ABSTRACT

Diabetes mellitus is one of the most challenging health problems of 21st century and is the fifth leading cause of death in developed countries. Asian patients are considered to have a higher risk of developing diabetes and potentially worse prognosis. By 2025, the number of individuals with diabetes is expected to be more than doubled. Urinary tract infection (UTI) poses serious future clinical repercussions, such as hypertension, anemia, kidney failure and death. Therefore, there is the need for the early detection and diagnosis of UTI. Currently, the use of the standard microbiological culture diagnostic method is hindered by the microbial tendency of being viable but non-cultural. On the other hand, molecular detection by direct Polymerase Chain Reaction (PCR) technique has proven to be more sensitive in detecting pathogens in various biological samples, this work was thus

INTRODUCTION

One of the common complications of diabetes is its effect on genito-urinary system. Diabetic patients are at increased risk of urinary tract infections. Diabetes causes several abnormalities in the host system that increases the risk of urinary tract infection. These include immunologic
impairments such as defective migration, phagocytic alteration of chemotaxis in polymorph nuclear leukocytes[1]. Diabetics are at increased risk of developing acute pyelonephritis, renal abscess, bladder scarring and myelitis. Emphysematous pyelonephritis is almost exclusively an infection of diabetic patients and carries a grave prognosis; papillary necrosis complicates 21% of cases. Treating UTIs with broad spectrum antibiotics is widely practiced; however, because of concerns about infection with resistant organisms, treating with a narrow spectrum antibiotic may be more appropriate[2]. It is more preferred to use fluoroquinolones as initial agent for empiric therapy of UTI in an area where there is a high concern about antibiotic resistance. The reason for this is their high bacteriological and clinical cure rates, and low resistance rates among most uropathogens.

**PROBLEM STATEMENT**

The conventional means of diagnosing UTI has long been the use of urine culture and antibiotic sensitivity testing. This microbiological technique has time limitation, requiring 2 to 3 days, which delays commencement of any treatment intervention. As a common practice, in the absence of supportive laboratory evidence, due to lack of a high resolving and fast laboratory diagnosis to detect microbial infection, clinicians usually initiate empirical antibiotic treatments. But such an approach could also unnecessarily expose patients to antibiotics with little or no success in the containment of infections. Therefore, a faster, more sensitive and specific diagnostic tool for microbial detection in urine samples for assessing UTI is required[3].

**JUSTIFICATION**

The PCR has proven to be a powerful and rapid tool for amplifying and detecting minute quantities of DNA in various biological samples. It takes a maximum of six hours to obtain results. Small amounts (micrograms or nanograms) of samples are needed to detect microorganisms present[4]. The diagnostic uniqueness of PCR is the ability to detect some pathogens that are undetectable by microbiological culturing methods. Specimens such as blood, sputum, cerebrospinal fluid and urine have been successfully screened for bacteria and other microbes, using the PCR technique[5].

**MAIN OBJECTIVES OF THE STUDY**

1. Isolation of pathogens from diabetic urinary tract infection patients.
2. Identification by using morphological and biochemical tests.
3. To use dipstick and classical microbiological culturing method to determine presence of E. coli in urine of subjects
4. To design a PCR assay based on dominant virulence genes (pap and usp) of uropathogenic E. coli strains for diagnosis of Urinary Tract Infection.
5. To determine the performance characteristics of the diagnostic methods.

MATERIALS AND METHODS

1- COLLECTION OF SAMPLES

First meeting was to get the participants informed about the objectives of the study and its significance. A questionnaire was administered and participants provided with the sample bottles to be used for the collection of the urine. Urine samples were collected from near hospitals. Early morning mid-stream urine samples of about 10-15 ml were collected; using UV sterilized plastic bottles with air-tight screw cap tops. Each urine sample bottle was labelled with a reference code, age, sex, and time of collection. The samples were placed in a cold box for transportation to the laboratory, where it was stored until analyses were carried out. All samples were analyzed with the microbial culture method and a 27 sub-population were analyzed with the dipstick, microbial culture and PCR methods.

2 URINE DIPSTICKS

Urine samples were preliminarily analyzed for UTI by use of DIRUI A10 urine test strips. This was done within the first two hours of sample collection. Each urine strip was dipped in urine and analyzed according to the manufacturer’s instructions.

MICROBIOLOGICAL CULTURE METHOD

MacConkey broth media was employed to determine the Most Probable Number (MPN) of microbes in all 27 samples. Serial dilutions of $10^{-6}$, $10^{-7}$ in addition to the raw sample, were used, with 1 ml of each solution added to 5 ml of the broth in triplicates and incubated at 44°C for 48 hours and add three drops of Kovac’s reagent were added to the test tubes. A positive test for E. coli presence was indicated by colour change from yellow to pink. A positive test for E. coli indicated a reddish ring formed at the surface of the broth. Positive E. coli test tubes were then plated, using SS agar and incubated at 37°C. E.coli presence was confirmed by identification of rose-red colonies (Stamm et al., 1982; Stark and Maki, 1984)[6].

PCR METHOD

1 PREPARATION OF BACTERIAL DNA.

This test was carried out on 27 of the urine samples among the one patient sample did PCR who showed positive both URINE
DIPSTICK and microbial method. DNA to be amplified was obtained from 400 μl aliquots of urine samples initially dispensed into sterile 1500 μl Eppendorf tubes and centrifuged at 11,000 rpm for 30 seconds[7]. Aliquots of 200 μl were pipetted from the supernatant into sterile 1500 μl Eppendorf tubes and incubated at 120°C for 15 minutes in a water bath, similar to the protocol described by Le Bouguenec et al (1992). Resultant template DNA solution was stored as template DNA stock and short-spinned for use in PCR reaction [8].

2 AMPLIFICATION PROCEDURES
PCR was done in a total volume of 10.5 μl, containing 1.5 μl of the template DNA, each of the primers at 10 μM, the four deoxynucleoside triphosphates (each at 250 μM), 10 mM Tris hydrochloride (pH 9), 1.5 mM MgCl2, 30 mM KCl and 1U of DNA polymerase (Bioneer, . PCR amplifications consisted of an initial denaturation at 94°C for 2 minutes and 30 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 1 min, and extension at 72°C for 2 minutes and finally held at 4°C for 7 minutes in a Thermal Cycler (Eppendorf mastercycler, Germany). Five microliters of the reaction mixture was then analyzed by electrophoresis on 2% agarose gels and 3 μL of ethidium bromide. The resulting electrophoretic band was compared to a DNA size ladder corresponding to the products of the amplification, while viewing with the aid of a UV-trans-illuminator (TFM-26, USA) and image captured with a digital camera (Samsung, 12.5 megapixels, Republic of Korea)

RESULTS
PREVALENCE OF UTI
A total of 27 urine samples were analyzed, using microbiological culture method to determine the total UTI prevalence (30.88%) by uropathogenic E. coli. Mean age of males (15 years) in Table 3 below was higher than that of females (14) with no significant difference (p= 0.2130). Conversely, females had a higher prevalence of 34.88% and 27.27% for males with no significant difference (p= 0.1903 the sub-populations used for comparing the three diagnostic tests had a mean age of 14.97±0.10 with males being significantly older (15.16±0.14 years) than females (14.74±0.14) with p value 0.0351. Leukocyte esterase parameter in dipstick analysis detected more females having UTI (40.90%) than males (24.30%) with p value 0.0320. In addition, the culture method detected more females significantly having UTI (39.77%) compared to males (25.23%) with a p value of 0.0320. The PCR method parameters (papC, usp, papC or usp and papC+usp) showed no significant differences between males and females. Males recorded higher percentages for papC (43.93%), papC or usp (79.44%) and papC+usp (20.54%) than females.
Table 1: Overall prevalence of UTI caused by uropathogenic E. coli, using the standard microbiological culture method.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>27</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>14.84±0.08</td>
<td>15.04±0.11</td>
<td>14.62±0.11</td>
<td>0.2330</td>
</tr>
<tr>
<td>No. of positives</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>30.88</td>
<td>27.27</td>
<td>34.88</td>
<td>0.1963</td>
</tr>
</tbody>
</table>

* = significant difference  
\( p < 0.05 \) at 95% confidence interval

Table 2: Various parameters determined for a sub-population of 27 subjects.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>27</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>14.97±0.10</td>
<td>15.16±0.14</td>
<td>14.74±0.14</td>
<td>0.0351*</td>
</tr>
<tr>
<td>Leukocyte esterase (%)</td>
<td>6(31.79)</td>
<td>2(24.30)</td>
<td>4(40.90)</td>
<td>0.0142*</td>
</tr>
<tr>
<td>Nitrite (%)</td>
<td>2(1.03)</td>
<td>0(0.00)</td>
<td>2(2.27)</td>
<td>0.2024</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>10(51)</td>
<td>10(51)</td>
<td>0(0.00)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Blood (%)</td>
<td>8(41.0)</td>
<td>4(3.74)</td>
<td>4(4.55)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Culture (%)</td>
<td>6(31.79)</td>
<td>3(25.23)</td>
<td>4(39.77)</td>
<td>0.0320*</td>
</tr>
<tr>
<td>( p_{C} ) (%)</td>
<td>16(38.97)</td>
<td>9(42.92)</td>
<td>7(32.65)</td>
<td>0.1406</td>
</tr>
<tr>
<td>( asp ) (%)</td>
<td>10(32.82)</td>
<td>4(30.47)</td>
<td>8(55.88)</td>
<td>0.4755</td>
</tr>
<tr>
<td>( p_{C} ) or ( asp ) (%)</td>
<td>14(76.41)</td>
<td>7(79.44)</td>
<td>7(67.72)</td>
<td>0.3108</td>
</tr>
<tr>
<td>( p_{C} ) and ( asp ) (%)</td>
<td>6(18.46)</td>
<td>4(26.56)</td>
<td>2(15.90)</td>
<td>0.4609</td>
</tr>
</tbody>
</table>

Figure 1: Urinalysis dipstick showing positive nitrite test.

Figure 2: Agar plate showing E. coli colonies growing on Salmonella Shigella agar.

Figure 3: Growths of E. coli as rose-red colonies (Figure 5) after streaking of urine samples on SS agar and incubating for 24 hours at 37°C.

**Dipstick test**

UTI prevalence of 31.79% was found using the dipstick method. Females had a higher prevalence (42.05%) than males (23.36%). The highest prevalence for females was recorded at age 14 (42.05%). Prevalence for the total and that of males were highest at age 15 with 23.36% respectively.

**PCR results**

A general prevalence of 75.88% was recorded for UTI using the PCR method. Females contributed a higher percentage of UTIs.
(42.05%) than males (23.36%). Figure 8 shows the different distribution of papC and usp genes in E. coli isolate from one of the female patients who have positive on urinary tract infection showed positive on PCR.

Figure 3: An ethidium bromide-stained agarose gel electrophoregram of amplified usp genes of uropathogenic E. coli DNA of different template preparation. [C= Control, A and A1= Denatured samples at 120°C, B= Sample without denaturation and M= DNA Marker]

DISCUSSION

The general prevalence of UTI caused by E. coli was 30.88% (Table 2), which is lower than the 52.77% value realized. The prevalence for this study is also lower than the 44.53% range observed for young adults between the ages of 14 to 15 years in the sub-region. This difference in prevalence could be based on differences in sanitary conditions and observed personal hygiene. Again, the higher prevalence of E. coli causing UTI in adult sth the observed value for a dolescent sample population may be due to sexual activity. According to Table 4, the mean age of males (15.16±0.14) was significantly higher than females (14.74±0.14) with p-value 0.0351. This suggests that males are more affected than females at the junior high school level. Females having statistically significant (p=0.0142) positive tests (40.90%) for leukocytes in the urine. The parameter of the study is the observation that the higher prevalence of UTI among females was 39.77% in the microbial culture, indicating a 39.77% prevalence in females compared to males (24.3%). This indicates the higher prevalence in the microbial culture method. This observation could be explained as being due to an increase in the number of uropathogenic organisms and infections which peaks at 14 and 15 years. Thus, these high numbers of uropathogenic E. coli were not detected in the present study.

Figure 4: An ethidium bromide-stained agarose gel electrophoregram of amplified papC genes in uropathogenic E. coli from UTI samples. [C= Control, 1-3= Denatured samples at 120°C and M= DNA Marker]
overall prevalence of 51.01% (25). This means that about half of the number of E. coli isolates employ fimbriae formation for adherence to epithelial cells to cause UTI. Moreover, it could be a result of most UTI detected being first-time infections, requiring pathogen adherence to epithelial cells, in order to colonize the urinary bladder. Hence, pathogens adapt through the production of adhesion structures like \( \text{papC} \). The \( \text{papC} \) prevalence of 51.01% is greater than the 21.1% detected in the Brazilian study and the 45% for immunocompromised populations in Slovenia (26). The observed differences might have arisen from both the sample populations and the composition number of males and females in these populations. It is also possible that differences due to geographic locations come because the factor that determines the deviation from the 94% formed on immunocompinor populations in literature (27). However, the difference in distribution with respect to gender could be justified by the fact that isolates from cystitis, a condition that predominates in females, have been found to have a lower prevalence of \( \text{papC} \), hence the smaller percentage of 32.95% determined in females (28). Moreover, some strains of E. coli isolates detected in males with UTI may be involved in first-time infections which require adhesion structures made possible by the \( \text{papC} \) gene, contributing to a higher prevalence of the gene, compared to females. Both males and females recorded \( \text{uspgene} \) as the predominant virulence gene, with 63.53% and 76.56%, respectively. Although there was a significant difference between genders. However, there was a statistical difference between \( \text{uspgene} \) and \( \text{papC} \) gene \((p=0.0020)(29)\). The overall prevalence of 52.82% for the \( \text{uspgene} \) was close to the 68.5% determined for women and the 79-93% range in mixed populations in Japanese studies (30). This indicates the importance of the \( \text{uspgene} \) for E. coli survival and persistence in the microbial community involved in urinary tract infections. Indeed, the higher percentage of \( \text{uspgene} \), compared to \( \text{papC} \) gene observed in isolates from females could be due to the fact that infection by different uropathogens can be greatly enhanced by the shorter urinary tract of females. This increase the diversity of the microbial community within the urinary tract, leading to the acquisition of the \( \text{uspgene} \) by E. coli to produce bacteria into eliminate competing uropathogens. It can also be argued that some of the UTI recorded might not have been first episode infection, but urinary infections made possible by reservoir uropathogens in the urinary tract. Thus, these E. coli forming part of the reservoir microbial community must have adapted by resorting to the use of the \( \text{uspgene} \), to eliminate other competing microbes, by the production of bacitracin. The two parameters of dipstick, i.e., leukocyte esterase and nitrite, and PCR were analyzed for their sensitivity, specificity and reliability in detecting UTI among schoolchildren in Ashanti region, using the microbial culture method as the gold standard. The leukocyte esterase sensitivity of 52% fell out of the 67-94% range for sensitivity components of dipstick urinalysis, employed by the WHO (WHO, 2005). This might have been due to the osmotic UTI cases detected being in the early stages in the urethra. As a result, these infections could not trigger enough influx of leukocytes, to produce high concentrations of leukocyte esterase for positive test ethnicity by colour generation on the dipstick. Moreover, the leukocyte esterase sensitivity (52%) could be attributed to dipstick defect in thereactive ingredients for detecting leukocyte esterase, leading to an increase in false negatives and decreasing its sensitivity. Value of 52% and negative predictive...
evaluation of 76% for leukocyte esterase is an indication that comparatively, the parameter better serves as an index for correctly eliminating a patient as not having UTI when the test result is negative (30).

Specificity value of 99% for nitrite which fell within the range of 90-100% (W.H.O., 2005) is a strong indicator of the parameter correctly testing negative for non-UTI samples. However, the extremely high value may be due to false positives caused by exposure of the dipstick to air. Low sensitivity (3%) for nitrite may have been primarily caused by low nitrite diets leading to a low level of nitrite by nitratereductase-negative microorganisms which could not show positive tests for UTI in positive samples. In addition, a positive predictive value of 1% is an indication of nitrite having a very low likelihood of a patient having the disease given that the test is positive. On the other hand, the negative predictive value of 68% is of relevance to physicians as it shows that nitrite has a higher probability for an individual not having UTI when the test is negative. PCR being a good technique for detecting the low presence of uropathogens in urine samples. However, the 29% of samples with E. coli undetected by PCR could probably have been due to sampling of DNA template from solution (31). Moreover, a lower specificity of 24% could be linked to false positives arising from a less sensitive culture standard, unable to detect some uropathogens or the PCR detecting the low presence of E. coli without satisfying the quantitative threshold comparable to the 10^4 CFU/mL cut-off in the culture standard for diagnosis. This result raises concern for a false positive predictive value of PCR as diagnostic test in UTI.

UTI prevalence of 30.88% obtained in this study raises concern, as it could have serious immediate or future repercussions. Fevers and pain associated with symptoms of UTI can disrupt the sound frame of mind needed by school-going adolescents and hinder their academic performance. Students infected with UTIs could also grow anaemic in advanced stages of the infection, due to kidney damage. This is a result of deficiency in reproduction of the hormone erythropoietin in the kidney which controls red blood cell formation. Currently, microbiological culture remains the standard method for UTI diagnosis and prevalence determination. However, established at the indicator that culture results could be contaminated by pathogenesis of faecal origin and this is a major shortcoming of the microbiological culture method (32). PCR method for diagnosing UTI, on the other hand can be strain-specific, depending on the set of primers used for diagnosis, as shown by this study. Moreover, performance comparison between PCR and microbiological cultures showed its superiority as a diagnostic method (33). Thus, PCR provides a more reliable result for UTI screening with respect to pathogen strain specificity.

REFERENCES


