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BACTERIA ASSOCIATED WITH URINARY TRACT INFECTIONS AND THEIR SUSCEPTIBILITY PATTERN TO ROUTINE ANTIBIOTICS.

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ABSTRACT

Forty samples of early morning urine were collected in the clinical pathology laboratory of a Military Reference Hospital, Kaduna, and analyzed for the isolation of bacteria associated with Urinary Tract Infection using standard bacteriological methods. The prevalence of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus and Saprophyticus sp* isolates from the urine samples were 48.49%, 21.21%, 15.15%, 9.09% and 6.06% respectively. The pattern of antibiotic sensitivity test varied with the concentration of the antibiotic used. All the isolates were sensitive to ciprofloxacin (5MCG) and resistant to amoxicillin (30MCG). Ciprofloxacin appeared to be the drug of choice for the treatment of urinary tract infection from this study.

Keywords: *Bacteria, Urinary tract, Infection, Susceptibility, Routine Antibiotics, Metropolis*

INTRODUCTION

The urinary tract is comprised of the kidneys, bladder,ureters and urethra. The Urinary Tract Infections (UTI) are caused by pathogenic organisms in any of the structures. Many other infections such as arthritis(urethral infection), cystitis (bladder infection), ureters infection and pyelonephritis (kidney infection) and other structures that eventually connect to or share close anatomical proximity to the urinary tract (for example, prostate,vagina and epididymis) are sometimes included in the discussion of UTIs. Urinary tract infection caused by bacteria affects any part of the urinary tract (Kunin, 2006). Urine contains a variety of fluids, salts and waste products and does not have bacteria in it. The entrance of bacteria into the bladder or kidney and its subsequent multiplication results to UTI. Cystitis and pyelonephritis can be symptomatic or asymptomatic, complicated or uncomplicated and sporadic or recurrent. The UTI symptoms and signs may vary according to age, sex and location of the infection in the tract. Some individuals will have *no* symptoms or mild symptoms and may clear the infections in about two to five days. Many people will not spontaneously clear the infection, some of the most frequent sign and symptoms experienced by most patients is a frequent urge to urinate, accompanied by pain or burning sensation on urination. The urine often appears cloudy and occasionally reddish if blood is present. The urine may develop an unpleasant odour. Women often have lower abdominal discomfort or feel bloated and experience sensation like the bladder in full. Women may also complain of a vagina discharge, especially if their urethra is infected. Men may complain of dysuria, frequency and urgency. Other symptoms may include rectal, testicular, penile or abdominal pain. Toddlers and children with UTIs often show blood in the urine, abdominal pain, fever and vomiting along with pain and urgency with urination. Location of the infection in the urinary tract usually gives certain symptoms. Urethral infection usually have dysuria

(pain or discomfort when urinating). Cystitis (bladder infection) symptoms include suprapubic pain usually without fever or flank pain. Ureter or kidney infections often have flank pain and fever as symptoms. Bacteriuria is acquired by the faecal- genital route, often via periurethral colonization in women. With the exception of patients who have rectovesical fistulas or other abnormal communications between the bladder and the intestine or vagina, anaerobic bacteria do not cause bacteriuria. The most common organism causing bacteriuria are: *Echerichia coli, Stapylococcus saprophyticus, Klebsiella* spp, *Enterobacter* spp, *Enterococcus* spp*, Proteus* spp*, Pseudomonas* spp*, Morganella morganii* and other organisms. Urinary tract infection is the common cause of medical consultations in both general and hospital practice (Nicholson, 1989). In the United Kingdom, Meers (1985) reported that 30.3% of all hospital acquired infections were urinary tract infections and 8.6% of the patients found to be catheterized during the study period, 21.2% had urinary tract infections compared with a rate of 2.9% in the non-catheterized patients population. Also, within the Kaduna metropolis alone, urinary tract infections had the highest percentage occurrence of (58.8%) among the pregnant women studied which gives cause for concern, as they are prone to several complication in labor as well as risk to the foetus (Olusi, *et al.*, 2004). Some of these have been observed to bring about miscarriages, prevent future conception and may cause blindness of the newborn (Olusi, *et al*., 2004). Urinary tract infection can be treated with antibiotics, however, these organisms are becoming resistant to the commonly used antibiotics such as the β-lactam antibiotics. It has been estimated that over 50% of the antibiotics prescriptions in hospitals are given without clear evidence of infection or adequate medical indication. Frequently, antibiotics are prescribed without culturing and identifying the pathogens or the bacterial sensitivity testing. Self medication may further increase the prevalence of drug resistant strains.

MATERIALS AND METHODS

Sample Collection

Forty clean catch mid stream urine samples were collected in sterile universal bottles. The samples were obtained from patients suspected to have urinary tract infections in the clinical pathology laboratory of the Reference Hospital in Kaduna and were analyzed in the Microbiology laboratory, Kaduna State University, Kaduna.

Media Preparation

The media (nutrient agar and MacConkey agar powder) used were prepared according to the manufacturers specification.

Isolation of Microorganisms

A loopful of clean catch mid-stream urine collected in sterile universal containers were aseptically streaked on the surface of the solidified Nutrient and MacConkey agar media respectively for isolation of the implicating agents. The plates were incubated at 37^{O}C for 24 hours in an incubator. After the incubation period, colonies on each plate were picked and sub-cultured on Nutrient agar slant, incubated at 37^{O}C for 24 hours. All tests were carried out following standard procedures (Prescott *et al*., 1999).

Gram Staining
All the microorganisms isolated were Gram stained according to the methods of Prescott *et al* (1999).

Biochemical Identification of the Isolates
The following biochemical tests were carried out for the identification of the suspected isolates according to the procedures of Odewumi (1981). These were: Coagulase, Indole, Catalase, Urease, Methyl red, Voges Proskauer, Citrate and Triple Sugar Iron Tests (TSI)

Motility Test
A fine slab about one to two centimeters from the bottom was made with the test organism on the motility medium. It was incubated at 37^{O}C for 24 hours. A cloudy medium only along the line of inoculation is negative, while a cloudy medium spread away from the line of inoculation is positive.

Gas Formation
This was determined by the appearance of one or several bubbles

Formation of Hydrogen Sulphide:
This was determined by the blackening of the whole butt or a streaked ring of blackening at the slant junction.

Sugar Fermentation
The absence of sugar fermentation was indicated by the absence of change in the TSI medium and it was represented as K/K meaning Alkaline/Alkaline or K/KG meaning Alkaline / Alkaline (gas) indicating the formation of gas.
A positive result was indicated by either A/A or K/A which represented Acid/Acid or Alkaline/Acid respectively. It was also noted that A/A indicated that the sample organisms were able to ferment their glucose and lactose or glucose and sucrose or glucose, lactose and sucrose, while K/A indicated that only glucose was fermented. The presence of gas formation was indicated as A/AG or K/AG.

Antibiotic Sensitivity Test
Antibiotic sensitivity test was carried out using Bauer-Kirby's (1996) method. Using a sterile wire loop, a colony of the isolates were streaked all over the surface of each nutrient agar plate and allowed to stand for few minutes at room temperature. The antibiotic multi –disc (oxoid) were then placed on the surface of the inoculated plates using a forceps and gently pressed. The plates were incubated at 37^{O}C for 18-24 hours. The diameters of zone of inhibition were measured in millimeters and the isolates were scored as sensitive or resistant to the antibiotics by comparing with values recommended by standard charts in this method above.

RESULTS AND DISCUSSION

Table 1. Shows the prevalence of Bacteria isolated from Urine samples in cases of UTI. Thirty three bacteria were isolated (33 out of the 40 samples investigated) and the frequency of isolation was very high (100%) when compared to the results (21%) obtained by Gleemon *et al.*, (1981) in Bangladesh and 20% by Mbakwem and Ene (2006) in Port Harcourt Nigeria. This disparity may be due to differences in the standard of living of the patients and possible due to an allergy to latex condoms, oral contraceptives, use of urinary catheters and vigorous sexual intercourse with a new partner may increase the risk of UTI. The isolation of these organisms is not surprising because they are part of the normal microbial flora of humans & can easily gain entry into the body and cause infections as opportunistic pathogens (Admokaya and Adebolu, 2005). Table 2. Shows the sex distribution and prevalence of UTI among patients. The occurrence of UTI was found to be statistically significant in females (66.7%) than in males (33.33%) when compared with the findings obtained by Gleemon et al., (1988) and Jeena *et al.*, (1996) suggested that anatomical differences between male and female such as shortness of the female urethra and its exit (or entry for pathogens) is close to the anus and vagina. There are reports that women who use diaphragm or who have sex partners that use condoms with spermicidal foam are at increased risk for UTIs. Other factors including improper cleaning of the perineum, the use of napkins, sanitary towels and female who become sexually active seem to have higher risk of UTIs; some investigators term these UTIs as "honeymoon cystitis"(medicineNet.com). Patients with chronic diseases such as diabetics or those who are immunosuppressed (HIV or cancer patients) are at higher risk of UTIs. Table 3. Shows the result of the biochemical identification of the bacteria isolated from cases of UTI. Table 4. Shows the number of sensitive, resistant and mean zone of inhibition of *E.coli, Klebsiella* and *Proteus* strains to individual antibiotics. Table 5. Indicates number of sensitive, resistant and mean zone of inhibition of *Staph. aureus & Staph. saprophyticus* to individual antibiotics .

TABLE 1: Prevalence of Bacteria Isolated from Urine Samples in Cases of UTI.

Organisms	No. of Isolates	Prevalence (%)
Escherichia coli	16	48.49
Klebsiella pneumonia	7	21.21
Proteus mirabilis	5	15.15
Staphylococcus aureus	3	9.09
Staph. saprophyticus	2	6.06
TOTAL	33	100%

TABLE 2: Sex Distribution and Prevalence of UTI among Patients

Sex	Number Examined	Number Infected	Percentage Infection
Male	15	11	33.33
Female	25	22	66.67
TOTAL	40	33	100.00

TABLE 3: Results of Biochemical Identification of the Bacteria Isolated from cases of UTI

Morphology and Gram staining reactions	Catalase Test	Coagulase Test	Indole Test	Methyl Test	Citrate Test	Voges Proskauer Test	TSI Test	Urease Test	Motility Test	Organisms
Negative rod	_	_	+	+	_	_	A/AG	_	+	*E.coli*
Negative rod	_	_	_	_	+	+	A/AG	_	_	*Klebsiella* spp
Negative rod	_	_	_	+	_	_	K/A	+	+	*Proteus* spp
Positive cocci	+	+	_	_	_	_	K/K	_	_	*Staphylococcus aureus*
Positive cocci	+	_	_	_	_	_	K/K	+	_	*Staph. Saprophyticus*

KEY:

A/AG indicates both slant and bottom yellow with gas production
K/A Indicates slant red and bottom yellow
K/K Indicates none of the three sugars is fermented (glucose, lactose and sucrose)
TSI means triple ion
+ means positive reaction
_ means negative reaction

TABLE 4: Number of Sensitive, Resistant and mean zone of Inhibition of *E.coli, Klebsiella* and *Proteus* strains to individual antibiotics

Antibiotics	No. of Sensitive			No. of Resistant			% Sensitive			% Resistant			Mean zone of inhibition(in millimeters)		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	A	B	C	A	B	C
Nitrofurantoin(N) (100MCG)	4	2	0	12	5	5	25	28.6	0	75	71.4	100	14	13	-
Ciprofloxacin(CIP) (5MCG)	8	6	5	8	1	0	50	85.7	100	50	14.3	0	21	20	17
Tetracycline (TE) (50MCG)	10	2	0	6	5	5	62.5	28.6	0	37	71.4	1oo	23	15	-
Norfloxacin (NB) (10MCG)	4	2	2	12	5	3	25	28.6	40	75	71.4	60	16	16	17
Amoxycillin (AX) (20MCG)	2	0	0	14	7	5	12.5	0	0	87.5	100	100	8	-	-
Oflaxacin (OF) (5MCG)	8	2	2	8	5	3	50	28.6	40	50	71.4	60	18	22	16
Chloramphenicol (C) (30MCG)	2	2	0	14	5	5	12.5	28.6	0	87.5	71.4	100	10	17	-
Cefuroxime (CF) (30MCG)	0	0	0	16	7	5	0	0	0	100	100	100	17	-	-
Gentamycin (GN) (25 MCG)	8	0	0	8	7	5	50	0	0	50	100	100	12	19	-
Ampicillin (AM) (10 MCG)	2	4	2	14	3	3	12.5	57.1	40	87.5	42.9	60	10	12	17

KEY:
= No zone of inhibition
A = *E.coli*
B = *Klebsiella pneumonia*
C = *Proteus mirabilis*

TABLE 5: Number of sensitive, Resistant and mean zone of inhibition of *S.aureus & saprophyticus* to individual antibiotics

Antibiotics	No. Sensitive		No. Resistant		% Sensitive		% Resistant		MZH(in millimeters)	
	D	E	D	E	D	E	D	E	D	E
Streptomycin (30MCG)	3	2	0	0	100	100	0	0	28-	29
Ciprofloxacin (25MCG)	3	2	0	0	100	100	0	0	24	28
Erythromycin (10MCG)	3	2	0	0	100	100	0	O	24	24
Amoxicillin (30MCG)	1	1	2	1	33	50	66.7	50	14	19
Septrin (30MCG)	1	0	2	2	33	0	66.7	100	15	-
Pefloxacine (10MCG)	3	2	0	0	100	100	0	0	19	19
Gentamycin (25MCG)	3	1	0	1	100	50	0	50	19	15
Zinacef (20MCG)	2	2	1	0	66.7	100	33	0	18	23
Ampiclox(30MCG)	0	1	3	1	0	50	100	50	-	11
Rocephin (25MCG)	3	2	0	0	100	100	0	0	18	20

KEY:
= No zone of inhibition
D *S. aureus*
E *S. saprophyticus*

Antibiotic sensitivity of *E. coli, Klebsiella spp* and *Proteus spp* correlates significantly with each other as earlier reported by Olusi *et al*. (2004). *E.coli* was highly sensitive to tetracycline followed by ciprofloxacin, ofloxacin and gentamycin. Resistance to amoxicillin, chloramphenicol, ampicillin and nitrofurantoin was also observed. Resistance is usually transferable especially amongst the members of the family Enterobacteriaceae (Gilman, 1996), and also are the predominant organisms implicated in urinary tract infections (Esan *et al*., 2004). Strains of *Klebsiella* and *Proteus* were highly sensitive to ciprofloxacin followed by ampicillin and resistance to amoxicillin cefuroxime, gentamycin, chloramphenicol, tetracycline and nitrofurantoin respectively. The resistance factors to these antibiotics is the ability of these organisms to acquire plasmid encoded genes i.e. extrachromosomal genetic element that is transferable from one bacterium to the other and it may also be due to indiscriminate

use of these drugs with or without prescription among the population studied(Chigbu and Ezeronye, 2003). The antibiotics sensitivity and resistance of *Staphylococcus aureus* and *Staphylococcus saprophyticus* agrees with what was observe by Olusi et al., (2004). *Staphylococcus aureus* and *Staphylococcus saprophyticus* shows greater sensitivity to ciprofloxacin, streptomycin, erythromycin, pefloxacine, gentamycin and rocephin respectively and highly resistance to septrin, amoxicillin and ampiclox. Generally, all species of the microorganisms isolated were susceptible to ciprofloxacin and resistance to amoxicillin. *E coli, Proteus* and *Klebsiella* were totally resistance to cefuroxime. The highly sensitivity of ciprofloxacin may be due to uncommon use of these drugs as opposed to the rampant use of amoxicillin & cefuroxime as broad spectrum antibiotics.

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