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Abstract

The prevalence rate of asymptomatic UTI among students of a community tertiary institution based in Evbuobanosa near Benin City, Nigeria was the focus of this work. A total of 390 freshly voided midstream urine samples collected into sterile plastic screw capped universal containers containing boric acid as preservative from students aged between 15 â?? 30 years were used for the study. Samples were screened for significant bacteriuria and puscells (neutrophil) counts. All positive samples with pyuria were aseptically cultured by standard methods on sterile Cystine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar and Sabouraud Dextrose agar plates and incubated appropriately. Antibiotic susceptibility testing was done on isolated and identified uropathogens by Kirby â?? Bauer agar diffusion disc method. While 231(59.2

Index terms—

1 Introduction

rinary tract infection(UTI) is the most common infection experienced by humans after respiratory and gastro-intestinal infections and also the most common cause of both community-acquired and hospital acquired (nosocomial) infections for patients admitted to the hospitals **??**Najaret al.,2009). UTI can be asymptomatic or symptomatic characterized by a wide range of symptoms from mild voiding irritation to bacteraemia, sepsis or even death **??**Ranjbar et al., 2009).

Infection of the urinary tract could manifest differently depending on the site of the infection and length of time involved **??**Takhar, 2011). Those that affect the lower urinary tract are called cystitis(i.e. involving the bladder alone with symptoms including painful urination, burning sensation, frequent urination or urge to urinate or both while those that affect upper urinary tract are referred to as pyelonephritis(i.e. involve the kidneys and other organs **??**Sarah,2010). The symptoms of the upper urinary tract infection include fever and flank pain during urination in addition to those of the lower urinary tract (Sarah 2010).

Urinary tract infection occurs more frequently in females than males due to the shortness and width of the female urethra to the vagina which makes it liable to trauma during sexual intercourse as well as bacteria being passed from the urethra into the bladder during pregnancy (Ebie et al., 2001). The moist environment of the female's perineum favours microbial growth and predisposes the female bladder to bacterial contamination (Ebie et al., 2001). In addition, urine of females was found to have more suitable pH and osmotic pressure for the growth of Escherichia coli than urine from males **??**Obiogbolu et al., 2004).

Most UTIs are caused by gram negative bacteria like Escherichia coli and Klebsiellaspp **??**Omonighoet al., 2001;Ebie et al.,2001) **??**Shankel,2007).

Drug resistance among bacteria causing UTI has increased since the introduction of UTI chemotherapy **??**Nerurkar et al.,2012; **??**ood and Gupta,2012; **??**ahadin et al.,2011; **??**aideret al.,2010). The aetiological agents and their susceptibility patterns vary in various regions and geographical locations (Mulugeta and Bayeh, 2014). Knowledge of the local bacterial aetiology and susceptibility pattern is required to trace any change that might have occurred with time so that updatedrecommendation for optimal empirical therapy of UTI can be made **??**Leegardet al., 2000).

A number of studies have been done on the prevalence and antimicrobial resistance patterns of UTIs **??**Onhet al., 2006; **??**bata,2007; **??**konko et al.,2009). Be that as it may, there is no documented UTI study that has been carried out in Ewobanosa community. Ewobanosa community is a bini speaking community situated about 20km from Benin City, Edo State of Nigeria. The community has a privately owned polytechnic situated tangentially on the express road that connects Benin City to Asaba (in Delta State of Nigeria). It was therefore to extend the frontiers of knowledge of UTI that this work aimed at studying community acquired urinary tract infection prevalence among students in a tertiary educational institution in Evbuobanosa, Nigeria was carried out with the following objectives:

? Determine the frequency distribution of microbial uropathogens in urine samples obtained from the students. ? Determine the sex distribution of uropathogens isolated. ? Determine the age distribution of the uropathogens isolated. ? Determine the antibiotic susceptibility profiles of isolated uropathogens.

2 a) Sampling

A total number of the three hundred and ninety (390) midstream urine samples were collected from students (both residential and non-residential) of Lighthouse Polytechnic situated in Evbuobanosa, a community in Orhionwon local government area of Edo State of Nigeria. Samples (which were freshly voided), were collected into sterile screw capped plastic universal containers containing a few crystals of boric acid as preservative. Recruited students were instructed on how to collect the samples. All samples were appropriately labeled and processed immediately in the Microbiology laboratory of Western Delta University, Oghara,Delta State. Students recruited were grouped into 15-20, 21-25 and 26-30 age brackets. The study designed was a descriptive cross sectional study. Samples were collected in March, 2014.

3 b) Ethnical Clearance

Ethnical clearance was sought and granted by the ethnical committee of the Polytechnic. In addition, the recruited students gave their verbal informed consent after thorough explanation of the rational for the study.

described by Mbata (2007). A standard bacteriological loopful of each urine sample (0.01ml) was spread over the surface of sterile Cystine Lactose Electrolyte Deficient (CLEDI) agar plates (LabM, UK). After inoculation, the plates were inverted and incubated at 37 o C for 18-24 hrs. The number of bacterial colonies was counted and multiplied by 100 to give an estimate of the number of bacterial organisms per millilitre of urine. A significant bacterial count was taken as any count equal to or in excess of 10 5 per millilitre.

4 d) Confirmation of Significant Bacterial Count by Microscopy

All samples that recorded significant bacterial counts were subjected to urine microscopy test to detect presence of five pus cells per high power focus (5PC/HPF) or 10 white blood cells (pus cells) /mm 3 in urine sediments or deposits (Smith, 2004) using x40 objective microscopically. All samples that were positive for significant bacterial count and also recorded 5PC/HPF or 10PC/mm 3 or more were cultured on suitable laboratory media.

5 e) Cultural Studies

Urine sample that recorded 5PC/HPF or 10PC/mm 3 and were positive for significant bacteriuria test were cultured aseptically on sterile CLED agar (Lab M, UK), MacConkey agar (Lab M, UK) and Saboraud Dextrose agar (Lab M, UK)plates according to standard methods. All inoculated plates were incubated at 37 0 C for 24hrs. Pure isolates were then obtained and identified according to schemes provided by Cowan and Steel (1993)and Alexopoulos **??**1996). All identified isolates were subjected to antibiotic sensitivity testing. Fungal isolates were excluded from antibiotic sensitivity testing. All urine samples that yielded no bacterial growth were noted.

6 f) Antibiotic Sensitivity Testing

Antibiotic susceptibility testing was carried out on confirmed uropathogens according to the agar diffusion disc technique described by **??**auer et al. (1966). A colony of each pure (axenic) isolate was streaked on sterile Mueller Hinton agar plates aseptically using sterile inoculating wire loop. The relevant multidiscs containing their respective minimum inhibitory concentrations (MICs) were used and included erythromycin (5ug), cefuroxime (30ug), gentamicin (10ug), nalidixic acid (30ug), cefixime (5ug), ceftazidime (30ug), ofloxacin

7 c) Processing of Samples

Samples were tested for significant bacteriuria by use of a modified semi quantitative technique surface of the dried plates using sterile forceps. The plates were left at room temperature for one hour to allow diffusion of the different antibiotics from the disc into the medium (Mbata, 2007).

8 II.

9 Materials And Methods

The plates were then incubated at 37 0 C for 18hrs. Interpretation of results was done using the length of diameter of zone of inhibition **??**NCCLS, 2000). Zones of inhibition greater than 10mm were considered sensitive, 5-10mm moderate sensitive and no zone of inhibition, resistant.

10 g) Statistical Analysis

Simple percentages were used throughout.

11 III.

12 Results

Table 1 shows the isolated identified uropathogens from the samples that yielded growth after 24hrs incubation period. A total of 390 midstream urine samples were processed of which 159 (40.8%) yielded no growth at the end of 48hrs incubation at 37 0 C. A total of 231 (59.2%) samples yielded growth of seven microbial uropathogens were identified as follows: Bacterial colonies that were in clusters, gram positive, catalase positive, coagulase positive, DNAase negative, phosphatase positive, mannitol fermenting, raised, round and smooth colonies were identified as Staphylococcus aureus. All gram negative raised entire, circular, motile, lactose, glucose fermenting, indole positive, methylred positive, voges praskauer negative, citrate negative and urease negative bacilli strains were identified as Escherichia coli.

Bacterial colonies that were positive for all the characteristics of Staph aureus colonies but were negative for slide and tube coagulase tests were identified as coagulase negative staphylococci. Gram negative mucoid non-motile, lactose, adonitol, inositol, glucose fermenting, voges praskauer positive, urease positive, citrate positive and indole negative bacilli strains were identified as Klebsiella aerogenes.Coliform organisms were mixed cultures made of Escherichia coli,Enterococcus faecalis and Clostridium perfringens. Gram positive yeast cells, pseudohyphae positive, chlamydospores positive, germtube positive, glucose, maltose galactose and sucrose assimilation positive strains were identified as Candida albicans.Gram negative, swarming, fish odour colonies on sodium chloride containing media, indole negative and urease positive strains were identified as Proteus spp. The frequency occurrence of the isolates and their strains therefore were as follows: Staphylococcus aureus (33.1%) Escherichia coli (20.8%), coagulase negative Staphylococci (15.6%), klebsiella aerogenes (7.9%), Coliform organism (7.9%),Candida albicans (7.9%) and Proteus app (6.8%). On the whole, total gram negative and gram positive bacilli isolated represented 43.4% and 48.7% respectively of which 43.4% and 56.6% belonged to enterobacteraceae and non enterobacteraceae respectively. The age distribution of the entire 231 uropathogen strains isolated is shown in Table 3 In Table 4, the antibiotic susceptibility patterns of all isolated uropathogens (with exception of Candida albicans and coagulase negative staphylococci) to some selected antibiotics are shown. The antibiotic sensitivity profiles of the 231 uropathogen strains to ciprofloxacin, cefuroxime, gentamicin, cefixime, ceftazidime, ofloxacin, streptomycin, erythromycin, tetracycline, cotrimoxazole, augmentin, nalidixic acid, nitrofurantoin, chloramphenicol are shown. Twenty four (32.0%), 11 (22.9%), 06 (33.4%) 09(50.0%) and 06(40.0%) strains of Staphylococcus aureus,Escherichia coli,Klebsiella aerogenes, Coliform organisms, and Proteus spp respectively were sensitive to all the 14 (fourteen) antibiotics used.

With respect to Staph aureus,12 antibiotics used were twelve because nalidixic acid and nitrofurantoin were not tested on it and whereas erythromycin and streptomycin were used for Staphaureus, they were not used for the other uropathogens. On the average, a higher percentage of pathogens resisted all fourteen antibiotics compared to those that were sensitive.

Hence, augmentin and nalidixic acid were the most In terms of effectiveness of each of the antibiotics used, 108(62.1%), 60(34.5%), 48(27.6%), 45(25.9%), 39 (22.4%), 24(13.8%), 24 (13.8%), 21 (12.1%), 15(8.6%), 15(8.6%), 12(6.9%), 12(6.9%), 06(3.5%) and 03(1.7%) strains of all isolated pathogens were sensitive to gentamicin, ofloxacin, streptomycin, nitrofurantoin, ciprofloxacin, tetracycline, cotrimoxazole, chloramphenicol, ceftazidime, cefixime, erythromycin, cefuroxime, augmentin and nalidixic acid in that decreasing order. The two most sensitive drugs in this study were therefore gentamicin and ofloxacin whereas augmentin and nalidixic acid were the least sensitive. resisted antibiotics with ofloxacin and gentamicin as the least resisted. Escherichia coli n = 48 - - - - - Coliform orgs n = 18 - - + - - Proteus spp - - - - +

IV.

13 Discussion

In this study, out of 390 urine sample processed, 159(40.8%) samples yielded no growth and no significant microbial growth combined. Hence, 231(59.2%) samples yielded growth and significant growth all together. The absence of bacterial growth in 40.8% of processed samples was established as such when microscopically, a non-significant pus cell count (less than 5) was observed (Otajevwo, 2014) (Akinyemi et al., 2000;Kolawole et al., 2009;Andriole, 1985).

13 DISCUSSION

Seven uropathogens were isolated in this study (Table 1) of which gram negative bacilli constituted 43.4% while gram positive bacteria accounted for 48.7%. Isolates that belonged to enterobacteriaceae and non-enterobacteriacease families were 43.4% and 56.6% respectively (Table1). Whereas Staphylococcus aureus and coagulase negative staphylococci were the only gram positive bacteria, Candida albicans represented the only member of the nonenterobacteriacease. Finding is consistent with the report of a previous author who isolated 86.1% gram negative bacilli and 13.9% gram positive bacteria of which enterobactereaceae accounted for 49.9% (Otajevwo, 2013). The report of this study does not agree with a report of Oluremi et al. 5 shows the frequency occurrence of multidrug resistance among the isolated bacterial uropathogens. All the bacterial uropathogens apart from Escherichia coli were resistant to more than three antibiotics at a time. For clarity and for the purpose of this study, a pathogen is described as multidrug resistant to any of the selected antibiotics if more than 50% of its strains are resistant to it and hence, a pathogen is multidrug resistant if it resists up to three drugs at a time. Therefore, only four out of the five bacterial uropathogens isolated were multidrug resistant. None was resistant to 3 drugs. Staph aureus strains were resistant to 4 drugs while Coliform organisms were resistant to 5 drugs. Klebsiella aerogenes and Proteus spp strains were resistant to 6 drugs each. 66.7%. The report of Otajevwo (2014) stating 65.2% and 34.8% gram negative and gram positive bacteria respectively as well as 55.7% and 44.3% enterobactereaceae and non -enterobactereaceae respectively as isolates is also not consistent with the findings in this study.

In this study, the most and second most occurring uropathogens were Staphylococcus aureus (33.1%) and Escherichia coli (20.8%). Other uropathogens isolated were coagulase negative staphylococcus (15.6%), Klebsiella aerogenes (7.9%), Coliform organisms (7.9%), Candida albicans (7.9%) and Proteus spp. This result suggests that the two least isolated uropathogens in the study area are Candia albicansand Proteus spp. The occurrence of Staphaureus and E.coli as the most commonly occurring uropathogens is consistent with the report of a previous study (Otajevwo, 2014) (Habibu, 2014) as the most and second most occurring uropathogens in UTI. In this study, Proteus spp, Klebsiella spp and Staph aureus were isolated and these organisms have been incriminated in hospital acquired infections often following catherization or gynaecological surgery (Cheesbrough 2000; **??**apsak et al., 1995). Proteus infection is also associated with renal stones (Cheesbrough, 2000). In this study and previous similar studies, it is yet again confirmed the prominent involvement of Overall, UTI prevalence rate was higher in males (57.1%) than in the female students (42.9%) of whichStaphaureus, E.coli and Coliform organisms occurred more in the male students than in the female students (Table 2). The earlier report of Otajevwo (2014) which recorded higher UTI prevalence rate does not agree with the finding of this report. The reason for a higher UTI prevalence rate in males than females in this study though not clear, may be due to lack of circumcision, receptive anal intercourse (as in homosexuals) and HIV infection(Orret and **??**avis, 2006) Also in this study, Candida albicans occurrence was higher in female students compared to the males. According to Ochei and Kolhatkar (2008), yeast cells appear in urine as a result of contamination from women with vaginal candidiasis (occasionally seen in the urine of men) or may be seen in the urine of diabetic patients due to presence of sugar in the urine. Yeasts may also cause recurrent infections in debilitated and immunecompromised patients (Cheesbrough, 2000;Ochei and Kolhatkar, 2008). The occurrence of Coliform organisms is crucial in pregnant women although its occurrence in females is lower compared to males. The isolation of Candida albicans and Coliform organisms in this study is consistent with the report of a previous work (Otajevwo and Eriagbor, 2014). It has been documented that Coliforms and Enterococcus spp can cause UTI when present in high numbers on the perineum (Behzardi and Behzardi, 2010; **??**oore et al., 2002).

This study, strangely, did not confirm reports of other studies which stated that UTI occurs more in females than in males except at the extremes of life (Ebie et al., 2001;Kolawole et al., 2009). In terms of age bracket, UTI occurred highest (44.2%) in the 21-25 age group followed by 37.7% occurrence rate of students in the 15 -20 age bracket. This finding does not agree with the reports of some previous authors which stated that UTI is more frequent in females than in males (Mbata, 2007;Ibeawuchi and Mbata, 2002; **??**sinobi, 2002;Olaita, 2006). Findings in this work however, agree with the reports of these same authors which stated that UTI is most prevalent during youth and adulthood as indicated by the 21 -25 age group occurring as the most implicated in UTI in this study. This age group as well as the 15 -20 group consist of teenagers, adolescents and young people who are characteristically vulnerable to increased sexual activities that predispose them to UTI **??**Oladeinde et al., 201, Oluremi et al., 2011).

There is considerable evidence of practice variation in the use of diagnostic tests, interpretation of signs or symptoms and initiation of antibiotic treatment such as drug selection, dose, duration and route of administration (Jamieson, 2006). For patients with symptoms of UTI and bacteriuria, the main aim of treatment is to get rid of bacteria causing the symptoms. Besides, there is need to obtain sensitivity reports before the start of antibiotic treatment to checkmate emergence of resistance strains and thus help in proper patient management. The decision to use a particular antibiotic however depends on its toxicity, cost and attainable level (Ibeawuchi and Mbata, 2002).

Therefore, antibiotic sensitivity testing was done on all seven isolates (minus Candida albicans -a fungal uropathogen in this study) and the resulting sensitivity profile (antibiogram) showed susceptibility reactions of 108 (62.1%), 60(34.5%, 48(27.6%), 45(25.9%), 39(22.4%), 24(13.8%), 24(13.8%), 21(12.1%), 15(8.6%), 12(6.9%), 06(3.5%) and 03(1.7%) for gentamicin, ofloxacin, streptomycin, nitrofurantoin, ciprofloxacin, tetracycline, cotrimoxazole, chloramphenicol, cefixime, ceftazidime, erythromycin, cefuroxime, augmentin and nalidixic acid

respectively. This suggests that more than 50% of the bacterial uropathogens implicated in UTI in this study were sensitive to gentamicin only. This finding with respect to gentamicin is consistent with the reports of some previous studies (The low sensitivity recorded for nitrofurantoin in this study is at variance with 100%, 97.6% and more than 50% sensitivities recorded by previous authors **??**Oluremi et al., 2011;Haruna et al., 2014;Alabi et al., 2014). The least sensitive antibiotics were augmentin and nalidixic acid. The low sensitivity of nalidixic acid is surprising because it is a drug that is not commonly and routinely used in medical practice. The less than 4% sensitivity of augmentin is very low and disturbing in view of its usefulness in the treatment of UTI's and other diseases. The almost total resistance of augmentin is alarming as it may have lost its value in the treatment of UTI **??**Oluremi et al., 2011).

Four out of the seven isolated uropathogens were resistant to more than three drugs and hence they were all multi-drug resistant (MDR). In this study, Staph aureus,kleb.aerogenes, Coliform organisms and Proteus spp were resistant to four drugs, seven drugs, five drugs and seven drugs respectively (Table 5). Multi drug resistance of Staph aureus may be due to betalactamase (penicillinase) encoding genes it carries on its plasmids as well as other extracellular and intracellular factors the organism elaborates. Besides, multi-drug resistant Staph aureus strains have been widely reported in some studies (Abubakar, 2009;Aiyegoro et al., 2007; **??**ales et al., 2000). Multi-drug resistance of Kleb. aerogenes and Proteus spp are documented. The prevalence of multiple antibiotic resistant strains in this study is a possible indication that very large population of bacterial isolates has been exposed to several antibiotics (Oluremi et al., 2011).

V.

14 Conclusion

A prevalence UTI rate of 59.2% in this study (though not very high), indicates that UTI may be a health problem among students (who are mainly residential) of Lighthouse Polytechnic, Evbuobanosa, near Benin City. The findings suggest that most (if not all) the uropathogens isolated in this study are excretable through urine to the environment of the study area, Ewobanosa and its environs. Besides, the students should be advised to step up their personal hygiene in their hostels and immediate environment. Where signs and symptoms of UTI are noticed notwithstanding the above, healthcare providers of the Polytechnic may administer doses of gentamicin only or ofloxacin or perhaps streptomycin for therapy. A prompt therapeutic intervention in this regard, will prevent asymptomatic UTI cases becoming symptomatic with the accompanying renal damage.

Figure 1: Community

Enterococcusspp formerly called Strept. faecalis (Kashefet al., 2010; Theodros, 2010;Mulugeta and Bayeh,2014) as well asProteus mirabilis, Pseudomonas aeruginosa, coagulase negative staphylococci, Acinectobacterspp and Serratiaspp

Figure 2: .

1

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Isolated Uropathogens	No of Strains	Frequency of Strains
Staphylococcus aureus	75	33.1%
Escherichia coli	48	20.8%
Coagulase negative Staph	36	15.6%
Klebsiella aerogenes	18	7.9%
Coliform organisms	18	7.9%
Candida albicans	18	7.9%
Proteus spp	15	6.8%
Total	231	100.0%

n=7
Gram negative bacteria: 43.4%
Gram positive bacteria: 48.7%
Enterobacteriaceae: 43.4%
Non-Enterobacteriaceae: 56.6%

Figure 3: Table 1 :

2

[Note: the sex distribution of the microbial strains isolated. Out of the total 231 strains isolated, 132 (57.1%) and 99 (42.9%) were obtained from male and female students respectively. In a deceasing order, the highest occurring uropathogens in male students were Staph aureus (34.1%), Escherichia coli (22.7%), coagulase negative staphylococci (18.2%), Coliform organisms (9.1%),Klebsiella aerogenes (6.8%),]

Figure 4: Table 2 shows

2

Isolated Uropathogens	No of Strains males (%)	No of Strains females (%)	Total %
Staphylococcus aureus	45 (34.1)	30 (30.3)	75(32.5)
Escherichia coli	30 (22.7)	18 (18.2)	48 (20.8)
Coag. Neg. Staph	24 (18.2)	12 (12.1)	36 (15.6)
Klebsiella aerogenes	09(6.8)	09 (9.1)	18 (7.8)
Coliform organisms	12 (9.1)	06 (6.1)	18 (7.8)
Candida albicans	06 (4.6)	12 (12.1)	18 (7.8)
Proteus spp	06 (4.6)	09 (9.1)	15 (6.5)
Total n=7	M 132 (57.1%)	F 99(42.9%)	231(100.0%)

Figure 5: Table 2 :

Hence, 102 (44.2%), 87 (37.7%) and 42 (18.2%) of the sampled students belonged to 21-25, 15-20 and 26-30 age groups respectively. Seventy two (70.6%), 45(51.7%) and 15(35.7%) students of 21-25, 15-20 and 26-30 age group respectively were males while 30 (29.4%), 42(48.3%) and 27(64.3%) students of the same age groups were females. Consequently, the highest number of uropathogen strains of 72(70.6%) was isolated from male students belonging to 21-25 age group. Fifteen (35.7%) uropathogen strains were the least isolated from male students who occurred in the 26-30 age bracket. The highest and lowest uropathogen strains of 42 (48.3%) and 27 (64.3%) respectively were isolated from female students of 15-20 and 26-30 age group respectively.

Figure 6:

3

Uropathogens Isolated	15 -20		21 -25		26 -30	
	M	F	M	F	M	F
Staph aureus	18 (40.0)	12 (28.6)	21 (29.2)	09 (30.0)	06 (40.0)	09 (33.3)
Escherichia coli	09 (20.0)	12 (28.6)				

Figure 7: Table 3 :

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Figure 8: Table 4 :

5

Bacterial Uropathogens	3 drugs	4 drugs	Resistance to 5 drugs	6 drugs	?6 drugs
Staph aureus n = 75	-	+	-	-	-

Figure 9: Table 5 :

Figure 10:

Figure 11:

The fluoroquinolone-
ofloxacin recorded below moderate sensitivity (i.e. 27.6%). Although this finding agrees with that of a previous study which recorded 30.2% (Otajevwo and Eriagbor, 2014), it is quite low when compared with 100% ofloxacin sensitivity recorded by Anigilaje and Bitto (2014) and more than 50% ofloxacin sensitivity reported by Okon et al. (2014). Sensitivity of streptomycin which was less than 30% was also very low when compared to 100% streptomycin sensitivity reported recently by an author (Habibu, 2014). Nitrofurantoin (22.4%) and ciprofloxacin (13.8%) sensitivities were quite low. The low sensitivities recorded for the fluoroquinolones (ofloxacin and ciprofloxacin) in this study was worrisome because these drugs are expensive and therefore are not readily accessible for abuse. In a similar study, Nakhjavani et al. (2007) reported that the widespread use of fluoroquinolones in medical centres is a possible cause of high level resistance to fluoroquinolones in UTI patients.

Figure 12:

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