007). UTI's occur in both acute and chronic forms. In the former, patients complain of severe and low back pain that may associate with fever due to the associated bacteraemia, while in the latter, a sensation of perennial fullness is felt. The common causative agent is *E. coli*,but *micrococcal*infections may account for up to 10-20% of cases in sexually active women.

UTI's are among the most common bacterial infections in humans, both in the community and hospital settings and have been reported in all age groups in both sexes.

It is a serious health problem, affecting millions of people each year and is the leading cause of Gram-negative bacteria called *Escherichia coli*.UTI accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria in particular.*Escherichia coli* remain the most frequent cause of UTI, although the distribution of pathogens that causes UTI is both males and females of any age, with bacterial counts as low as 10^6 colony forming units (CFU) per millimeter in urine (Akinyemi*et al*., 1997). This is common in patients with symptoms of acute urethral syndrome, males with chronic prostatitis and patients with indwelling catheters (Karen *et al*., 2006). Females are however believed to be more affected than males except at the extremes of life (Smith *et al*., 2003). Recently published studies have added to the body of knowledge concerning the pathogenesis, diagnosis and management of UTI's (Michael *et al*., 2007). Usually, a UTI is caused by bacteria that can also live in the digestive tract, in the vagina, or around the urethra, which is at the entrance to the urinary tract. Most often these bacteria enter the urethra and travel to the bladder and kidneys. Usually, the body removes the bacteria, and shows no symptoms. The signs and symptoms include burning feeling during urination, frequent or intense urges to urinate, even when one have little urine to pass, pains in the back or lower abdomen, cloudy, dark, bloody, or unusual-smelling urine, fever or chills (Njoku *et al*., 2005).

Women tend to have UTIs more often than men, because, bacteria can reach the bladder more easily in women. This is partially due to the short and wider female urethra and its proximity to anus. Bacteria from the rectum can easily travel up the urethra and cause infections (Walls *et al.*, 2011). Moreover, the main factors predisposing married women to bacteriuria are pregnancy and sexual intercourse (Nicolle 2008). Sexual activity increases the chances of bacterial contamination of female urethra. Having intercourse, may also cause UTIs in women, because, bacteria can be pushed into the urethra. This anatomical relationship of the female urethra to 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 (Kolawole *et al*., 2009). Using a diaphragm can also lead to UTIs because diaphragms push against the urethra and make it more difficult to completely empty the bladder. The urine that stays in the bladder is more likely to allow growth of bacteria and cause infections (Njoku *et al.*, 2005).

However, the importance of coliform bacilli in UTI among pregnant women has long been known in developed countries. Health care practitioners regularly have to make decisions.about prescription of antibiotics for urinary tract infections. UTI is the second most common clinical indication for empirical antimicrobial treatment in primary and secondary care, and urine samples constitute the largest single category of specimens examined in most medical microbiology laboratories (Morgan and McKenzie, 1993). UTI is

challenging, not only because of the large number of infections that occur each year, but also because the diagnosis of UTI is not always straight forward (Kolawole *et al*., 2009). Criteria for the diagnosis of UTI vary greatly depending on the patients and context. According to Ronald*et al*.(2001), there is not one best way of performing urine cultures. Guidelines for the diagnosis of UTI includes the use of sheep blood agar and either MacConkey agar or a similar selective medium for routine urine culture. The plates should be incubated overnight (at least 16 hours) at 37°C in ambient air; alternatively, the blood agar plate can be incubated in elevated (3–8%) CO_2 (Clarridge *et al*., 1988). For fastidious microorganisms, chocolate agar can be added to the MacConkey agar and the plates incubated in 5% Co_2 for 2 days (Clarridge *et al*., 1988). Co_2 can play a role in the growth of microorganisms for instance, *E. coli* as a substrate for carboxylation reactions (Kozliak *et al*., 1995). There is considerable evidence of practice variation in use of diagnostic tests, interpretation of signs or symptoms and initiation of antibiotic treatment such as drug selection, dose, duration and route of administration. For patients with symptoms of UTI and bacteriuria, the main aim of treatment is to get rid of infectious bacteria, most especially *E.coli* causing the symptoms. Secondary outcomes are adverse effects of treatment or recurrence of symptoms.

Materials and Methods

Sample collection

A total of 100 urine samples were collected randomly from in and out patients attending Umaru Sanda Ndayako General Hospital, Bida. Questionnaires were administered to determine the age of the patients before each sample was collected, Clean catch urine (early morning or midstream urine) was collected, following the laboratory rules of fresh urine sample collection, The female patients were advised to clean their vaginal area properly with water, then dry the surface with sterile cotton wool, then held the vaginal labial apart before passing the urine into the container, while male patients were also advised to washs their hand properly before holding their penis to pass the urine in the container. The containers were labeled with patient's name, date of sample collection, patient's hospital number and time of collection.

Test for the presence of microorganism in urine (bacteriuria) using combi 9 test strips.

One of the Biochemical tests which is helpful in investigating UTI include, Nitrate detection in urine using reagent test strip known as combi urine (9), which consists of nine(9) biochemical molecules including nitrate.Urine sample were collected in a dry sterile containers, the test strip was removed carefully from the container, and dip briefly in the urine, making sure that the test area is fully immersed inside the urine .Excess urine was removed from the strip by running the edge of the strip along the rim of the urine container. The test strip was allowed to stand for 30secs which was read carefully in a good light. The reading was carried out by holding the strip close to the color chart on the container label. Color change from yellow to any other color in the container means positive. (Cheesbrough, 2000).

Microscopic Examination of Urine.

Urine samples were centrifuged and the deposits were examined microscopically for the presence ofsignificantpuria, i.e. WBCs in excess of 10cells/cfu of urine, Red blood cells, Cast, Yeast and Bacteria (Provided the urine was freshly collected). Exactly 10ml of a well-mixed urine sample was aseptically transferred to a well labeled test tube; Centrifuged at 500g for 5minute, the supernatant was poured into another container. The sediment was remixed by tapping the bottom of the bottle; 1 drop of this well mixed sediment was then transferred to a slide which was covered with a cover slip. The prepared slide was mounted on the microscope which was examined using x10 andx40 objectives with the condenser iris closed sufficiently to the slide in other to give a good contrast (Cheesbrough, 2000).

Isolation of Bacteria from the urine sample

Culturing of the urine sample: Two different media were used to culture the urine specimen i.e. MacCkonkey agar and Eosin methylene blue agar.A sterileinoculating loop was used to inoculate the media with the urine specimen, which was properly mixed by rotating before inoculation. The urine sample was then streaked on the surface of themacConkey agar which was spread evenly in other to obtain distinct colony. The plates were then incubated aerobically overnight at 37^0C for 24hours. Distinct colonies were sub- cultured on eosin methylene blue for selective isolation of *E.coli*, then incubated for another 24hours at 37^0C. The plates were observed for cultural characteristics of *E.coli*. before subjecting the isolates to the following biochemical tests for proper identification: Gram Staining, Indole test, Methyle red/Vogesproskauer test, Citrate utilization test, Catalase and coagulase tests.

Antibiotic Sensitivity Testing

Sterile nutrient agar plates were prepared and standardized as instructed by the manufacturer. It was poured into petridishes and allowed to solidify. The inoculum was prepared in a sterile tube in conformity to 0.5 McFarland's standard. The prepared Nutrient agar plates were inoculated with the test isolates using a sterile swab stick. The antibiotic discs were impregnated into the inoculated plates using a sterile forceps. The plates were incubated at 37^0C for 24hours. Zones of inhibition were measured to the nearest mm. (Cheesbrough, 2000).

Results

The prevalence of bacteria isolated from patients with UTI is as shown in Table 1. *Escherichia coli* with thirty-six (36) isolates have the highest percentage prevalence of 43.6, while *Staphylococcus aureus* and *Enterobacter* sp. with seven (7) isolates each has the least percentage prevalence. Pure cultures of the bacterial isolates from the patients' were subjected to different biochemical tests as reflected in table 2 for proper identification. It is on this basis that the true identity of the different bacteria isolated from the patients' with UTI was carried out. The result of antibiotic susceptibility testing in table 3 showed that out of the 36(43%) positive *E. coli* isolated, 29(81%) were sensitive to ciprofloxacin, Trimethoprim chloramphenicol and nitrofurantion. Apart from *E. coli*, other bacteria sensitive to these antibiotics include, *Enterobacter* sp, *Proteus mirabilis* and *klebsiella* sp. Seven (7) (19.4%) of *E. coli* , 5(50%) of *Proteus mirabilis* and 3(33%) *Klebsiella* sp were resistance to ceporex, Amoxicillin and Tetracycline.

The distribution of bacteria among male and female UTI patients' indicated *E. coli* as the dominant bacteria isolated from all age groups. However, the female category of the UTI patients' has more isolates of *E. coli* than their male counterparts, with the highest figure recorded between 31-40 age brackets. The percentage prevalence of UTI was found out to be 49 among females and 36 among males.The highest positive Bacteriuria were however found among the adult women, those of which were of 21-30 (16.8%) age bracket, closely followed by those between 31-40(13.3%) age bracket. The least positive Bacteriuria occurred among the males of age bracket 1-10(3.6%).

Table 1. Prevalence of Bacteria Isolated From UTIs In Umaru Sanda Ndayako General Hospital,Bida.

Bacteria	Total Number	Percentage
Escherichia coli	36	43.4
Staphylococcus aureus	07	8.4
Streptococcus sp	08	9.6
Klebsiella sp	15	18.2
Enterobacter sp	07	8.4
Proteus mirabilis	10	12.0
Total	83	100

Table 2: Biochemical Characterization of the isolates

Gramstain	Morphology	Indole	MR	VP	Citrate utilisation	Catalase	Coagulase	Organisms isolated
-	R	+	+	-	-	+	-	*E. coli*
+	C	-	-	-	-	+	+	*Staphylococcus aureus*
+	C	-	-	-	+	-	-	*Streptococcus*sp
-	R	-	-	+	+	-	-	*Enterobacter*sp
-	R	-	-	-	+	-	-	*Proteus mirabilis*
-	R	-	-	+	+	-	-	*Klebsiella*sp

Key:
+ Positive, - Negative, + R = Positive rod, + C = Positive cocci, *S.aureus* = *Staphylococcus aureus*, - R= Negative rod, - C = Negative cocci, *Strept*sp. = *Streptococcus specie*

Table 3: Percentage Susceptibility of the isolates to the antibiotics.

Antibiotics	***E. coli*** **N=36**	***S. aureus*** **N = 7**	***Streptococcus* sp** **N = 8**	***Proteus mirabilis*** N = 9	***Klebsiella* sp** **N = 6**	***Enterobacter* sp** **N = 7**
STR	72.8	39.9	12.2	10.1	18.2	10.8
CPX	80.9	0.0	90.1	29.1	0.0	0.0
TMP	98.2	81.1	80.0	30.3	30.3	99.9
CHL	90.2	70.2	70.0	12.2	67.6	100.0
PEN	0.0	11.0	12.1	0.0	0.0	0.8
TET	39.6	40.0	40.1	70.3	40.2	98.0
NIT	96.9	0.0	0.0	0.0	0.0	70.1
SEP	18.8	22.3	14.3	70.0	20.0	0.0
AMX	0.0	17.2	10.0	0.0	0.0	12.0
CEP	20.0	60.1	0.0	10.0	40.1	100

Key:
STR = Streptomycin
NIT = Nitrofurantion
SEP = Septrin
CHL = Chloramphenicol
PEN = Penicillin
0.0 = Not sensitive
CEP = Ceporex
AMX = Amoxycillin
TMP = Trimethoprim
CPX = Ciprofloxacin
TET = Tetracycline

Table 4: Distribution of Bacteria isolated from male and female patients with UTIs

AGE	***E. coli***		***S. aureus***		***Enterobacter* sp**		***Klebsiella* sp**		***Streptococcus* sp**		***Proteus mirabilis***	
	M	F	M	F	M	F	M	F	M	F	M	F
1- 10	1	3	-	-	-	-	-	-	-	-	-	-
11- 20	2	4	1	-	-	-	-	-	-	1	-	1
21- 30	1	5	2	1	1	3	1	-	2	-	1	-
31- 40	2	9	1	1	-	-	-	1	-	1	1	-
41- 50	2	4	-	-	-	-	1	2	1	-	2	1
>50	3	2	-	-	-	-	-	3	2	1	1	-

Key: M= Male F= Female

Table 5: Prevalence of Positve Bacteriuria Among Different Age Groups of UTI Patients' In Umaru Sanda Ndayako, General Hospital, Bida.

Age group	Samples Collected	Positive result N0	UTI(s) (%)	Male prevalence No	(%)	Female prevalence No	(%)
1-10	12	8	9.61	3	8.82	5	10.2
11-20	16	14	16.9	7	20.5	7	14.3
21-30	30	22	26.5	8	23.5	14	28.6
31-40	20	18	21.6	7	20.5	11	22.4
41-50	12	11	13.3	5	14.7	7	14.3
≥50	10	10	12.0	6	17.6	5	10.2
Total	100	83	100	36	100	49	100

Discussion

This study implicated Seven (7) microorganisms as possible etiological agents of the UTI cases observed. These organisms include *Escherichia coli, Proteus* sp, *Klebsiella* sp, *Proteus mirabilis. Staphylococcus* sp., *Streptococcus* sp. *Enterobacter* sp and *Candida albicans. C. albicans* is a fungal isolated from UTI.This result is in line with the earlier report of Ronald *et al.* (2001) and Nicolle, (2008), who reported that UTI may be due to Fungal or bacterial infection. *E.coli* and other associated bacteria such as *Staphylococcus saprophyticus*, *Klebsiella, Proteus*, and *Enterobater* spp are the most common causative agents of UTI's. National committee for clinical laboratory standard in USA (2000) reported that all these microorganism are common and typically related to the abnomalites of urinary tract infection. Barisc *et al.*(2009) reported that UTI's due to *Staphylococcus aureus* typically occur as a result of secondary blood born infection. *Escherichia coli* is the most common organism isolated with the incidence of 36(43.4%) in this research study, this agrees with the study of Ronald *et al (*2005), in his study on UTI, he stated that *E. coli* causes 80 to 85% of UTI's cases.This study is also in line with the earlier report by (Leffer and Svanborg, 2005) that isolated *E.coli* with the highest prevalence of microorganism responsible for UTIs.Walls *et al.* (2011) reported *Escherichia coli* (*E. coli*) as the major etiological agent causing UTI, which accounts for up to 90% of UTI cases. The higher prevalence of *E. coli 36*(43.4%) reported in this study may be due to faecal contamination and other virulent factors which enable them to adhere to the bladder wall. It was observed that the susceptibility of the isolates to the ten antibiotics differs with the species. Out of the 36(43%) positive *E. coli* isolated, 29(80%) were sensitive to ciprofloxacin, Trimethoprim, chloramphenicol and nitrofurantion, apart from *E. coli*, other microorganisms sensitive to these antibiotics include, *Enterobacter*, *proteus mirabilis* and *Klebsiella spp*.This agrees with the work of Ronald *et al*.(2001) and Cubeddu *et al.* (2004). whostated that frequently used of antibiotics such as nitrofurantion and trimethoprim can serve as agents that reduces bladder infection due to low acidity produce by these antibiotics. Oral antibiotics such as trimethoprim/Chloramphenicol,cephalosporinan dnitrofurantoin,substantially shorten the time to recovery with all being equally effective (Lane and Takhar, 2011). A three-day treatment with trimethoprim, TMP/SMX, or a fluoroquinolone is usually sufficient, whereas nitrofurantoin requires 5–7 days. With treatment, symptoms should reduce within 36 hours (Nicolle, 2008). Earlier researches also revealed that the success of ciprofloxacin could be due to its broad

spectrum activities, its bactericidal activity on organisms both in replicating and resting state and its ability to disrupt DNA functions leading to the death of the bacterium (Vorland, 2001). This result is in contrary to previous reports on some antibiotics where most isolates were revealed to be sensitive to Chloramphenicol, which could be due to development of new metabolic pathway by the microoganisms.

Resistance to antimicrobial agents has been noted since the first use of these agents and is an increasing world-wide problem (Bhat, 2000). This study revealed that 7(19.4%) of *E. coli*, 5(50%) of *proteus mirabilis* and 3(33%) *Klebsiella* spp were resistance to ceporex, Amoxicilline and Tetracycline. This is in with line the findings reported by Micheal *et al.* (2007),that 30% of *E. coli* and *Proteus* spisolates were resistant to ceporex and Amoxicillin. This is of great importance and implies that these antibiotics cannot be used as empirical therapy for urinary tract infection particularly in the study area. A comparative rate of resistance to these antibiotics has been reported for these drugs in previous studies done in Ethiopia (Benjamin *et al*., 2009), in Kosovo (Leung , 2010), in Iran and in South Korea (Barisc *et al.*, 2009). Low resistance was observed for these drugs because they are not easily accessible and relatively expensive in price compared to others.

Table five (5) gives a demographic situation of positive UTI cases examined by age groups. It was observed that majority of the positive cases fall between age 21-30 years for both male and female patients, taking about 38 (50%)of the total sample collected, population falls within this bracket inferring that most of the patients within this age group are sexually active. Sexual activity enhances better transmission of UTI especially in this age range, and in females, who usually have higher prevalence of UTI than males (Nicolle, 2002). This result is in conformity with the statement of Ronald *et al.* (2001), in young sexually active women, sexual activity is the cause of 75–90% of bladder infections, with the risk of infection related to the frequency of sex. The term "honeymoon cystitis" has been applied to this phenomenon of frequent UTIs during early marriage. As revealed in this study, women of different age group were observed to be very much prone to UTIs, out of 100 samples screened, women account for about 56% positive cases of UTIs, where men take about 39% only. The highest positive numbers were found among the adult women, those of which were 21-30 (16.8%) years old and those 31-40 (23.4%) years. In support of this finding, Health care providers each year reported that women are more prone to UTIs than men due to some anatomical reasons (Nicolle, 2008). One of these reasons is that women urethras are shorter than that of men, therefore allowing bacteria to strive easily to the bladder. National committee for clinical laboratory standard of USA (2000) reported thatWomen are more prone to UTIs than men because, in females, the urethra is much shorter and closer to the anus (Leung *et al.*, 2010). As a woman, the estrogen levels decrease with menopause therefore increase the risk of urinary tract infections and increases due to the loss of protective vaginal flora.

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