

FREQUENCY, URINALYSIS AND SUSCEPTIBILITY PROFILE OF PATHOGENS CAUSING URINARY TRACT INFECTIONS IN ENUGU STATE, SOUTHEAST NIGERIA

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SUMMARY

Objective: This study was designed to determine the frequency and causative agent(s) of urinary tract infections (UTIs) in individuals with symptoms of urinary tract infections in Enugu State of Southeast Nigeria, and to determine the antibiotic susceptibility pattern of microbial agents isolated from urine culture. **Methods:** The study involved 211 individuals (149 females and 62 males) clinically suspected for UTI. Urine samples were collected by the mid-stream 'clean catch' method and tested using standard procedures. Antibiotic susceptibility of the isolated pathogens was tested using the Kirby-Bauer technique according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** Microscopy of centrifuged urine samples showed 16 patients had pyuria while 54 had pus cells. Calcium oxalate crystals were found in 14 samples. Urinalysis performed with urine samples showed 17 had protein; seven were nitrite positive and three had moderate to high glucose concentration. Fifty-four urine samples (36.2%) from females and 12 (19.4%) from males showed significant growth upon culture. Gram stain and biochemical tests identified nine different organisms with *Escherichia coli* as the most common isolated species. Forty three randomly selected strains were further tested for their susceptibility against a panel of antibiotics. Thirty isolates (81.08%) were resistant to four or more antibiotics with the highest resistance shown by *E. coli* (76.67%). All the Gram-negative isolates were resistant to Ampicilox, Cefuroxime and Amoxicillin. **Conclusion:** Urinary tract infections were found more in females in the area under study. As found in other studies, *E. coli* was the most predominant isolate, although other organisms seem to be on the increase.

KEYWORDS: Urinary tract infection; Pathogen; Southeast Nigeria; Etiology; Antibiotic susceptibility.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common infections diagnosed in both hospitalized and outpatients¹². About 150 million UTIs are reported worldwide every year⁹ costing the world economy over six billion US dollars². UTIs are reportedly the most common bacterial infections in women and account for significant morbidity and increase in the cost of healthcare. In young sexually active women, the incidence of UTI exceeds 0.5 episodes per year with about 30% of women experiencing recurrent infections. In developed countries such as the USA, UTIs alone account for over eight million physician visits per year⁷. In developing countries, especially in many resource poor settings, the situation is worse and many clinicians often initiate empiric antibiotic treatment even before the final laboratory diagnostic test results are available. This is occasioned by the long duration, high cost and non-availability of experienced personnel and equipment needed for reporting accurate diagnosis by culture and susceptibility testing. Currently, there is an increasing problem of resistance to antibiotics especially in developing countries, prompting the need for a good knowledge of antibiotic resistance patterns of these pathogens so as to ensure efficient treatment and eradication. Moreover, area-specific studies

are required in monitoring the trend and gaining useful knowledge about the type of pathogens responsible for UTIs. The etiology and resistance patterns of community-acquired UTI pathogens have not been studied extensively in many settings⁸. Moreover, even in areas where a few studies have been done, there is need to continue to monitor the trend in terms of the etiology and antibiotic resistance patterns of these uropathogens. Although a few studies have been carried out previously in Southeast Nigeria^{5,6,14}, most of them investigated UTIs in association with other diseases in certain specific populations. Thus, there is not much recent data specifically on the etiology and antimicrobial resistance patterns of uropathogens among individuals specifically presenting with UTIs in Enugu State, Southeast Nigeria. This information will be very useful to clinicians so as to facilitate empirical treatment and management of patients with symptoms of UTIs. We therefore carried out this study to ascertain the current etiology and antimicrobial susceptibility patterns of uropathogens in Enugu State, Nigeria.

MATERIALS AND METHODS

Sample collection and analysis: Urine samples were collected from three randomly selected centers: Bishop Shanahan Hospital, Nsukka,

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McChuks Medical Diagnostic Laboratory, Enugu and Dynamic Medical Diagnostic Laboratory, Enugu all located in Enugu State, Southeast Nigeria. The patients who came for consultation and whose urine samples were collected were all outpatients. They were given sterile wide-mouthed containers and instructed on how to collect clean, mid-stream, voided urine samples. Those who were unable to collect their own samples were aided. Consent approvals were obtained from all those who participated in the study. The study was approved in each center where samples were collected. Patients who had no symptoms suggestive of UTI at the time of observation were excluded from the study. Those suffering from diabetes mellitus, renal disorders, HIV positivity or on corticosteroid or antibiotic therapy were also excluded. Those who refused to give consent or were non-cooperative were not included in the study.

Urinalysis: The urine samples were examined macroscopically for color and turbidity. They were then centrifuged and the sediment examined microscopically for the presence of red blood cells, white blood cells (pus cells), protozoa, *Schistosoma* eggs, yeasts and crystals. They were inspected for pH and the presence of glucose, protein and nitrite using Combi 9 (Medi-Test, Machery-Nagel GmbH, Duren) dipstick.

Isolation and identification of bacterial isolates from urine samples: A loopful of each fresh urine sample was inoculated onto Cysteine Lactose Electrolyte-Deficient (CLED) agar (Oxoid, UK) and incubated for 18-24hrs at 37 °C. Urine samples, that had yeast cells upon microscopic examination, were inoculated onto Sabouraud Dextrose Agar (SDA) (Oxoid, UK) and incubated at 37 °C for 18-24h. Discrete colonies were placed in slants and kept in the refrigerator and characterized as previously described⁴.

Antibiotic Susceptibility Test: Sterile Mueller-Hinton Agar plates were seeded with 0.1mL of 0.5 MacFarland (approximately 10⁷CFU/mL) standardized isolates, spread out with a bent sterile glass rod and left to dry on the bench for 30 minutes. Antibiotic discs were placed aseptically on the plates using sterile forceps. Plates were then incubated at 37 °C for 16-18h. The diameters of the zones of inhibitions were measured, recorded and interpreted according to CLSI approved standard guidelines¹³. The antibiotics tested against Gram positive organisms were Septrin (30 µg), Erythromycin (10 µg), Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxicillin (30 µg), Rocephin (30 µg) and Ciprofloxacin (10 µg) while those tested against Gram negative organisms were septrin (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Pefloxacin (30 µg), Tarivid (10 µg) and Streptomycin (30 µg).

RESULTS

Isolation and characterization of microbial isolates: A total of 211 urine samples (149 from females, 62 from males) were collected from all three centers located in Enugu State, Nigeria. Microscopy of centrifuged urine samples showed 16 (11 females and five males) had significant number of pus cells (pyuria) while 54 (46 female and eight males) had fewer pus cells. Calcium oxalate crystals were found in 14 urine samples (four with growth and 10 without growth). One male urine sample contained tyrosine crystals; another contained cysteine crystals with microbial growth on inoculation, while another had *Schistosoma* eggs and a lot of red blood cells. One female urine sample had triple

phosphate crystals (with no microbial growth); and another seven had numerous epithelial cells, also without any growth. Urinalysis carried out with combi-9 (Medi-Test) showed 17 urine samples (10 with growth and seven without growth) had protein; seven urine samples (all with growth) were nitrite positive and three urine samples had moderate to high glucose concentration. Fifty-four urine samples (36.2%) from females and twelve urine samples (19.4%) from males showed significant growth. The isolates were identified using Gram stain and biochemical tests as *Escherichia coli* (17), *Staphylococcus* species (12), *Staphylococcus aureus* (7), *Proteus* species (8) *Pseudomonas aeruginosa* (6), *Candida* species (7), *Enterobacter* species (5), *Klebsiella* species (3), and *Candida albicans* (2).

From the above isolates, 43 strains - *Escherichia coli* (12), *Staphylococcus* species (10), *Proteus* species (5), *Staphylococcus aureus* (4), *Candida* species (4), *Klebsiella* species (3), *Pseudomonas aeruginosa* (3), and *Candida albicans* (2) were used for the study.

Antibiotic susceptibility profile of the recovered urine isolates: Out of the 43 clinical bacterial isolates used in the study, 30 (81.08%) were resistant to four or more antibiotics with the highest resistance shown by *E. coli* (76.67%), followed by *Klebsiella* species (63.33%), *S. aureus* (57.5%), *Pseudomonas aeruginosa* (53.33%), *Staphylococcus* species (51%), and the least was *Proteus* species (38%). All the Gram-negative isolates were resistant to Ampiclox, Cefuroxime and Amoxicillin. However, most isolates were less resistant to Ciprofloxacin and it was thus used as the antibiotic control (Tables 1 and 2).

DISCUSSION

Microscopy of the centrifuged urine samples showed presence of moderate to many pus cells (white blood cells) in fewer urine samples which showed significant microbial growth on culture; presence of crystals such as calcium oxalates, tyrosine, cystine and triple phosphate; epithelial cells and *Schistosoma* eggs with numerous red blood cells. Pyuria, which is the presence of pus cells in this quantity, is an indication of microbial infections since pus cells are white blood cells that have succumbed in defense of the body against pathogens that invade it. Few are singly excreted in normal urine but in clumps in urinary infections. Pyuria with significant microbial growth ($\geq 10^5$ organisms/mL) in 16 urine samples is a good indication of urinary tract infection while significant microbial growth (bacteriuria) without pyuria in 54 urine samples screened in this study may be an indication of diabetes, enteric fever, bacterial endocarditis or contaminants from the perineum.

Crystals are formed from chemicals in normal urine and are usually refractive in appearance. The crystal types found indicate the presence or absence of a disease. Calcium oxalate crystals are formed from oxalic acid abundance in diet rich in leafy vegetables or coffee and calcium excreted into urine due to chronic dehydration or hyperparathyroidism. The crystals usually form stones (calculi) in the kidneys, ureters or bladders. Therefore, the presence of calcium oxalate crystals might be an indication of calculi in the urinary tract, a predisposing factor to urinary tract infection. Tyrosine crystals presence might indicate a possible severe liver disease while cystine crystals are found in cystinuria, a rare congenital metabolic disorder. Presence of numerous epithelial cells may indicate inflammation of the urinary tract or vaginal contamination of the specimen; triple phosphate crystals in urine is not harmful but

Table 1
Antibiotic susceptibility test result against Gram - positive UTI isolates

Urine isolates (No.)	ANTIBIOTIC TESTED										% resistance
	S	SXT	E	PEF	CN	APX	Z	AM	R	CPX	
<i>S. aureus</i> (1)	+	+	+	+	+	+	+	+	+	-	90
<i>S. aureus</i> (2)	+	-	-	-	-	+	+	+	-	-	40
<i>S. aureus</i> (3)	-	+	+	-	+/-	+	+	+	-	-	60
<i>S. aureus</i> (4)	-	-	+	-	-	+	+	+	-	-	40
<i>Staphylococcus</i> spp (1)	-	-	+	+	-	+	+	+	+	+	70
<i>Staphylococcus</i> spp (2)	-	-	+	-	-	+	+	+	-	-	40
<i>Staphylococcus</i> spp (3)	-	-	-	-	-	+	+	+	+	-	40
<i>Staphylococcus</i> spp (4)	+/-	+	+	-	-	+	+	+	-	-	60
<i>Staphylococcus</i> spp (5)	+	+	+	+	+	+	+	+	+	-	90
<i>Staphylococcus</i> spp (6)	-	-	-	-	-	+	+	+	-	-	30
<i>Staphylococcus</i> spp (7)	+	-	+	-	-	+	+	+	-	-	50
<i>Staphylococcus</i> spp (8)	-	-	-	-	-	+/-	+	+	-	-	30
<i>Staphylococcus</i> spp (9)	-	+	+	-	+/-	+	+	+	-	-	60
<i>Staphylococcus</i> spp (10)	-	-	+	-	-	+	+	+	-	-	40
Total resistance (%)	35.71	35.71	71.43	21.43	28.57	100	100	100	28.57	7.14	

- = susceptible; +/- = intermediate; + = resistant; sp = species; S = Septrin 30 µg, E = Erythromycin 10 µg, PEF = Pefloxacin 10 µg, CN = Gentamycin 10 µg, APX = Ampiclox 30 µg, Z = Zinnacef 20 µg, AM = Amoxicillin 30 µg, R = Rocephin 30 µg and CPX = Ciprofloxacin 10 µg.

indicates the pH is highly alkaline while presence of *Schistosoma* cells and numerous red blood cells indicates the infection, schistosomiasis^{10,11}. Urinalysis results showed the presence of protein, nitrite and moderate to high glucose concentration in urine samples. Presence of protein in urine known as proteinuria is usually an indication of kidney disorders including glomerulonephritis and urinary tract infection. Nitrates are found in normal urine and are as a result of feeding on a diet containing vegetables. They are reduced to nitrites by pathogens such as *E. coli*, *Proteus* species and *Klebsiella* species when in sufficient concentration in the urine. On the other hand, presence of glucose in urine is an indication of diabetes mellitus^{11,19}. The different urine isolates recovered were *Escherichia coli* (24.6%), *Staphylococcus* species (17.4%), *Staphylococcus aureus* (11.6%), *Proteus* species (11.6%), *Pseudomonas aeruginosa* (10.1%), *Candida* species (10.1%), *Enterobacter* species (7.2%), *Klebsiella* species (4.3%) and *Candida albicans* (2.9%). This agrees with the findings of LAUPLAUD *et al.*¹¹ which gave the percentage of *E. coli* implicated in urine of patients in Calgary Health Region Intensive Care Unit in Canada as 23%. Only 2.9% of *Candida albicans* were recovered compared to 20% found by LAUPLAUD and co-workers¹¹ suggesting that they were not nosocomial infections. However, the percentage of *E. coli* (24.6%) disagrees with the findings of AIYEGORO *et al.*¹ and ZEIGHAMI (2008)²¹ which were respectively 52.6% and 55.9%. AIYEGORO *et al.*¹ working with out-patients aged 5-18 years visiting Obafemi Awolowo University Teaching Hospital Complex Ile-Ife in Nigeria also isolated *Klebsiella* spp. (25%), *Proteus* spp. (3.9%) and *P. aeruginosa* (2.8%). ZEIGHAMI (2008)²¹ working with urine of patients that had kidney transplant and asymptomatic bacteriuria also isolated *Klebsiella* spp. (22.4%), *P. aeruginosa* (8.6%), and *Proteus* spp. (5.2%). These organisms

have been said to be common cause of nosocomial infections^{3,4}. We also isolated these organisms though the percentage of *Klebsiella* species (4.3%) was smaller than those reported above. *Staphylococcus* species and *Staphylococcus aureus* are normal body flora and must have come from the environment. The fact that more urine samples from females (36.2%) showed significant microbial growth as against males (19.4%) is in line with the findings of AIYEGORO *et al.*¹ who reported bacteriuria only in urine from females. KWOK *et al.*¹⁰ sampled children aged 0-18 years and showed that UTI in girls was (34.4 episodes/1000 persons) as against males (4.4 episodes/1000 persons) and this agrees with our finding. The nature and proximity of the urinary system and the anus in women may be the reason why women had more bacteria growth in their samples than men since many organisms from feces can easily infect or contaminate the urinary system in females.

The antibiotic susceptibility profile of the urine isolates showed that *E. coli* (76.7%), *Klebsiella* species (63.3%), *S. aureus* (57.5%), *P. aeruginosa* (53.3%), *Staphylococcus* (51%) and *Proteus* (38%) species were resistant (not susceptible) to conventional antibiotics used. BROOKS *et al.*³ stated that antibiotics such as derivatives of penicillin (ampicillin and amoxicillin), sulphonamides, and the quinolones (nitrofurantoin and nalidixic acid) are effective urinary antiseptics. The genera *Escherichia*, *Klebsiella* and *Pseudomonas* from urine show multiple resistances to antibiotics with *Klebsiella* species and *E. coli* noted to be resistant to broad-spectrum β -lactam drugs like penicillins and third generation cephalosporins²⁰. All *S. aureus*, *Klebsiella* species, *P. aeruginosa* and 83.33% of *E. coli* in our study were resistant to four or more antibiotics. Among the *E. coli*, 83.33% were resistant to septrin

Table 2
Antibiotic susceptibility test result against Gram – negative UTI isolates

Urine isolates (No.)	ANTIBIOTIC TESTED										% resistance
	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	
<i>E. coli</i> 1	+	+	+	+	+	+	+/-	+	+	+/-	100
<i>E. coli</i> 2	+/-	-	+/-	-	+/-	-	-	-	-	+/-	40
<i>E. coli</i> 3	-	-	+	-	-	-	-	-	-	-	10
<i>E. coli</i> 4	+	+	+	+/-	+	+	+/-	+	+/-	+	100
<i>E. coli</i> 5	+	+	+	+	+	+	-	+	+	+	90
<i>E. coli</i> 6	+	+	+	+	+	+	+	+	+	+/-	100
<i>E. coli</i> 7	+	+	+	+	+	+	+	+	+	+/-	100
<i>E. coli</i> 8	+	+	+	+	+	+	+/-	+	+	+/-	100
<i>E. coli</i> 9	-	-	+	+	+	-	-	-	-	-	30
<i>E. coli</i> 10	+	+	+	-	-	-	-	+/-	+/-	+/-	60
<i>E. coli</i> 11	+	-	+	+	+	+	+	+	+	+/-	90
<i>E. coli</i> 12	+	+	+	+	+	+	+	+	+	+/-	100
<i>Klebsiella</i> spp 1	+	-	-	-	+/-	+	+	-	-	+	50
<i>Klebsiella</i> spp 2	+	+	+	+/-	+	+	+/-	-	-	+/-	80
<i>Klebsiella</i> spp 3	+	+	+	-	+	+	-	-	-	+	60
<i>Proteus</i> spp 1	-	-	-	-	-	+	-	-	-	-	10
<i>Proteus</i> spp 2	+	+	+	-	+	+/-	-	+	+	-	70
<i>Proteus</i> spp 3	+	+	+	-	+	+	-	-	-	-	50
<i>Proteus</i> spp 4	+/-	+	-	-	-	+	-	-	-	-	30
<i>Proteus</i> spp 5	+/-	+	-	-	-	+	-	-	-	-	30
<i>P. aeruginosa</i> 1	+	+	+	-	-	+	-	-	-	-	40
<i>P. aeruginosa</i> 2	+	+	+	-	-	+	-	-	-	-	40
<i>P. aeruginosa</i> 3	+	+	+	+	+	+	-	+	+	-	80
% resistance	87.0	73.9	82.6	47.8	69.6	82.6	39.1	47.8	47.8	56.5	

- = susceptible; +/- = intermediate; + = resistant; spp = species, SXT = 30 µg septrin; CH = 30 µg chloramphenicol; SP = 10 µg sparfloxacin; CPX = 10 µg ciprofloxacin; AM = 30 µg Amoxicillin; AU = 30 µg Augmentin; CN = 10 µg Gentamycin; PEF = 30 µg perfloracin; OFX = 10 µg tarivid; S = 30 µg streptomycin.

(cotrimoxazole), amoxicillin and streptomycin; 75% to ciprofloxacin (a quinolone mostly prescribed currently), tarivid and perfloracin (also quinolones) etc. These findings validate the data from WILLEY *et al.*²⁰. It also falls in line with the findings of SAHM *et al.*¹⁸ where 95.8% of *E. coli* isolated was resistant to ampicillin and 92.8% to trimethoprim-sulfamethoxazole (drug of choice in United States). The 83.3% *E. coli* resistant to cotrimoxazole and amoxicillin is supported by the data from ODUYEBO *et al.*¹⁵ where all *E. coli* showed resistance to amoxicillin-clavulanic acid and 87.5% to cotrimoxazole and also in OLSON *et al.*¹⁶ in which a total of 80.1% *E. coli* tested was resistant to ampicillin.

All *S. aureus* isolates were resistant to Amoxicillin Clavulanate. In ONANUGA *et al.*¹⁷, all *S. aureus* tested were resistant to ampicillin. Similarly, *Klebsiella* species were all resistant to cotrimoxazole and augmentin while all *P. aeruginosa* were also resistant to cotrimoxazole, chloramphenicol and augmentin. High resistance of *Klebsiella* species to cotrimoxazole was observed by ODUYEBO *et al.*¹⁵ where it was 87.5%

while that of *P. aeruginosa* to amoxicillin/clavulanate (Augmentin) and cotrimoxazole is supported by findings of AIYEGORO *et al.*¹. Our findings and those of others seem to contradict the statements of BROOKS *et al.*³ who reported that excretion of high percentage of ampicillin and amoxicillin in urine as a basis for their preference in treatment of acute urinary tract infections. This contradiction might have resulted from abuse of these drugs in the environment leading to urinary pathogens becoming more resistant to them.

In conclusion, we observed high resistances of isolates to antibiotics which is consistent with the information in literature. Furthermore, test results with broad spectrum antibiotics showed that Gram-negative isolates are showing higher resistances than Gram-positives.

Some of the limitations of our study include the non-determination of the MICs of the tested antibiotics and the non- inclusion of control strains in the susceptibility studies because they were not available.

RESUMO

Perfil da frequência, análise urinária e suscetibilidade de patógenos causando infecções do trato urinário no estado de Enugu, Sudeste da Nigéria

Objetivo: O estudo teve como objetivo determinar a frequência e agentes causadores das infecções do trato urinário (UTIs) em indivíduos com sintomas desta infecção no estado de Enugu, Sudeste da Nigéria e determinar a suscetibilidade antibiótica dos agentes microbianos isolados de cultura da urina. **Métodos:** O estudo envolveu 211 indivíduos (149 mulheres e 62 homens) clinicamente suspeitos para UTI. Amostras urinárias foram coletadas pelo método de meia corrente "clean catch" e testadas por procedimentos standards. Suscetibilidade aos antibióticos dos patógenos isolados foi testada usando a técnica de Kirby-Bauer e de acordo com as diretrizes do "Clinical and Laboratory Standards Institute" (CLSI). **Resultados:** Microscopia das amostras de urina centrifugadas mostraram que 16 pacientes tinham piúria enquanto que 54 tinham células do pus. Cristais de oxalato de cálcio foram encontrados em 14 amostras. Análise de amostras da urina mostraram que 17 tinham proteína; sete eram positivas para nitrito e três tinham concentração de glicose de moderada para alta. Cinquenta e quatro amostras de urina (36.2% de mulheres) e 12 (19.45% de homens) mostraram crescimento significante após cultura. Coloração pelo Gram e testes bioquímicos identificaram nove organismos diferentes com a *Escherichia coli* sendo a mais comum das espécies isoladas. Quarenta e três espécies selecionadas ao acaso foram testadas posteriormente para sua suscetibilidade contra um painel de antibióticos. Trinta amostras isoladas (81.08%) foram resistentes a quatro ou mais antibióticos sendo que a maior resistência foi da *E. coli* (76.67%). Todas as amostras Gram negativas isoladas foram resistentes a Ampicilox, Cefuroxime e Amoxicilina.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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