Antimicrobial Susceptibility Profile of Urinary Tract Pathogenic Infections in Diabetic Patients Attending a Health Facility in Kumasi, Ghana

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Authors' contributions

This work was carried out in collaboration between both authors. Author MAP performed data curation, investigated the data, wrote the protocol and the first draft of the manuscript. Author MGA presented the conceptualization, supervised the work, checked the validation, review, editing and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study seeks to investigate the antimicrobial susceptibility profile of urinary tract pathogenic infections in diabetic patients attending a health facility in Kumasi, Ghana.

Study Design: A total of 285 patients were recruited using Cochran’s formula at a prevalence of 26.4% for this study from patients attending the University Hospital from April 2018 to October 2018. Data were collected using a structured questionnaire.

Methodology: Clean-catch midstream urine samples were screened for the presence of pathogenic bacteria and their antimicrobial susceptibility pattern using recommended culture methods.

Results: Out of the 285 patients, 125 (43.9%) were diabetic with 90 (72%) being female and 35 (28%) male. There was no association between UTI’s and gender (P=0.5799) with diabetic patients recording higher bacteriuria compared to non-diabetics (P< 0.001). Isolates from 113 (39.4%) of the samples were identified and these included, *Escherichia coli*, *Pseudomonas species*, *Klebsiella species*, *Proteus species* and *Staphylococcus aureus*. The most predominant was *E. coli* 62.

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1. INTRODUCTION

Diabetes Mellitus is rated among the top ten causes of deaths in the entire world and hence is a big public health concern [1]. It occurs as a result of increasing blood glucose levels due to improper functioning of the hormone insulin which regulates blood glucose levels. It is a great threat to the quality of life throughout the world with about 200 million people estimated to be living with diabetes mellitus by 2020. International Diabetes Federation estimated that 10.8 million people have diabetes mellitus in sub-Saharan Africa in 2006, and this would rise to 18.7 million by 2025 [2]. Diabetes in itself does not kill rapidly but once accompanied by other complications such as immunosuppression, urinary tract infections, hypertension and hyperglycemia, can be very fatal [3]. Globally, the incidence of urinary tract infection among diabetic patients is well known and account for several deaths.

Studies have shown that urinary tract infection are the most prevalent form of infection among diabetic patients [4]. In 2012, the United States of America revealed that over $2.3 billion dollars was used in the management of urinary tract infections (UTIs) in about 22 million patients with diabetes [4]. Also, the tremendous increase of urinary tract infection in the diabetic patients is known to have a direct influence on the occurrence of diabetic kidney infections and several other uropathogens worldwide [5].

There have been several reported cases of resistance to antibiotics by many pathogens that are associated with urinary tract infections. Tao et al. [1] in a study stated that some organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* species have developed resistance strategies against some conventional antibiotics. Borj et al. [6] and Hamdan et al. [7] report that *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Enterococcus* spp., *Staphylococcus aureus*, and coagulase negative staphylococci (CoNS) are the most common bacteria associated with UTI in diabetics. An increase in resistance to antibiotics could lead to about 10 million deaths globally each year as projected by O'neil et al. [8]. The successful treatment of UTI in diabetic patients depends on the proper identification of the bacteria responsible and the selection of effective antimicrobial agents against them [9].

Most pathogens associated with diabetic patient's urinary tract infections are highly prone to changing patterns and though studies have been conducted in this area, yet the situation still persists and hence needs a pragmatic study that will give current information on the topic. There is not much data on the relation of UTIs among patients with diabetes on the African continent. This study seeks to identify pathogens associated with urinary infections among diabetic patients and the antibiotic susceptibility patterns of these microbes. This will provide information on the above which will guide the trend of treatment therapeutics.

2. MATERIALS AND METHODS

2.1 Study Site and Design

The study was conducted in the University Hospital of the Kwame Nkrumah University of Science and Technology, Kumasi, in the Ashanti Region of Ghana from April 2018 to October 2018. Geographically, the Hospital is located at the middle zone of Ghana with 6°40'N. 1°37'W coordinates. A total of 285 people were recruited...
for this study using a simple random sampling technique.

2.2 Sampling
The sample size was calculated using Cochran’s formula at a prevalence of 26.4%. This included all patients who were directed to the lab for routine urine examinations. Diabetic patients on any form of antibiotics for at least two (2) weeks were excluded from the study. Participants were selected at random and after assenting to take part in the study and was instructed how to collect a clean-catch midstream urine specimen (i.e. how to collect at least 10ml of early morning mid-stream urine into a sterile container). The samples were correctly labelled with participant’s ID and transported to the microbiology laboratory of the Theoretical and Applied Biology Department for further microbial analysis.

2.3 Culturing and Identification of Isolates
The urine samples were aseptically inoculated onto a MacConkey/CLED agar (Oxoid, Hampshire, UK) and incubated at 37°C for 24–48h using a standard calibrated loop. Growth of bacteria colonies on the media were observed and the bacteria count determined. Significant bacteriuria was defined as urine culture greater than $10^5$ cfu/ml [10]. Morphological appearance of the colonies as well as Gram staining were used for identification of the bacteria. Various biochemical tests such as the catalase test, coagulase test, indole test, citrate test and oxidase test were used to confirm the presence of the bacteria.

2.4 Antimicrobial Susceptibility Pattern
For each of the uropathogens isolated, antimicrobial Susceptibility Test (AST) was done using the modified Kirby–Bauer disc diffusion method to identify resistant and sensitive isolates using recommended guidelines (CLSI, 2020). Sterile cotton swab was dipped into the suspension forming a uniform lawn over the entire surface of Mueller-Hinton agar (Oxoid, Ltd, UK). A total of 12 antimicrobials which include the following were used on the bacteria isolates. gentamicin (GEN; 10µg), tetracycline (TET; 30µg), ciprofloxacin (CIP; 5µg), amikacin (AMK; 10µg), nalidixic acid (NA; 30µg), norfloxacin (NIT; 10µg), ceftoxazone (CPZ; 10µg), ceftriazone (CTR; 30µg), piperacillin (PIL; 100µg), cefotaxime (CFX; 30µg), levofloxacin (LEV; 5µg) and nitrofurantoin (NIT; 50µg). The zone of inhibition was measured to the nearest millimeter after an overnight incubation at 37°C. The isolates were then classified based on CLSI [11].

2.5 Data Analysis
The data obtained were exported into the Statistical Package for Social Sciences software version 21.0 (SPSS Inc, Chicago, IL) for analysis. Descriptive statistics were used to summarize the findings. Tables and charts were used to show frequencies where appropriate. The chi-square test was used to look for the association between diabetics and Urinary Tract Infections. Those variables with a p value < 0.05 at 95% confidence interval were considered statistically significant.

2.6 Ethical Clearance
Ethical clearance was obtained from the committee on Human Research, Publication and Ethics of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Written informed consent was obtained from all study participants. Infected patients were treated based on their laboratory result.

3. RESULTS

3.1 Characteristics of Patients
Out of the 285 patients recruited, 199 (69.8%) were females and 86 (30.2%) were males. A total of 125 (43.9%) of the patient were diabetic with 90 (72%) being female and 35 (28%) being male (Fig. 1). Bacteria were isolated from the urine of 113 patients with the majority (39.6%) between 25-29 years and the least were samples from patients below 15 years (0.9%) (Table 1).

3.2 Bacterial Isolates
Of the total 285 urine samples analysed, bacterial isolates from 113 (39.4%) of the samples were identified and these isolates included, *Escherichia coli*, *Pseudomonas species*, *Klebsiella species*, *Proteus species* and *Staphylococcus aureus*. Out of the total 113 isolates, 89(78.8%) were Gram negative while 24(21.2%) were Gram-positive bacteria. The most predominant was *Escherichia coli* 62 (54.9%) followed by *Staphylococcus aureus* 24 (21.2%), *Klebsiella species* 14(12.4%), *Pseudomonas species* 12(10.6%) and *Proteus species* 1(0.9%).

3.3 Isolates in Diabetic and Non-diabetic Patients
Bacteria were isolated from 82(72.6%) samples taken from diabetic patients with the distribution
as follows: *Escherichia coli* 48(42.5%), *Staphylococcus aureus* 18(15.9%), *Klebsiella species* 9(8.0%), *Pseudomonas species* 6(5.3%) and *Proteus species* 1(0.9%), (Fig. 2). The prevalence of distribution of bacterial isolates in the non-diabetic samples 31(27.4%) showed the same pattern as that of the diabetic samples with *Escherichia coli* recording the highest even though the numbers were significantly lower compared to the former (Fig. 2).

### 3.4 Factors Associated with UTIs

Out of the 113 patients with Urinary tract infections (UTIs), 81 (72%) of them were females and 32 (28%) were males. From the study, it was seen that gender was not associated with UTIs (p > 0.05). From the 113 urine samples, 82 (72.6%) were diabetic and 31 (27.4%) were non-diabetic. Being diabetic was significantly associated with the presence of bacteria in the urine (p< 0.05) (Table 2).

### 3.5 Antibiotic Susceptibility Pattern

A total of 12 antimicrobials were used on the bacterial isolates and the sensitivity and resistance patterns of the various bacteria showed that the organisms were multi drug resistant. *E. coli* and *Proteus* spp. which are Gram-negative pathogens were sensitive to most of the antibiotics, whereas *Klebsiella* and *Pseudomonas* spp. were less sensitive (Table 3). *E. coli* which was the most prevalent pathogen, showed a relatively high antimicrobial sensitivity rates against most of the tested antibiotics, that is, gentamycin (90.3%), amikacin (98.4%),

| Age group | Percentage (%) isolates |
|-----------|-------------------------|
| <15       | 1 (0.9)                 |
| 15-19     | 2 (1.8)                 |
| 20-24     | 6 (5.3)                 |
| 25-29     | 18 (15.9)               |
| 30-34     | 7 (6.2)                 |
| 35-39     | 14 (12.4)               |
| 40-44     | 15 (13.3)               |
| 45-49     | 7 (6.2)                 |
| 50-54     | 5 (4.4)                 |
| 55-59     | 10 (8.8)                |
| 60-64     | 12 (10.6)               |
| >Above 64 | 16 (14.2)               |
| Total     | 113 (100)               |

![Fig. 1. Gender and diabetic status of patients](image-url)
nalidixic (34%), cefotaxime (80.6%) and nitrofurantoin (93%). Proteus spp. on the other hand, showed 100% sensitivity to all the antibiotics except tetracycline, amikacin and cefotaxime. It was observed that *Escherichia coli* was mostly resistant to tetracycline (96.8%), norfloxacin (69.4%) and cefotaxime (61.4%). Similarly, *Klebsiella species* was most resistant to tetracycline (100%), norfloxacin (71.4%) and cefotaxime (75%). *Pseudomonas* were also shown to be most resistant to tetracycline (91.7%), norfloxacin (66.7%), ceftriaxone (66.7%), piperacillum (75%) and cefotaxime (75%). *Staphylococcus aureus*, which was the only Gram-positive bacteria isolates showed a higher level of resistance to tetracycline (100%) followed by norfloxacin (70.8%), cefotaxime (66.7%) and piperacillum (62.5%) in that order but were most resistant to gentamycin (100%) and nitrofurantoin (100%) (Table 3).

4. DISCUSSION

Studies have shown that Urinary tract infection (UTI) remains one of the most common infectious diseases diagnosed in the community [12,13]. The present study showed bacterial isolates were at a prevalence of 39.6%. Our results are relatively comparable with some earlier work, but was clearly higher than the 20–30% usually reported in diabetic patients [14]. Ranjar et al. [15] in their studies had found out that the percentage of bacteria isolated were 40% whilst studies conducted in certain states in Nigeria also had similar findings [16,17,1]. The high prevalence rate in our study may be due in part to factors such as unsanitary activities and low economic status. It is also reported that, in diabetic patients, specific risk factors for UTI are usually the duration of diabetes and the presence of long-term complications [18].

Analysis of the results showed that the age group 25-29(15.9%) had the highest number of bacterial isolates closely followed by 64+ (14.2%) age group. Similar studies conducted elsewhere, also showed that a high prevalence of bacteria isolated was observed for young adults between the ages of 21 to 30 years in the sub-Saharan region [16,19]. The differences in prevalence observed among the various age groups in this study might be due to the differences in sanitary conditions and observed personal hygiene. Our study contradicts that of Nicolle et al. [20] and
| Antibiotics         | Escherichia coli (%) | Staphylococcus aureus (%) | Klebsiella species (%) | Pseudomonas species (%) | Proteus species (%) |
|---------------------|----------------------|---------------------------|------------------------|-------------------------|---------------------|
|                     | S        | R      | S         | R      | S         | R      | S         | R      | S         | R      |
| Gentamicin (GEN)    | 56(90.3) | 6(9.7) | 24(100)   | 0(0)   | 10(71.4)  | 4(28.6) | 8(66.7)   | 4(33.3) | 1(100)    | 0(0)   |
| Tetracycline (TET)  | 2(3.2)   | 60(96.8)| 0(0)      | 24(100)| 0(0)      | 14(100)| 1(8.3)    | 11(91.7)| 0(0)      | 1(100) |
| Ciprofloxacin (CIP)| 41(66.1) | 21(33.9)| 14(58.3)  | 10(41.7)| 8(57.1)   | 6(42.8) | 9(75)     | 3(25)  | 1(100)    | 0(0)   |
| Amikacin (AMK)     | 61(98.4) | 1(1.6) | 23(95.8)  | 1(4.2) | 12(85.7)  | 2(14.3) | 10(83.3)  | 2(16.7)| 0(0)      | 1(100) |
| Nalidixic acid (NA)| 50(80.6) | 12(19.4)| 19(79.2)  | 5(20.8)| 10(71.4)  | 4(28.6) | 8(66.7)   | 4(33.3)| 1(100)    | 0(0)   |
| Norfloxacn (NIT)   | 19(30.6) | 43(69.4)| 7(29.2)   | 17(70.8)| 4(28.6)   | 10(71.4)| 4(33.3)   | 8(66.7)| 1(100)    | 0(0)   |
| Cefoperazone (CPZ) | 50(80.6) | 12(19.4)| 16(66.7)  | 8(33.3)| 13(92.9)  | 1(7.1)  | 7(58.3)   | 5(41.7)| 1(100)    | 0(0)   |
| Ceftriazone (CTR)  | 31(50)   | 31(50) | 13(54.2)  | 11(45.8)| 8(57.1)   | 6(42.8) | 4(33.3)   | 8(66.7)| 1(100)    | 0(0)   |
| Piperacillin (PII)  | 50(80.6) | 12(19.4)| 9(37.5)   | 15(62.5)| 7(50)     | 10(70)  | 3(25)     | 9(75)  | 1(100)    | 0(0)   |
| Cefotaxine (CFX)   | 24(38.7) | 38(61.7)| 8(33.3)   | 16(66.7)| 4(25)     | 10(75)  | 3(25)     | 9(75)  | 0(0)      | 1(100) |
| Levofloxacin (LEV) | 48(77.4) | 14(22.6)| 22(91.7)  | 2(8.3) | 11(75)    | 3(25)   | 9(75)     | 3(25)  | 1(100)    | 0(0)   |
| Nitrofurantoin (NIT)| 58(93.5)| 4(6.5) | 24(100)   | 0(0)   | 9(64.3)   | 5(35.7) | 10(83.3)  | 2(16.7)| 1(100)    | 0(0)   |

S= Sensitivity; R = Resistant
Foxman [21] who observed that bacteriuria is more present in adult men over the age of 64 but 30 times more common in younger adult women than men. Their explanation was that elderly men experience prostatic secretions, prostate enlargements, and reduced bactericidal activity. Many studies have also shown that, diabetes mellitus is associated with an increase in the prevalence of bacteriuria [21,22] and this is consistent with our findings, where 72.6% of them had bacteriuria (p < 0.001). In this study 72% of those with UTIs were females but there was no association between gender and UTIs. This prevalence is in accordance with studies conducted in Ethiopia and the United States of America by Worku, et al. [23] and Simkhada [24] respectively who reported that UTIs were found to be 3.6 times higher among diabetic females than males. Contrary to this study, Masinde et al. [25] and Nitzan et al. [26] reported that the risk of females in contracting UTIs is higher as compared to males due to the decrease in oestrogen levels which dries up the vagina affecting the vaginal microflora.

In this study, the general observation was that *E. coli* and Proteus spp. which are Gram-negative pathogens showed variable sensitivity to most of the antibiotics, whereas Klebsiella and Pseudomonas spp. were less sensitive. Even though *E. coli* is known to be the most common Gram-negative bacteria associated with UTI, other bacteria such as *Klebsiella*, *Pseudomonas*, *Proteus* and *Staphylococcus aureus* are also known to be highly prevalent and associated with UTI [27]. The study demonstrated that the bacterial isolates were most sensitive to amikacin (93.81%), nitrofurantoin (90.7%), gentamicin (87.6%), levofloxacin (80.53%), nalidixic acid (77.88%), cefoperazone (77%), ciprofloxacin (64.6%) and piperacillin (61.06%). However, they were mostly resistant to tetracycline (97.20%), norfloxacin (69.03%) and cefotaxime (65.49%) which supports a similar study conducted by Mama et al. [28]. Most antibiotics especially tetracycline is known to be very affordable and easily available hence, its abuse leading to its resistance. The resistance activity of norfloxacin and cefotaxime is alarming because these antibiotics are usually used in the treatment of urinary tract infection.

*S. aureus*, the only Gram-positive isolate, showed a 100% sensitivity to gentamycin and Nitrofurantoin with 100% resistance to tetracycline. The data for resistance of tetracycline obtained from this study were similar to the data previously published for studies conducted in Ethiopia (Yismaw et al. [29]). Reasons for the high resistance could be attributed to the use of antibiotics as a prophylactic treatment, easy availability, and indiscriminate use of antimicrobials. Based on sensitivity, gentamicin (87.6%), amikacin (93.81%), ciprofloxacin (64.6%), nalidixic acid (77.88%), piperacillin (61.06%), nitrofurantoin (90.7%), levofloxacin (80.53%) and cefoperazone (77%) were found to be the most effective. This indicates that these drugs are effective in treating UTI and their use should be varied to prevent development of resistant strains of a pathogen. Even though some studies have found some of these drugs to be ineffective, management and proper treatment of UTI is very essential in dealing with these infections.

5. CONCLUSION

The prevalence of this study indicated that UTI among diabetic patients was relatively comparable with other studies and that, there was no association between urinary tract infections (UTI’s) and gender. The most common isolates were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella species*, *Pseudomonas species* and *Proteus species*. Amikacin (93.81%) and nitrofurantoin (90.7%) were found to be the most effective even though gentamicin (87.6%) and levofloxacin (80.53%) were also effective. Tetracycline, norfloxacin and cefotaxime with resistivity of (97.20%), (69.03%) and (65.49%) respectively, should not be used in the treatment of UTI.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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