academicJournals

Vol. 5(10), pp. 460-466, October 2013
DOI: 10.5897/IJMMS2013.0958
ISSN 2006-9723 ©2013 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Prevalence and antibiotic susceptibility of uropathogens among patients attending University of Abuja Teaching Hospital, Gwagwalada, Abuja

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Accepted 28 August, 2013

A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract become infected by the presence and growth of microorganisms that overcome the structures strong natural defenses. This study reports the examination of bacterial organisms implicated in urinary tract infections among 180 patients suspected to have UTI that attended University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja for a period of 3 months and the antibiotic susceptibility patterns of the bacterial isolates. First morning mid stream urine sample cultures were done by the calibrated loop technique delivering 0.002 ml of urine plated on Cystine Lactose Electrolyte Deficient (CLED) agar and blood agar medium for isolation of uropathogens which were further analyzed for drug susceptibility by disk diffusion method. Result showed that 104 (57.78%) patients had urinary tract infection. The uropathogens isolated were *Escherichia coli* as the most abundant (43.27%) followed by *Staphylococcus aureus* (35.57%), *Klebsiella* species (11.54%), *Proteus* species (5.77%) and *Pseudomonas* species (3.85%) as the least occurring. *In vitro* antibiotic susceptibility test revealed that the Gram negative bacteria were most sensitive to quinolones (ofloxacin and levofloxacin), gentamycin and nalixidic acid while the Gram positive isolates were sensitive to erythromycin, chloramphenicol and quinolones (ofloxacin and levofloxacin). The most effective drug in both cases was the quinolones–ofloxacin (78.32%) and levofloxacin (76.98%), while the least sensitivity pattern was observed with ampicillin (14.29%).

Key words: Urinary tract, uropathogens, antibiotics susceptibility.

INTRODUCTION

The urinary tract includes the organs that collect and store urine and release it from the body which include kidneys, ureters, bladder and urethra. A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract (that is, kidneys, ureters, bladder and urethra) become infected by the presence and growth of microorganisms that overcome the structures strong natural defenses. UTI have become the most common hospital-acquired infections, accounting for as many as 35% of nosocomial infections, and are the second most common cause of bacteria in hospitalized patients (Stamm, 2002; Kolawole et al., 2009).

Even though several different microorganisms, that is, protozoan, parasites, fungi and viruses can cause UTI, bacteria are the major causative organisms and are accountable for more than 95% of UTI cases (Bonadio et al., 2001). Common pathogens that have been implicated in UTIs are primarily Gram negative organisms with *Escherichia coli* having a higher prevalence than other Gram negative pathogens. Other pathogens include *Klebsiella* species, *Enterobacter* species, *Proteus mirabilis, Pseudomonas aeruginosa* and *Citrobacter*

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species (McLaughlin and Carson, 2004; Blair, 2007). These bacteria usually enter the system from external sources. Bacteria that live in the digestive tract, vagina or around the urethra, which is at the entrance to the urinary tract have also been implicated in UTI. The bacteria usually multiply at the opening of the urethra and travel up to the bladder (known as ascending route) much less often, as reported by Simon (2002).

There are several factors and abnormalities of UTI that interfere with its natural resistance to infections. These factors include sex and age diseases, hospitalization and obstruction (Kolawole et al., 2009). Numerous reports have also suggested that UTI can occur in both males and females of any age, with bacterial counts as low as 100 colony forming units (CFU) per milliliter in urine (Ebie et al., 2001). Females are however believed to be more affected than males. This is due to the short and wider female urethra and its proximity to the anus. The female urethra is also adjacent to the genital and intestinal tracts. Bacteria from the rectum can easily travel up the urethra and quickly gain access to the bladder and cause infections (Ebie et al., 2001; Kolawole et al., 2009).

The traditional guideline that urine containing more than 100,000 bacteria per milliliter is an indication of a urinary tract infection has been modified to counts as low as 100 ml^{-1} of any single bacteria type or as few as 100 ml^{-1} of coliform (intestinal bacteria such as *E. coli*) are now considered as an indication of significant infection especially if leukocytes, appear in the urine. Before therapy is initiated, urine bacteria are usually cultured to antibiotic sensitivity (Tortor and Funke, 2004).

The aim of this research is to study the prevalence rate of urinary tract infections among symptomatic patients attending University of Abuja Teaching Hospital (UATH) in terms of sex, to determine the causative agents and their susceptibility patterns to commonly used antibiotics in the treatment of UTIs.

MATERIALS AND METHODS

Study population

The study population was drawn from patients attending University of Abuja Teaching Hospital Gwagwalada, Abuja. One hundred and eighty patients (between 18 and 45 years) with suspected urinary tract infection consisting of 85 males and 95 females were screened in the study. Those excluded from the study were patients already on antibiotic therapy.

Specimen

With the help of trained nursing staff, the first morning urine samples were collected from one 180 patients and were transported to the laboratory in an ice pack and analyzed within 6 h of collection.

Collection of samples

Early morning, clean catch, mid-stream urine samples were collected in sterile universal containers as described by Karlowsky et al. (2006) and Solberg et al. (2006) into sterile, wide mouthed bottles with screw cap tops. The following details were indicated on the urine sample bottles as per standard laboratory procedures; name, sex and time of collection.

Sample processing

Macroscopy

The samples were examined, observed and recorded for colour and cloudiness on arrival at the laboratory (Anochie et al., 2001).

Culture

Culture was done by the calibrated loop technique as described by Cheesbrough (2000). A loop full of the well mixed urine sample was inoculated and plated on Cystine Lactose Electrolyte Deficient (CLED) and blood agar using streaking technique. The loop used transfers 0.002 ml of urine sample. All plates were then incubated at 37°C aerobically for 24 and 48 h in negative cases. The plates were further examined for bacterial growth. Observations were made on plates for growth, size of colony, morphology, consistency, aeration, color and odor (Mbata, 2007).

A ten-fold serial dilution was also carried out by transferring 1.0 ml of the urine sample into 9.0 ml of sterile distilled water. Using a sterile Pasteur pipette, 0.02 ml of the third dilution was inoculated onto the already prepared CLED agar plates in duplicates and spread with a sterile hockey stick-shaped glass rod; inoculated plates were incubated at 37°C for 24 h.

A significant bacteriuria count was taken as any count equal to or in excess of 100,000 cfu/ml (Smith et al., 2006). Colonies isolated were subcultured and identified by macroscopy, microscopy and biochemical tests.

Dipstick test

Urine test strips were used to detect the presence of protein, nitrite and leukocyte in the urine. The urine samples were properly mixed and transferred into test tubes. The strip was subsequently inserted into it. The development of an immediate colour change was noted and compared with the colour chart on the urine strip container (Smith et al., 2006; Bachur and Harper, 2001).

Antibiotic susceptibility testing

Antimicrobial susceptibility of isolates was tested by the Kirby Bauer disk diffusion method (Bauer et al., 1966), using commercially available disc. Antimicrobial agents tested were levofloxacin Antimicrobial susceptibility of isolates was tested by the Kirby Bauer nitroflurantoin (30 µg), nalixidic acid (30 µg), ampicillin (30 µg), augmentine (30 µg), streptomycin (30 µg), chloramphenicol (15 µg), tetracycline (30 µg), gentamycin (10 µg), erythromycin (15 µg), cotrimoxazole (10 µg), cloxacillin (30 µg) and floxacin (10 µg). With the use of a sterile wire loop, colonies of the test organism were emulsified in 2 ml of sterile physiological saline. The turbidity of the suspension was matched with the turbidity standard (0.5 McFarland Solution); this was viewed against a sheet of paper for easier comparison. Using a sterile syringe, 2 drops of the suspension was placed on the surface of a nutrient agar plate, with the use of a sterile glass rod; the suspension was evenly spread on the surface of the medium, rotating the plate approximately 60° to ensure even distribution. With the Petri dish lid in place, it was allowed to stand for about 5 min for the surface of the agar to dry. Using a sterile

Table 1. Bacterial load of urine samples.

Number of sample	Colony forming unit (cfu)/ml
82	$0 - 9.5 \times 10^5$
8	$1.0 \times 10^6 - 1.95 \times 10^6$
5	$2.0 \times 10^6 - 2.95 \times 10^6$
3	$3.0 \times 10^6 - 3.95 \times 10^6$
2	$4.0 \times 10^6 - 4.95 \times 10^6$
2	$5.0 \times 10^6 - 5.95 \times 10^6$
12	$6.0 \times 10^6 - 6.95 \times 10^6$
21	$7.0 \times 10^6 - 7.95 \times 10^6$
16	$8.0 \times 10^6 - 8.95 \times 10^6$
14	$9.0 \times 10^6 - 9.95 \times 10^6$
9	$1.0 \times 10^7 - 1.095 \times 10^7$
2	$1.1 \times 10^7 - 1.195 \times 10^7$
4	$1.2 \times 10^7 - 1.295 \times 10^7$
Total	180

forceps, the antibiotic disc was placed on the surface of the plate. Within 30 min of applying the disc, the plate was inverted and incubated aerobically at 35°C for 18 h. After overnight incubation, a ruler was used to measure the zone of inhibition in mm underside of the plate (Cheesbrough, 2000).

Interpretation of results was done using the zone sizes. Zone of inhibition of greater than and equal to 18 mm was considered sensitive, 13 to 17 mm intermediate and less than 13 mm resistant (NCCLS, 2000).

RESULTS

Over a period of three months (June to August), 180 mid-stream urine samples from patients attending University of Abuja Teaching Hospital (UATH) were analyzed, of which 104/180 were positive, while 76/180 were negative. The prevalence was found to be higher in females with a percentage rate of 62.50%, while the males had a percentage rate of 37.50%.

The colony forming unit (CFU) as was determined is shown on Table 1. Counts less than 100000 (10^5 ml^{-1}) were regarded as insignificant while counts greater and equal to 100000 (10^5 ml^{-1}) were indicated as significant bacteriuria.

Dipstick test of the uncentrifuged urine revealed that 46/180 of the specimen were positive for protein, while 134/180 were negative. Of the 46 positive protein samples, 22 yielded significant bacterial growth, 15/46 yielded no bacterial growth and 9/46 had an insignificant bacterial growth. For nitrite 28/180 of the specimens were nitrite positive while 152/180 showed negative. Of the 28 nitrite positive samples, 21 yielded significant bacterial growth, 3/28 yielded no bacterial growth while 4/28 had an insignificant bacterial growth. Dipstick test was also used to detect the presence of leukocyte (white blood cells) in the sample. Of the 180 samples examined, 63 were positive for leukocyte, while 117/180 were negative. Of the 63 positive samples, 51 showed significant bacterial growth, while 5/63 showed no bacterial growth, whereas 7/63 showed an insignificant growth (Table 3).

Of the 104 isolates obtained, Gram negative bacteria had a higher frequency of occurrence than Gram positive with *E. coli* having the highest frequency rate of 43.27%. Other Gram negative organisms isolated include *Klebsiella* spp. 12 (11.54%), *Proteus* species 6 (5.77%) and *Pseudomonas* species 4 (3.85%). The only Gram positive organisms isolated were *S. aureus*, which had a prevalence rate of 35.57%. It was found that the rate of occurrence of *E. coli, Klebsiella* spp. and *Proteus* spp. were higher in females than males. However, *Staphylococcus aureus* was found to be slightly higher in males than females (Table 2).

In vitro antibiotic susceptibility patterns of isolates to common antimicrobial agent are shown in Table 4. It was found that *E. coli* were most sensitive to levofloxacin (75. 56%), ofloxacin (71.11%), gentamycin (62.22%), chloramphenicol (57.78%) and nitroflurantoin (53.33%). It however showed a high resistant rate to cloxacillin (86.67%), cotrimoxazole (88.89%), ampicillin (80.00%), erythromycin (62.22%) tetracycline (77.78%) and nalixidic acid (53.33%). *S. aureus* were found to be highly sensitive to erythromycin (86.49%), ofloxacin (62.16%), streptomycin (54.1%), chloramphenicol (70.27%) and levofloxacin (67.68%). They showed resistance to ampicillin (81.08%), nitroflurantoin, (51.35%) nalixidic acid (51.35%) and augmentin (54.1%). *Klebsiella* spp. were found to be highly sensitive to levofloxacin (83.33%), ofloxacin (75%), nalixidic acid (58.33%), chloramphenicol (58.33%), gentamycin (66.67%) and erythromycin (50.00%). They showed resistance to cloxacillin (58.33%), augmentine (50.00%), streptomycin (66.67%), ampicillin (66.67%) and nitroflurantoin (50.00%). *Proteus* spp. were found to be sensitive to levofloxacin (83.33%), ofloxacin (83.33%), gentamycin (66.67%), erythromycin (50.00%) and nalixidic acid (50.00%). They showed high resistance to cloxacillin (66.67%), cotrimoxazole (100%), augmentine (100%), chloramphenicol (66.67%), and ampicillin (66.67%).The susceptibility pattern of *Pseudomonas* spp. showed a high sensitivity rate to levofloxacin (75%), gentamycin (50%), chloramphenicol (50%), ofloxacin (100%) and nalixidic acid (50%) and a resistant rate of 100% to cloxacillin, cotrimoxazole, augmentine, streptomycin, and ampicillin. It was also resistant to tetracycline by 75% and nitroflurantoin by 50%.

DISCUSSION

Urine is one of the sterile fluids in the body, but when it is colonized with bacteria, all the structures of the urinary tract are at risk of being invaded. Infections of the urinary tract are one of the most common infectious diseases and they affect all age groups and people (including men, women and children) worldwide (Llenerrozos, 2004;

Table 2. Prevalence of isolates in relation to sex of patients.

Isolate	Isolates from males	Isolates from females	Percentage
Escherichia coli	12	33	45 (43.27)
Staphylococcus spp.	19	18	37 (35.57)
Klebsiella spp.	3	9	12 (11.54)
Proteus spp.	1	5	6 (5.77)
Pseudomonas spp.	4	0	4 (3.85)
Others	-	-	-
Total	39	65	104 (100)

Table 3. Relationship between significant bacteriuria and protein, nitrite and leucocytes in urine samples of patients.

Parameter	Protein	Nitrites (%)	Leukocytes
Positive	25.56	15.56	35.00
Negative	74.44	84.44	65.00
Positive with growth	47.83	75.00	80.95
Positive without growth	32.61	10.71	7.94
Positive with insignificant growth	19.57	14.29	11.11

Blair, 2007). The antibiotic sensitivity patterns of organisms change rapidly over a short period. It is especially true for developing countries where antibiotics are not sold by only the medical practitioners but are also purchased directly from the pharmacists without prescription (Hooton and Stamm, 1997). Periodic evaluation of sensitivity pattern is therefore essential for rational and appropriate use of antibiotics (Karlowsky et al., 2006).

In this study of 180 urine samples collected, only 104 gave significant growth and thus had a bacterial urinary tract infection, while 76/180 showed no growth and no significant bacteria growth. This figure is higher than the prevalence rate of 11.9% reported by Aiyegoro et al. (2007) among children and adolescents in Ile-Ife and 35.5% rate recorded by Ebie et al. (2001) among children and adults in Rukuiba Military Cantonment Jos. It is slightly lower than the 77.9% recorded by Mbata (2007) among prison inmates in Nigeria. It however, agrees with the 52.83% reported by Maripandi et al. (2010) and 58% recorded by Onifade et al. (2005) among pregnant women. The high prevalence may be due to genuine population susceptibility, because factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status are common among Nigerian men and female (Mbata, 2007).

Protein result from dipstick test showed that 46/180 of the samples were positive for protein out of which 22 yielded bacteria growth. This corresponded with the works of Sandberg et al. (2005) and Zhang and Bailey (1995) which showed 8 (53.33%) and 24 (42%) positive protein samples with bacteria growth, respectively. This does not correspond with the result obtained by Sultana et al. (2001) which showed a relatively low number of 87 (21.75%) of protein positive samples with bacteria growth.

Dipstick test showed that 28/180 of the samples were positive for nitrite; of this few, 21 yielded bacteria growth. This is similar to the report of Russell et al. (2007) where 29 (85.7%) of the positive samples yielded bacteria growth.

Similarly, 65/180 of the samples were positive for leukocyte, while 51 of the positive samples yielded bacteria growth. This is relative to the result obtained by Kolawole et al. (2009) where 125 out of 130 samples positive for leukocyte yielded bacteria growth.

The most common organisms isolated were *E. coli* (43.27%), *S. aureus* (35.57%), *Klebsiella* spp. (11.54%), *Proteus* spp. (5.77%) and *Pseudomonas* spp. (3.85%). This finding is similar to other reports which indicate that a Gram negative bacterium, particularly *E. coli* is the commonest pathogen isolated in patients with UTI (Ebie et al., 2001; Njoku et al., 2001). In other similar studies, the commonest isolates reported were also *E. coli*, *S. aureus* and *Klebsiella pneumoniae* (Kolawole et al., 2009). However, the 35.5% incidence rate of *S. aureus* in this study brings to light the fact that *Staphylococcus* species are becoming more predominant as aetiological agents of UTI than previously reported in reports by Amin et al. (2009) where it had a prevalence rate of 2.2%. Relative high incidences of 27.3 and 28.9% have also been reported by Nwanze et al. (2007) and Okonkwo et al. (2009). The high incidence of *S. aureus* recorded in this study could be due to virulent nature of the organism, which gives it the ability to overcome body defence mechanism and resistance to antibiotics (Ojiegbe and

Table 4. Antibiotic sensitivity patterns of isolates.

Isolate	Levo (%)	Clox (%)	Cotr (%)	Eryt (%)	Gent (%)	Augm (%)	Strep (%)	Tetra (%)	Chlo (%)	Ampi (%)	Oflo (%)	Nitro (%)	Nalix (%)
Escherichia coli													
S	75.56	11.11	8.89	31.11	62.22	22.22	15.6	17.78	57.78	13.33	71.11	53. 33	28.89
I	6.67	2.22	2.22	6.67	15. 56	11.11	26.67	44.44	13. 33	6. 67	11. 11	15. 56	17.78
R	17.78	86.67	88.89	62.22	22.22	66.67	57.78	77.78	28.89	80.00	17.78	28.89	53.33
Staphylococcus aureus													
S	67.68	35.14	37.84	86.49	48.65	40.54	54.1	43.24	70.27	8.11	62.16	27. 03	33.43
I	8.11	45.95	24.32	8.11	18.82	5.41	29.73	24.32	10. 81	10. 81	13. 51	21. 62	16.22
R	24.32	18.92	37.84	5.41	32.43	54.1	16.22	32.43	18.92	81.08	24.32	51.35	51.35
***Klebsiella* species**													
S	83.33	25.00	33.33	50.00	66.67	16.67	33.33	41.67	58.33	33.33	75.00	25.00	58.33
I	-	16.67	25.00	8.33	-	33.33	-	41.67	16.67	-	18.33	25.00	16.67
R	16.67	58.33	41.67	61.67	33.33	50.00	66.67	16.67	25.00	16.67	66.67	50.00	25.00
***Proteus* species**													
S	83.33	16.67	-	50.00	-	66.67	33.33	50.00	-	83.33	16.67	33.33	50.00
I	-	16.67	-	33.33	-	-	16.67	16.67	33.33	16.67	16.67	50.00	16.67
R	16.67	66.67	100.00	16.67	33.33	100.00	33.33	33.33	66.67	66.67	-	16.67	33.33
***Pseudomonas* species**													
S	75.00	-	-	25.00	50.00	-	-	25.00	50.00	-	100.00	25.00	50.00
I	25.00	-	-	50.00	50.00	-	-	-	-	-	-	25.00	25.00
R	-	100.00	100.00	25.00	-	100.00	16.22	75.00	50.00	100.00	-	50.00	25.00

Levo, Levofloxacin; Tetra, Tetracycline; Clox, Cloxacillin; Chlo, Chloramphenicol; Cotr, Cotrimoxazole; Ampi, Ampicillin; Eryt, Erythromycin; Oflo, Ofloxacin; Gent, Gentamycin; Nitro, Nitroflurantoin; Augm, Augmentine; Nalix, Nalixidic Acid; Strep, Streptomycin S=Sensitive I=Intermediate R=Resistant.

Nworie, 2000).

The prevalence of UTI occurred more often in females than in males. Of the 104 isolates obtained, 65 were from females, while 39 were from males. These results also agree with other reports, which showed that UTIs are more frequent in females, than males during adolescence and adulthood (Ibeawuchi and Mbata, 2002; Mbata, 2007). This high prevalence rate in females has been reported to be due to the shorter and wider urethra of females than those of males. Also, the anatomical relationship of the female urethra and the vagina makes it susceptible to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy and child birth (Arthur et al., 2005).

The most useful antibiotics in this study were quinolones (ofloxacin and levofloxacin), gentamycin, nalixidic acid, erythromycin and chloramphenicol (in Gram positive), because they

inhibited most commonly isolated UTI pathogens. This concurred to other reports where quinolones are the most effective (Ebie et al., 2001; Mbata, 2007).

Nitroflurantoin, ampicillin and cotrimoxazole (septrin) which are commonly used antibiotics were poorly effective against majority of the organisms isolated in this study. The resistance observed with these drugs used may be due to antibiotics being used for a long period and must have been abused and as a result the organisms must have developed mechanisms of circumventing their mode of action (Kolawole et al., 2009). In the evaluation of the efficiency of the quinolone drugs used, these drugs now appear as promising therapeutic agents for the treatment of acute urinary tract infection (Mbata, 2007).

Conclusion

This study shows that there is a high prevalence rate of UTI among symptomatic patients attending University of Abuja Teaching Hospital, especially among the females. It also shows the necessity of obtaining sensitivity pattern reports before the start of antibiotics treatment in case of suspected urinary tract infection. However, the decision to use a particular antibiotic depends on its toxicity, cost and attainable level. Even though the susceptibility pattern shown in this study buttressed the need for *in vitro* sensitivity reports before antibiotics therapy initiation, it should be borne in mind that *in vitro* antimicrobial sensitivity is only a guide and that conditions *in vivo* may be quite different (Ibeawuchi and Mbata, 2002).

ACKNOWLEDGEMENTS

The authors thank all members of staff in the Microbiology Laboratory of the University of Abuja Teaching Hospital for their help in obtaining the samples used for this work and to Mrs. Bevan, D. of the Blood Bank Department of the hospital for her help in obtaining the blood used in this research work.

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