

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3
AJCEM/1824 [Shttp://www.ajol.info/journals/ajcem](http://www.ajol.info/journals/ajcem)
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AFR. J. CLN. EXPER.MICROBIOL. 19 (3): 171-176

A SURVEY ON URINARY TRACT INFECTION ASSOCIATED WITH TWO MOST COMMON UROPATHOGENIC BACTERIA

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ABSTRACT

The aim of this study was to determine the prevalence of urinary tract infection (UTI) caused by the uropathogens – *Escherichia coli* and *Klebsiella pneumoniae* among outpatients attending a hospital in Benin and the antibiotic susceptibility pattern of both isolates. Mid-stream urine samples were collected from 96 patients, analysed microscopically and streaked on the surface of prepared plates of blood and MacConkey Agar. Isolates resulting from incubated plates were identified by standard microbiological techniques. Susceptibility testing was done using the disk diffusion technique on Mueller Hinton Agar. The prevalence of UTI was 22.9%. It was significantly more prevalent in females (30.6%) than males (8.8%) ($p < 0.05$) and in age group 31 – 40 years (45.5%). *Escherichia coli* was the most commonly isolated and more prevalent (14.6%) followed by *Klebsiella pneumoniae* (5.2%). High level resistance (100%) to amoxicillin/clavulanic acid, ampicillin and ceftazidime was observed among the isolates. The active drugs against the bacteria were nitrofurantoin, ofloxacin and imipenem. Routine diagnostic procedure/screening is necessary to avoid complications.

Keywords: Infection, prevalence, resistance, uropathogens.

UNE ENQUÊTE SUR L'INFECTION DES VOIES URINAIRES ASSOCIÉS À DEUX PRINCIPALES BACTÉRIES UROPATHOGÈNES

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Résumé

Le but de cette étude était de déterminer la prévalence de l'infection d'appareil urinaire (UTI) causée par *Escherichia coli* uropathogènes - et *Klebsiella pneumoniae* chez les patients fréquentant un hôpital au Bénin et le modèle de sensibilité aux antibiotiques des isolats des deux. Les échantillons d'urine mi-jet ont été recueillis auprès de 96 patients, analysés au microscope et de rayés sur la surface des plaques préparées du sang et de la gélose MacConkey. Les plaques d'isolats résultant d'incubés ont été identifiées par des techniques microbiologiques standard. De sensibilité a été réalisée en utilisant la technique de diffusion sur gélose Mueller Hinton. La prévalence de l'UTI était de 22,9 %. Il a été significativement plus élevée chez les femmes (30,6 %) que chez les hommes (8,8 %) ($p < 0,05$) et dans le groupe d'âge 31 - 40 ans (45,5 %). *Escherichia coli* a été le plus souvent isolés et plus fréquente (14,6 %) suivi par *Klebsiella pneumoniae* (5,2 %). La résistance de haut niveau (100 %) à l'amoxicilline-acide clavulanique, à l'ampicilline et la ceftazidime a été observée parmi les isolats. Les médicaments actifs contre les bactéries ont été nitrofurantoin, l'ofloxacine et l'imipénème. Procédure de diagnostic/dépistage systématique est nécessaire pour éviter les complications.

Mots clés : Infection, prévalence, résistance, uropathogènes.

INTRODUCTION

Bacteria are the commonest cause of both community and hospital-acquired infections globally. Infection of the urinary tract is the second most prevalent infection (after respiratory tract infection), but the most hospital-acquired infection accounting for 39 – 40% of total number of reported cases by acute health

care hospitals (1, 2, 3). Urinary tract infection (UTI) refers to the presence, growth and multiplication of microbial pathogens within any organ that make up the urinary system, including the two kidneys, two ureters, single bladder and urethra (4).

The system is concerned with the filtering out of excess fluid and other substances from the blood stream in the form of urine (which is a good growth medium for many species of bacteria though normally sterile) (5, 6).

Infections of the kidney (pyelonephritis), bladder (cystitis) and urethra (urethritis) are among the commonly reported infections (7, 8), often leading to the presence of bacteria in the urine and could be asymptomatic or symptomatic depending on the host immune status and virulence of the invading pathogen. UTIs caused by bacteria are mostly common while that by fungi or viruses are rare (6), but related to abnormalities of the urinary system or its instrumentation (catheterisation) (9, 10). *Escherichia coli* is the cause of 80 - 85% of community acquired and 50% of hospital acquired UTIs (11, 12). *Klebsiella* and *Proteus* species account for 5 - 10%, and the Gram positive species such as *Staphylococcus saprophyticus*, accounts for another 5 - 10% of UTIs in young women (5). Several studies conducted in Nigeria and other parts of the world have reported *Escherichia coli* as the major organism isolated in significant numbers from urine followed by *Klebsiella pneumoniae* (in some studies) (13, 14, 15, 16). The frequency of these pathogen depends on age, sex, hospitalization and urethral catheterisation and thus are predisposing factors (17) as they could affect the prevalence of urinary tract infections (UTIs) (18).

Strains that cause UTIs often originate from the gut/intestinal flora of the patient, with infection occurring in an ascending manner. Uropathogenic *Escherichia coli* adheres to uroepithelial cells by means of adhesins, pili, type 1 and P fimbriae - all of which induce cytokine production and chemotaxis of neutrophils, initiating an inflammatory response (19, 20). Other known virulence factors produced by Gram negative uropathogens include haemolysins, capsule and proteases which contribute to damaging host cells and evading the immune system (21, 22).

Some strains that cause UTIs are resistant to the drugs used for treatment. This creates the need for their detection and susceptibility profile on a regular basis as it would enhance therapeutic decision. Thus, the aim of this study was to determine the prevalence of UTI due to *Escherichia coli* and *Klebsiella pneumoniae* among outpatients attending the Central Hospital, Benin, and the susceptibility pattern of both isolates.

Subjects and Method

A total of 96 outpatients attending the General Outpatients Department of the Central Hospital,

Benin were included in this study after obtaining relevant ethical clearances from the Hospital Management Board and the Central Hospital's management. Subjects who were on antibiotic therapy were excluded. Data of subjects including age and sex were obtained from laboratory record/request form.

Specimen collection and bacterial isolation

Voided mid-stream urine specimens were collected in sterile universal containers. Samples were examined microscopically and loopful (0.001ml) streaked onto the surface of dried plates of blood and MacConkey Agar (Lab M, UK). Culture plates with bacteria counts greater than or equal to 10^5 cfu/ml of urine were considered as positive for UTI according to Cheesbrough (7). Isolates were identified by standard microbiological techniques including morphological (Gram staining) and biochemical (catalase, coagulase, indole, citrate, voges-proskauer and sugar fermentation) tests.

The prevalence/isolation rate of *Escherichia coli* or *Klebsiella pneumoniae* was determined by the formula:

$$= \frac{\text{Number of isolates recovered}}{\text{Total number of samples screened}} \times \frac{100}{1}$$

Susceptibility testing

Sensitivity of the isolates to 16 antibiotics was performed using the disk diffusion technique on Mueller Hinton Agar (Lab M, UK). Zones of inhibition diameter measured in millimeter (mm) were interpreted using the interpretive chart published by the Clinical and Laboratory Standards institute (CLSI, 2012) and reported as sensitive (S), intermediate (I) and resistant (R).

Statistical analysis

Data were analysed using SPSS 16.0. Chi-square test was used to test for significance.

RESULTS

A total of 96 urine samples were studied. The patients included 34 (35.4%) males and 62 (64.6%) females giving a male to female ratio of 1: 1.82. Their age range was from 21 - 80 years with a mean age of 33.7 years. A total of 22 patients had UTI with majority of the isolates recovered from age groups 31 - 40 years (45.4%) and 21 - 30 years (31.8%) but was most prevalent in the former age group (45.4%) (Table1). The prevalence was also higher in females (30.6%) compared to males (8.8%) (Table2). The total number of *Escherichia coli* and *Klebsiella pneumoniae* recovered were 14 (63.7%) and 5 (22.7%) respectively with *Escherichia coli* being most prevalent (14.6%) (Table3). No mixed culture was encountered.

TABLE 1: PREVALENCE OF UTI IN RELATION TO AGE AND GENDER

| Age | No. of specimen collected | Number positive | | Total positive(%) | Prevalence rate (%) |
|--------------|---------------------------|-----------------|-----------|-------------------|---------------------|
| | | Male | Female | | |
| 21 - 30 | 54 | 1 | 6 | 7 (31.8) | 12.9 |
| 31 - 40 | 22 | 2 | 8 | 10 (45.5) | 45.4 |
| 41 - 50 | 10 | 0 | 3 | 3 (13.6) | 30 |
| 51 - 60 | 5 | 0 | 1 | 1 (4.55) | 20 |
| 61 - 70 | 3 | 0 | 1 | 1 (4.55) | 33.3 |
| 71 - 80 | 2 | 0 | 0 | 0 (0) | 0 (0) |
| Total | 96 | 3 | 19 | 22 (100) | 22.9 |

TABLE 2: DISTRIBUTION OF BACTERIAL ISOLATES BY GENDER OF PATIENTS

| Organisms | Gender | | Total (%) |
|------------------------------|-----------------|------------------|-----------------|
| | Male (n = 34) | Female (n = 62) | |
| <i>Escherichia coli</i> | 2 (14.3) | 12 (85.7) | 14 (100) |
| <i>Klebsiella pneumoniae</i> | 0 (0) | 5 (100) | 5 (100) |
| <i>Staphylococcus aureus</i> | 1 (50) | 1 (50) | 2 (100) |
| <i>Proteus mirabilis</i> | 0 (0) | 1 (100) | 1 (100) |
| Total recovered (%) | 3 (13.6) | 19 (86.4) | 22 (100) |
| Prevalence rate (%) | 8.8 | 30.6 | 22.9 |

TABLE 3: FREQUENCY OF BACTERIAL ISOLATES IN URINE (N = 96 SAMPLES)

| Organisms | Number isolated (%) | Prevalence rate (%) |
|------------------------------|---------------------|---------------------|
| <i>Escherichia coli</i> | 14 (63.6) | 14.6 |
| <i>Klebsiella pneumoniae</i> | 5 (22.7) | 5.2 |
| <i>Staphylococcus aureus</i> | 2 (9) | 2.1 |
| <i>Proteus mirabilis</i> | 1 (4.55) | 1 |
| Total | 22 (100) | 22.9 |

The comparative antibiotic susceptibility pattern of isolates revealed a high level resistance to amoxicillin/clavulanic acid (100%), ampicillin (100%) and ceftazidime (100%) by both groups of organisms (Table 4). *Escherichia coli* isolates were more resistant to cefpodoxime (71.4%), cefotaxime (64.2%) and

ceftriazone (71.4%) than *Klebsiella pneumoniae* isolates with 20% resistant to each drug respectively. Nitrofurantoin, ofloxacin and imipenem were the active drugs against the isolates as indicated by the sum of percentage sensitive and intermediate.

TABLE 4: COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *Escherichia coli* AND *Klebsiella pneumoniae* ISOLATES

| Antibiotic (µg) | <i>Escherichia coli</i> (n = 14) | | | <i>Klebsiella pneumoniae</i> (n=5) | | |
|----------------------------------|----------------------------------|----------|-----------|------------------------------------|--------|---------|
| | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Amoxicillin/clavulanate (20/10) | 0 (0) | 0 (0) | 14 (100) | 0 (0) | 0 (0) | 5 (100) |
| Ampicillin (10) | 0 (0) | 0 (0) | 14 (100) | 0 (0) | 0 (0) | 5 (100) |
| Cefuroxime (30) | 0 (0) | 0 (0) | 14 (100) | 0 (0) | 0 (0) | 5 (100) |
| Cefpodoxime (10) | 3 (21.4) | 1 (7.1) | 10 (71.4) | 4 (80) | 0 (0) | 1 (20) |
| Cefotaxime (30) | 4 (28.5) | 1 (7.1) | 9 (64.2) | 4 (80) | 0 (0) | 1 (20) |
| Cefoxitin (30) | 6 (42.8) | 0 (0) | 8 (57.1) | 2 (40) | 1 (20) | 2 (40) |
| Ceftazidime (30) | 0 (0) | 0 (0) | 14 (100) | 0 (0) | 0 (0) | 5 (100) |
| Ceftriaxone (30) | 2 (14.2) | 2 (14.2) | 10 (71.4) | 0 (0) | 4 (80) | 1 (20) |
| Ciprofloxacin (5) | 1 (7.1) | 5 (35.7) | 8 (57.1) | 1 (20) | 1 (20) | 3 (60) |
| Cloxacillin (1) | 0 (0) | 0 (0) | 14 (100) | 0 (0) | 0 (0) | 5 (100) |
| Gentamicin (10) | 8 (57.1) | 0 (0) | 6 (42.8) | 1 (20) | 0 (0) | 4 (80) |
| Nitrofurantoin (300) | 9 (64.2) | 2 (14.2) | 3 (21.4) | 3 (60) | 2 (40) | 0 (0) |
| Ofloxacin (5) | 9 (64.2) | 4 (28.5) | 1 (7.1) | 3 (60) | 1 (20) | 1 (20) |
| Piperacillin (100) | 3 (21.4) | 4 (28.5) | 7 (50) | 1 (20) | 1 (20) | 3 (60) |
| Piperacillin/tazobactam (100/10) | 8 (57.1) | 4 (28.5) | 2 (14.2) | 1 (20) | 2 (40) | 2 (40) |
| Imipenem (10) | 5 (35.7) | 8 (57.1) | 1 (7.1) | 3 (60) | 1 (20) | 1 (20) |

Key: S =sensitive, I = intermediate, R = resistant, µg = microgram

DISCUSSION

The genitourinary system is one of the portals of entry for pathogens and infection can occur anywhere along the tract. Urinary tract infections (UTIs) due to *Escherichia coli* and *Klebsiella pneumoniae* are among the widely encountered in clinics worldwide as both organisms have displayed marked predominance in the invasion of the tract. Infections due to the former organism are more common compared to the latter which is often devastating in immunocompromised patients (23).

In this study, the overall prevalence of UTI was 22.9%. This is higher than 2% (Abakaliki), 9.2% (Ethiopia) and 13% (Abuja) (16, 4, 24). On the other hand, the rate recorded is comparatively lower than 25% (Bangladesh), 39.69% (Okada), 43.1% (Jordan) and 48% (Rivers) (25, 14, 26, 27). The reason for the slightly low prevalence observed in this study may be due to the fact that only outpatients were studied. Outpatients are at lower risk of infection than inpatients who may have been predisposed by urethral catheters (28, 29) or other debilitating diseases. This is

well buttressed by a study where the prevalence of UTI among inpatients (36%) to be three times that among outpatients (11%) (24).

In a related development, the prevalence/isolation rate of *Escherichia coli* and *Klebsiella pneumoniae* as observed in this study was 14.6% and 5.2% respectively. Thus, *E. coli* is the most prevalent and predominant cause of UTI in our environment ($p < 0.05$) – a situation that is linked to its myriad of virulence factors. This trend has also been respectively observed in similar studies: 22% and 13.7% (24); 16.5% and 1.7% (14); 15.4% and 5.7% (26). Slight deviation from this pattern was recorded with *K. pneumoniae* being the most prevalent (17.3%) compared to *E. coli* (12.7%) (27). *Staphylococcus aureus* was also reported to be the most prevalent (28%) in a different study in Benin (30).

Also in this study, majority of the isolates were recovered from age group 31 – 40 (45.5%) followed by 21 – 30 (31.8%) but the highest prevalence was seen in the former age group (45.4%). This is partly in line

with a similar study where UTI was most prevalent in age group 38 - 42 (66.7%) (26). This finding is however contrary to two separate studies that reported highest prevalence in age groups 21 - 30 (44.67%) (14) and 18 - 29 (46%) (27), though there is a level of closeness in these age groups. Nevertheless, the high prevalence recorded in the above age groups is related to the indiscriminate sexual activity and high pregnancy rate (among females) that characterize these groups - all which are predisposing factors.

With respect to gender, UTI was significantly more prevalent among females (30.6%, 1 in 3) compared to males (8.8%, 1 in 11) ($p < 0.05$). Females were thus 3.4 times more likely to develop UTI than males. This finding has been observed in other studies (11, 13, 30, 27). Higher prevalence of UTI in females has been attributed to physiological and anatomical differences in both sexes. UTIs occur more frequently in women than men because the shorter, wider, female urethra appears to be less effective in preventing access of bacteria to the bladder (8). Different studies have also demonstrated that women who are prone to UTIs possess epithelial cells with significantly more receptors for uropathogenic bacteria than healthy controls (31, 32). The similarities, disparities and variations in prevalence rates recorded in different studies may be partly due to population difference and criteria (inclusion/exclusion) used by different researchers (14).

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The comparative antibiotic susceptibility pattern revealed a high level resistance by *Escherichia coli* and *Klebsiella pneumoniae* isolates to amoxicillin/clavulanic acid, ampicillin, cefuroxime and ceftazidime. Suffice to add, these isolates were multidrug resistant which might have been due to the expression of several resistance mechanisms (including production of multiple beta-lactamases) arising from selective pressure (33, 34). This makes the choice of drug for empirical treatment a challenging task. Similar observations have also been reported in other studies (4, 14, 35). However, the active drugs were nitrofurantoin, ofloxacin and imipenem. Low resistance to the last drug (imipenem) is related to its high cost and difficulty in accessibility which have often discouraged its abuse. Nevertheless, organisms causing UTI in different patient population differ in their pathogenic potentials and susceptibility to antimicrobials (22).

Conclusion

The human urinary tract is normally sterile except for the distal end of the urethra closest to the external environment. Microbial invasion of this tract is quite common leading to a wide range of clinical conditions in millions of people worldwide. This study has implicated *Escherichia coli* as the major cause of UTI especially in females of reproductive age. Some of the aetiologic agents were also found to be multidrug resistant to the penicillins and cephalosporins. Regular fluid intake and routine diagnostic procedure/screening are necessary to avoid complications.

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