

Prevalence of Uropathogenic *Escherichia coli* among Adult Male Patients 40 Years and above with Haematuria and Impaired Kidney Attending General Hospitals in Benue State

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Abstract

Haematuria is the presence of red blood cells in urine. It is most often caused by urinary tract infections of which *Escherichia coli* is frequently implicated. Impairment of kidney functions could occur as a result of infection or other complications of the kidney. The aim of the study was to determine the prevalence of uropathogenic *Escherichia coli* among adult male patients with haematuria and impaired kidneys attending a general hospital in Benue state. Three hundred and sixty-eight (368) samples of urine were collected from 368 male patients (≥ 40 years) attending the 23 general hospitals in Benue state. Each of the urine samples was divided into two parts for haematuria and isolation and identification of *Escherichia coli*. Blood samples (368) were also collected from the patients and used for quantitative determination of creatinine and estimation of glomerular filtration rate. The presence of haematuria was 45.1% and ranges from 12.5% to 100%. Prevalence of haematuria with respect to age shows that patients within the age group of 90 - 99 years had the highest rate (100%) and the least were those within the ages of 40 - 49 years (20.0%). Isolation rate of uropathogenic *Escherichia coli* was 16.3% and ranged from 6.3 to 37.5%. Patients within the age group of 90 - 99 years had the highest elevated impaired renal function of 4 (80%), followed by patients within the ages of 80 - 89 years [17 (77.3%)] and the lowest were those within the ages of 40 - 49 [6 (10.0%)]. The overall presence of haematuria in the patients was high (45.1%) with similar high *Escherichia coli* isolation rate and impaired renal function which could mean that acute or chronic kidney disease may set in.

Keywords

Uropathogens, Haematuria, *Escherichia coli*, General Hospital, Impaired

Kidney Function

1. Introduction

Escherichia coli are a normal gastrointestinal tract flora of human infants found within a few hours of birth [1]. *Escherichia coli* are found in water, soil and vegetables [2]. Uropathogenic *Escherichia coli* (UPEC), one of the members of extra-intestinal pathogenic *E. coli* (EXPEC) is a predominant pathogen causing urinary tract infections (UTIs). It is one of the main causes of community (80% - 90%) and nosocomial acquired urinary tract infections (30% - 50%) [3].

Certain *Escherichia coli* mediate various diseases, including intestinal and extra-intestinal disorders in humans and animals worldwide [4]. The clinical manifestations of infection with *Escherichia coli* depend on the site of the infection and cannot be differentiated by symptoms or signs from processes caused by other bacteria [5].

Urinary tract infections are the most common human bacterial infections and are responsible for substantial morbidity and mortality [6]. In most cases of UTI caused by *Escherichia coli*, the host fecal flora is the source of the infecting *Escherichia coli* strain and spread via the perineal, vaginal, and periurethral areas to the lower urinary tract where they may establish colonization [7]. A variety of host factors, such as age, gender, pregnancy, or immunological status, may predispose to UTI and allow less virulent pathogens to become very virulent [7]. If the infection confines to the lower urinary tract, with symptoms such as dysuria and frequent urination, the infection is cystitis. If the infection spreads to the upper urinary tract with symptoms such as flank pain, fever, and malaise, the infection is defined as acute pyelonephritis [6]. Different virulent mechanisms help uropathogenic *Escherichia coli* to colonize the bladder. Such virulence factors play critical roles in the pathogenesis of urinary tract infections. The factors include but are not limited to surface structural components, such as polysaccharide capsules, lipopolysaccharide (LPS), outer-membrane vesicles, flagella, pili, other adhesins, outer-membrane proteins (OMPs), as well as secreted toxins, secretion systems, and TonB-dependent iron-uptake receptors, including siderophore receptors [8].

Haematuria means there is blood in urine. In some haematuria conditions, there is blood urine seen macroscopically known as gross haematuria. Microscopic haematuria occurs when there is a presence of blood in the urine seen only microscopically. Haematuria is a common diagnosis in individuals who visit urologists [9] [10]. Haematuria may be found in urinary schistosomiasis (usually with proteinuria), bacterial infections, acute glomerulonephritis (inflammation of the glomeruli of the kidneys), sickle cell disease, leptospirosis, infective endocarditis, calculi (stone) in the urinary tract, malignancy of the urinary tract, and hemorrhagic conditions [11].

The aim of this study was to determine the prevalence of uropathogenic *Escherichia coli* among adult male patients 40 years with haematuria attending a general hospital in Benue state, Nigeria. Adult male patients' urine samples from the 23 general hospitals in Benue state were collected and analysed for haematuria. The urine was also inoculated on appropriate media for *E. coli* isolation and identification. Blood samples of the same patients were used for the analyses of urea creatinine and estimation of glomerular filtration rate. Data analysis for significant relationships between or among variables at a 95% confidence interval was conducted.

2. Materials and Methods

2.1. Sample Size

Sample size was determined using the formula [12]:

$$S = \frac{Z_1 - \alpha/2^2 - P(1 - P)}{d^2}$$

where;

- S = Sample size been sought;
- $Z_1 - \alpha/2^2$ = Standard normal variant (at 5% type 1 error $p < 0.05$) it is 1.96;
- p= Expected proportion in population based on previous or pilot study (62.2%) it 0.622;
- d = Absolute error or precision (0.05) [12]. The sample size was 361, but for fair representation 368 were collected, that is 16 samples per general hospital.

2.2. Ethical Approval, Inclusion and Exclusion Criteria

Ethical approval was obtained from the ethical committee of the Benue State Hospitals Management Board (HMB/OFF/215/VOL.II453).

Adult male patients from forty years (40 years) and above with hematuria (those urinating with visible blood in the urine), who were not on any antibiotic therapy for the past two weeks (2 weeks) before the sample was collected were included. Patients who were not on any antibiotic therapy for the past two weeks (2 weeks) before the sample was collected were included. Patients excluded from the study were those on antibiotic therapy or any local anti-microbial agents prior to sample collection and those male patients below forty years (40 years). Excluded were also those who failed to give their urine. Those male patients though forty years (40 years) and above but showed no visible sign of blood in the urine were also excluded.

2.3. Media Preparation

Media used for the isolation and identification of *E. coli* were obtained commercially and were prepared according to manufacturers' instruction. The media included Cysteine Lactose Electrolytes Deficient Medium (CLED) (Oxoid, CM 0398), Eosin Methylene Blue Agar (EMB) (Oxoid, CM 0069), Muller Hinton Agar

(MHA) (Oxoid, CM00339), Simmons Citrate agar (Oxoid, CM 0155) and Triple Sugar Iron agar (TSI) (Oxoid, CM0277).

2.4. Sample Collection and Inoculation

The study was done in Benue state, Nigeria. The samples were collected from the twenty-three (23) General Hospitals in the State from 1st November, 2021 to 30th April, 2022. Urine and blood specimens were collected from the patients. The blood samples were separated and dispensed into “cryo” vials and were ice packed. The urine was collected in sterile urine containers and triple packed, transported to the microbiology laboratory of Quality Assurance (QA) Diagnostics, High Level Makurdi. The urine was inoculated on cysteine-lactose-electrolyte-deficient (CLED) agar. Each sample of urine was inoculated onto a CLED plate, and incubated at 37°C for 24 h. Plates were examined for bacterial growth. Bacterial growth of $\geq 10^5$ colony-forming units was taken to be significant. Suspected colonies were sub-cultured repeatedly on eosin methylene blue (EMB) agar to obtain pure cultures.

2.5. Identification of Isolates

Colonies of *Escherichia coli* on CLED were identified by colonial morphology, Gram staining, motility test, oxidase test, citrate test, triple sugar iron agar and indole test.

2.6. Molecular Identification of *Escherichia coli* Using Polymerase Chain Reaction (PCR)

Successive washing in nuclease-free water and centrifugation as described by Sambrook and Russell [13] were used for the extraction and purification of bacterial DNA for *Escherichia coli* identification. Polymerase chain reaction (PCR) was carried out to amplify the 16SrRNA of the *Escherichia coli* gene using the primer pair F: CGT GAT CAG CGG TGA CTA TGA C and R: CGA TTC TGG AAA TGG CAA AAG. The PCR reaction was carried out using the Solis BioDyne ready to load master mix (5×). Polymerase chain reaction was performed in 25 μ l of a reaction mixture. The reaction concentration was brought down from 5× concentration to 1× concentration containing 1× blend master mix buffer (Solis Biodyne). One and half (1.5 mM MgCl₂, 200 μ Mol) of each deoxy nucleoside triphosphates (dNTP) (Solis Biodyne), 25 pMol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proof-reading Enzyme, 5 μ l of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in a TECHNE 3 Prime thermal cycler for an initial denaturation of 95°C for 5 minutes. Thereafter followed by denaturation at 95°C for 30 seconds, annealing at 61°C for 1 minute (the annealing temperature is determined by the primer used), and extension at 72°C for 2 minutes, and a final extension step of 10 minutes at 72°C. The total number of cycles was 35 cycles.

Agarose gel electrophoresis for the *Escherichia coli* 16SrRNA genes was performed on the amplification products using 1.5% agarose gel at 80 V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining.

The amplicon generated from the 16srRNA was Sanger sequenced at Epoch Life Science Inc., Texas (USA) and the corresponding nucleotide sequences were BLAST in the NCBI GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the representative isolate.

2.7. Phylogenetic Tree

The maximum likelihood method and Tamura-Nei model [14] were used. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value.

2.8. Determination of Haematuria Using Urit Automated Urine Analyzer

The urine samples collected in sterile urine containers were used for the determination of blood in the urine. “Urit’ urinalysis stripe” (Urit 50 urine analyzer, Urit medical electronic Ltd. SN 5011817E) were dipped into each of the urine sample after gentle inversion for 8 times and excess urine shaded off by gentle tapping. The stripes were then separately inserted one after the other into “urit” Urine analyzer. The urit urine analyzer read the stripes automatically and displayed the results on the screen. Although other parameters like protein, ascorbic acid, bilirubin and urobilinogen were usually part of the result; our interest was only the presence of blood. Outcome of the results were recorded semi-quantitatively in plus such as +, ++, +++ depicting the degree of positivity.

2.9. Analysis and Estimation of Glomerular Filtration Rate

Serum and creatinine levels were determined using MNCHIP 4 automated chemistry analyzer (Tianjin MNCHIP Technologies Co. Ltd. Model: Pointcare M4). The reagents and the cartridges were brought to ambient temperature before butting and calibration. The bar code of the cartridge was scanned to get the kit details such as lot number, expiration date and kit constituents. The cartridge was placed on the analyzer cartridge hold and the blue film cover removed and pressed “ok”. The analyzer displayed a window for patient details. The analyzer run automatically and printed the results out, and the results were documented. The glomerular filtration rate (eGFR) was calculated using glomerular filtration rate calculation software. Normal glomerular filtration rate is approximately 120 mL/min/1.732m² [15].

2.10. Statistical Analysis

Statistical analyses were done using the Statistical Package for Social Sciences

(SPSS) version 17 (2008) Pearson's Chi-square test was used to determine associations between variables at 95% confidence level with $p \leq 0.05$ being considered to be indicative of a statistically significant relationship between two or more variables.

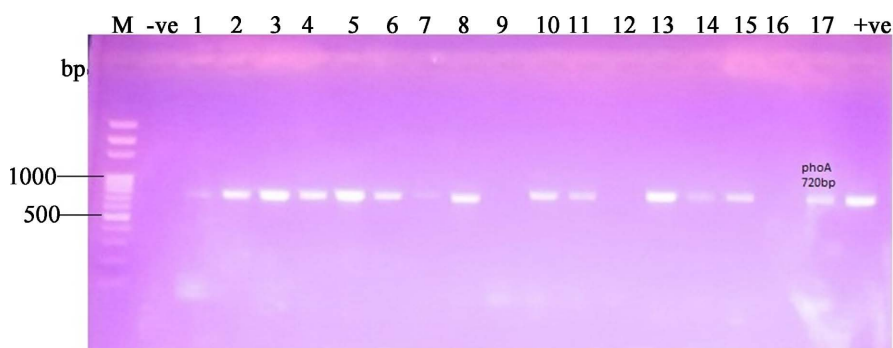
3. Results

The morphological and biochemical characteristics of *Escherichia coli* isolated showed that the colonies were yellowish on cysteine-lactose-electrolyte deficient agar (CLED) and pinkish on MacConkey agar. They were Gram-negative rods, motile, fermented lactose by producing acid with or without gas, methyl red positive and indole positive. They were Voges-Proskauer, citrate utilization, oxidase, urease, and gelatin liquefaction negative (Table not shown).

Figure 1 shows a gel image of the alkaline phosphatase gene (*PhoA*) for the molecular identification of *Escherichia coli* isolates. The presence of intense bands in lanes 2 (BU4), 3 (BU12), 4 (GJ8), 5 (GJ15), 6 (AL2), 8 (TA3), 10 (AD5), 11 (UA3), 13 (OT14), 14 (OJ13), 15 (WA6), 17 (VA11) and 18 (MK4) confirmed the presence of *Escherichia coli* at 720 bp.

Table 1 shows the sequence results of analysis of the *E. coli* isolates. Isolate NK5 with accession number CP110978.1, chromosomal DNA, had a complete genome sequence, 99% similarity and nucleotide length of 626. Isolate TA10 with accession number CP107197.1, chromosomal DNA, had a complete genome sequence, 98% similarity and nucleotide length of 619. Isolate MK7 with accession number AP023237.1, chromosomal DNA, had a complete genome sequence, 99% similarity and nucleotide length of 655, isolate VA3 with accession number CP097721.1, chromosomal DNA, had complete genome sequence, 99% similarity and nucleotide length of 654 and isolate MK15 with accession number AP026937.1, chromosomal DNA, had complete genome sequence, 99% similarity and nucleotide length of 663 (**Table 1**).

Figure 2 shows the results of analysis of phylogenetic tree constructed for the *E. coli* isolates using 16SrRNA gene sequences. Many strains of *E. coli* were identified

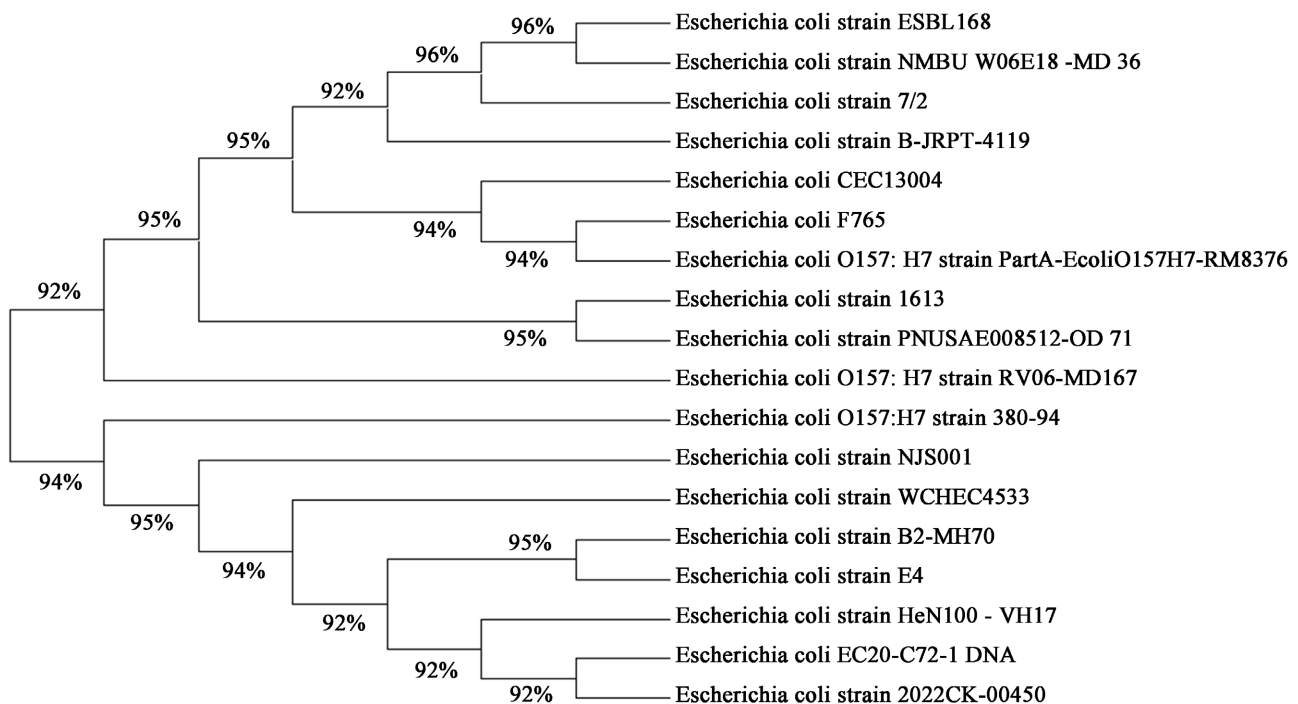


Key: M = DNA ladder, 1 - 18 *E. coli* isolates, -ve = Control (PCR reaction without DNA), PhoA = alkaline phosphatase gene (housekeeping gene for *E. coli*).

Figure 1. Agarose gel electrophoresis image for alkaline phosphatase gene in *Escherichia coli* isolates.

Table 1. Results of sequence analysis of *E. coli* isolates

Isolate ID No	Accession number	Bacteria isolate identified	Type of genome	% Similarity	Nucleotide length
NK5	CP110978.1	<i>E. coli</i> strain XYEH3934	cDNA, Complete genome	99	626
TA10	CP107197.1	<i>E. coli</i> strain KR001-HIC-0034	cDNA, Complete genome	98	619
MK7	AP023237.1	<i>E. coli</i> F070	cDNA, complete genome	99	655
VA3	CP097721.1	<i>E. coli</i> strain MS1665	cDNA, complete genome	99	654
MK15	AP026937.1	<i>E. coli</i> strain EC20-4B-2	cDNA, complete genome	99	663

**Figure 2.** Phylogenetic tree constructed based on 16S rRNA gene sequences.

with very high degree of relatedness ranging from 92% - 96% similarity.

The isolation rate of *Escherichia coli* in the 23 general hospitals is presented in **Table 2**. The total number of *Escherichia coli* isolated was 60 (16.3%). Patients from general hospital Gbajimba had the highest number of *Escherichia coli* isolate six (37.5%), followed by patients from Adikpo, Makurdi and Vandeikya; they had 4 (25.0%). General hospitals Igumale, Obagaji, Buruku, Aliade, Naka, Katsina-ala, Otukpa, Oju and Otukpo had the same number of isolate 3 (18.8%). General hospitals Tse-Agbaragba, Ugba and Obarike-Ito had the same number isolate 2 (12.5%). Patients from general hospitals Ugbokpo, Idekpa, Okpoga, Wannune, Sankera and Lessel were the least with isolate of 1 (6.3%). The rate of isolation of *Escherichia coli* in the 23 General Hospitals ranged from 6.3% to 37.5% while the overall *Escherichia coli* isolation rate was 16.3%. The differences in the isolation rate of *Escherichia coli* across the 23 General Hospital states were statistically significant ($\chi^2 = 60$, $df = 22$, $p < 0.05$).

Table 2. Isolation rate of *Escherichia coli* among adult male patients 40 years and above.

General hospital	Number investigated	Number positive (%)	Number negative (%)
Igumale	16	3 (18.8)	13 (81.3)
Obagaji	16	3 (18.8)	3 (81.3)
Ugbokpo	16	1 (6.3)	15 (93.8)
Buruku	16	3 (18.8)	13 (81.3)
Gboko	16	3 (18.8)	13 (81.3)
Gbajimba	16	6 (37.5)	10 (62.5)
Aliade	16	3 (18.8)	13 (81.3)
Naka	16	3 (18.8)	13 (81.3)
Katsina ala	16	3 (18.8)	13 (81.3)
Tse agbaragba	16	2 (12.5)	14 (77.8)
Adikpo	16	4 (25.0)	12 (75.0)
Ugba	16	2 (12.5)	14 (77.8)
Makurdi	16	4 (25.0)	12 (75.0)
Obarike ito	16	2 (12.5)	14 (77.8)
Otukpa	16	3 (18.8)	13 (81.3)
Idekpa	16	1 (6.3)	15 (93.8)
Oju	16	3 (18.8)	13 (81.3)
Okpoga	16	1 (6.3)	15 (93.8)
Otukpo	16	3 (18.8)	13 (81.3)
Wanune	16	1 (6.3)	15 (93.8)
Sankera	16	1 (6.3)	15 (93.8)
Lessel	16	1 (6.3)	15 (93.8)
Vandeikya	16	4 (25.0)	12 (77.8)
Total	368	60 (16.3)	308 (83.7)

χ^2 (% *E. coli* occurrence vs general hospitals) = 86.60, df = 22, p = 0.000 (p < 0.05).

Table 3 shows the prevalence of *Escherichia coli* isolation with respect to age. The highest isolation was among patients within the age range of 80 - 89 years 8 (36.4%). In descending order, the isolation rate is as follows: patients within the ages 70 - 79 years had 20 (21.3%), 90 - 99 years had isolation rate of 1 (20.0%), 60 - 69 years a rate of 20 (18.2%), 50 - 59 years had rate of 7 (7.1%), and the least being those within the ages 40-49 years with isolation rate of 4 (6.7%). The differences in the prevalence of *Escherichia coli* across the age groups were statistically significant. ($\chi^2 = 57.80$, df = 5, p < 0.05).

The prevalence of haematuria among the 368 patients in the 23 general hospitals in Benue state is given in **Table 4**. The prevalence of haematuria was

Table 3. Prevalence of *Escherichia coli* with respect to age (≥ 40 years).

Age group (years)	Urine examined	Positive (%)	Negative (%)
40 - 49	60	4 (6.7)	56 (93.3)
50 - 59	77	7 (7.1)	70 (90.9)
60 - 69	110	20 (18.2)	90 (81.8)
70 - 79	94	20 (21.3)	74 (78.7)
80 - 89	22	8 (36.4)	14 (63.6)
90 - 99	5	1 (20.0)	4 (80.0)
Total	368	60 (16.3)	308 (83.7)

χ^2 (% *E. coli* occurrence vs Age groups) = 57.80, df = 5, p = 0.000 (p < 0.05).

Table 4. Prevalence of haematuria among adult male patients.

General hospital	Number of urine samples tested	Number positive (%)	Number negative (%)
Igumale	16	8 (50)	8 (50)
Obagaji	16	7 (43.8)	9 (56.3)
Ugbokpo	16	8 (50)	8 (50)
Buruku	16	7 (43.8)	9 (56.3)
Gboko	16	7 (43.8)	9 (56.2)
Gbajimba	16	12 (75)	4 (25)
Aliade	16	15 (93.8)	1 (6.3)
Naka	16	16 (100)	0
Katsina Ala	16	14 (87.5)	2 (12.5)
Tse Agbaragba	16	12 (75.0)	4 (25.0)
Adikpo	16	5 (31.2)	11 (68.8)
Ugba	16	4 (25.0)	12 (75.0)
Makurdi	16	3 (12.5)	13 (81.3)
Obarike Ito	16	0	16 (100)
Otukpa	16	6 (37.5)	10 (62.5)
Idekpa	16	3 (12.5)	13 (81.3)
Oju	16	3 (12.5)	13 (81.3)
Okpoga	16	7 (43.8)	9 (56.3)
Otukpo	16	6 (37.5)	10 (62.5)
Wanune	16	5 (31.3)	11 (68.8)
Sankera	16	9 (56.3)	7 (43.8)
Lessel	16	5 (31.3)	11 (68.8)
Vandeikya	16	4 (25.0)	12 (77.8)
Total	368	166 (45.1)	202 (54.9)

Key: GH = general hospitals, χ^2 (% Distribution of Haematuria vs Gen Hospital) = 373.46, df = 22, p = 0.000 (p < 0.05).

45.1%, N = 368. Patients from general hospital Naka had the highest haematuria rate of 16 (100%), followed by patients from Aliade with a percentage haematuria of 15 (93.8%). Those from Katsina-Ala had haematuria rate of 14 (87.5%), those patients from Tse-Agbaragba had haematuria rate of 12 (75.0%) and patients from Gbajimba had haematuria rate of 12 (75.0%), those from Sankera had haematuria rate of nine (56.3%), while patients from Igumale and Ugbokpo had haematuria rate of eight (50.0%). Patients from general hospitals Gboko, Obagaji, Buruku and Okpoga all had a haematuria rate of seven (43.8%), those patients from Otukpo and Otukpa had a haematuria rate of six (37.5%). Patients from general hospitals Ugba, Wannune, Adikpo and Lessel had a haematuria rate of five (31.3%). Those patients from Vandeikya had a haematuria of four (25.0%), with the least from Makurdi, Idekpa and Oju with a haematuria rate of three (12.5%) each. All the patients from Obariko-Ito were haematuria negative.

Table 5 shows the prevalence of haematuria with respect to age. Patients between the ages of 90 - 99 years had the highest haematuria rate of 5 (100%), followed by those between the ages 70 - 79 years with a haematuria rate of (71.3%). Those between the ages 80 - 89 years had a haematuria rate of 15 (68.2%), Patients between the ages 60 - 69 years had a haematuria rate of 46 (41.8%) those between the ages 50 - 59 years had a haematuria rate of 21 (27.3%) and the least are those patients between the ages 40 - 49 years with a haematuria rate of 12 (20.0%). A total of 45.1% of the patients had haematuria while 54.9% were haematuria negative.

The prevalence of impaired renal function with respect to age is presented in **Table 6**. Patients within the age group of 90 - 99 years had the highest elevated impaired renal function of 4 (80%), followed by patients within the ages of 80 - 89 years with an elevated impaired renal function rate of 17 (77.3%). Those between the ages of 70 - 79 years had an elevated impaired renal function rate of 41 (43.6%), and patients between the ages of 60 - 69 years had an elevated impaired renal function rate of 37 (33.6%). Patients between the ages 50 - 59 years had an elevated impaired renal function rate of 12 (15.6%) and the least were those between the ages of 40 - 49 years with an elevated impaired renal function of six (10.0%). The overall impairment was 117 (31.8%). The difference in impaired

Table 5. Prevalence of haematuria with respect to age (≥ 40 years).

Age group (years)	Urine samples examined	Positive (%)	Negative (%)
40 - 49	60	12 (20.0)	48 (80.0)
50 - 59	77	21 (27.3)	56 (72.7)
60 - 69	110	46 (41.8)	64 (58.2)
70 - 79	94	67 (71.3)	27 (28.7)
80 - 89	22	15 (68.2)	7 (31.8)
90 - 99	5	5 (100)	0
Total	368	166 (45.1)	202 (54.9)

χ^2 (% distribution of haematuria vs Age group) = 84.56, df = 5, p = 0.000 (p < 0.05).

Table 6. Distribution of impaired renal function with respect to age.

Age group (years)	Serum examined	Impaired (eGFR < 60 mL/min/1.73m ²) (%)	Not impaired (eGFR > 60 mL/min/1.73m ²) (%)
40 - 49	60	6 (10)	54 (90)
50 - 59	77	12 (15.6)	65 (71.4)
60 - 69	110	37 (33.6)	73 (66.4)
70 - 79	94	41 (43.6)	53 (56.4)
80 - 89	22	17 (77.3)	5 (22.7)
90 - 99	5	4 (80)	1 (20)
Total	368	117 (31.8)	251 (68.2)

Key: eGFR= estimated glomerular filtration rate 1.73 m² =average surface area of an adult. mL/min = milliliter/minute. χ^2 (% distribution of impaired renal function versus age) = 51.50, df = 5, p = 0.001 (p < 0.05).

renal function with age groups was significant ($\chi^2 = 51.50$, df = 5, p < 0.05).

4. Discussion

In the study, 368 adult male patients' urine from the 23 general hospitals in Benue state were collected and analysed for the presence of haematuria. The urine was also inoculated on Cysteine lactose electrolytes deficient medium (CLED) for *E. coli* isolation and identification. From the urine samples, 16.3% were confirmed to be *Escherichia coli*. This slightly differs from the report of Menyfah *et al.* [16]. The researchers reported the work conducted by the emergency department of KAMC in Riyadh, Saudi Arabia. They reported a higher percentage of *Escherichia coli* (60.24%). Patients from general hospital Gbajimba had the highest rate of *Escherichia coli* isolation (37.5%), followed by Adikpo, Makurdi and Vandeikya patients with a 25.0% isolation rate. The rate of *Escherichia coli* isolation in the 23 general hospitals ranged from 6.3% to 37.5% with a statistically significant (p < 0.05) difference across the local governments. The isolation rate of *Escherichia coli* was compared with the age of the patients; the highest *Escherichia coli* isolation rate of 36.4% was seen among patients within the ages of 80 - 89 years. The implication of this finding is that there will be more burdens on their caregivers since most patients within this age group depend on other persons to foot their bills. This agrees with the work of Baudron *et al.* [17] carried out in 15 French Hospitals in Paris, France between January to December 2005. They reported that the majority of patients hospitalized for *Escherichia coli* bacteremia were aged > 65 years. Nicolle [18] carried out a study of urinary tract infections in a long-term care facility in a health Sciences Center, in Winnipeg, Ontario Canada; Marik and Zaloga [19] did their work on patients in 105 hospitals in the USA and Canada; Opal *et al.* [20] worked on "The immunopathogenesis of sepsis in elderly patients in the USA". They all stated that high infection rate in adults may be a result of several predisposing factors for infection

encountered in the elderly such as immunosenescence, denutrition, anatomical modification favoring bacterial colonization and frequent comorbidities, such as diabetes mellitus, chronic obstructive pulmonary disease, heart failure and renal insufficiency. Although the isolation rate in patients between the ages of 90 - 99 years was zero. This could result from the limited number of patients within this study age group at the time of the study.

The presence of haematuria in the patients was 45.1%. The implication of this is that continuous leaking of blood into the urine could drop the packed cell volume and subsequently anemia. This is in agreement with the work of Nikhil *et al.* [21]. Out of 1730 patients who attended a "One stop" Haematuria Clinic at Freeman Hospital, Newcastle-upon-Tyne between April 2003 and March 2006, from 1730 patients, 1061 male patients (61.3%) were recognized as having haematuria secondary to benign prostatic hyperplasia and no other pathology defined. Although patients from general hospital Naka had the highest haematuria rate of 100%, followed by patients from Aliade with a haematuria rate of 93.8% and patients from Kastina-Ala with a haematuria rate of 87.5%, patients from the other general hospital also had high haematuria rates. The implication of this high haematuria rate among the subjects is that it may escalate the economic burden on the patients and their relatives and patients' increased anxiety could follow. However, all the patients from Obariko-Ito were haematuria negative. The differences in the haematuria rate among the patients were significant ($p < 0.05$).

Older patients within the age group of 90 - 99 years had the highest rate of (100%) of haematuria. The implication of this is that the higher rate of haematuria in older patients could worsen their health conditions especially those with other co-morbidity like diabetes. A total of 45.1% had haematuria while 54.9% were haematuria negative. There were occasions where the presence of occult blood did not result in the presence of the target organism (*Escherichia coli*). This does not exclude the possibility of other causes of urinary tract (UTI).

One striking thing this study found out was the prevalence of impaired renal function (low glomerular filtration rate = eGFR) in most age groups. Patients between the ages of 90 - 99 years had the highest elevated impaired renal function (eGFR) at a rate of 80.0%. There were significant differences ($p < 0.005$) in impaired renal function among the various age groups. Patients with higher impaired renal function markers were of the older age groups. The implication of elevated renal impairment in this study is that acute kidney disease could emerge. The overall prevalence of impaired renal function was 31.8%.

The present study had some limitations including the inability to study genetic diversity of the different *Escherichia coli* from different patients. In addition, assessment of resistance genes could not be done on the basis of locations or geographical areas in the state owing chiefly to some financial bottlenecks. History of past medications that can affect the kidney was not known and could also be responsible for the blood in the urine. History of other conditions that can cause kidney damage such as type I and type II diabetes, heart diseases and obes-

ity were not known. Outside bacterial infection, other causes of hematuria such as trauma, vigorous exercise, and sexual activities and even parasitic infection were not part of the present study.

5. Conclusion

The overall prevalence of *Escherichia coli* isolated among adult male patients was 16.3% and ranged from 6.3% to 37.5%. The isolation rates were higher in older patients 80 - 89 years (36.4%). The presence of haematuria in the patients was 45.1%. Overall, this study recorded a higher incidence of haematuria in older age. The higher incidence of occult blood in the urine sample as well as the isolation of *Escherichia coli* from these patients concludes that there is infection and inflammation. The overall prevalence of impaired renal function was 31.8% implying that acute kidney disease could emerge.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] James, B.K., James, P.N. and Harry, L.M. (2004) Pathogenic *Escherichia coli*. *Journal of Nature Reviews*, **2**, 124-139.
- [2] Ochei, J. and Kolhatkar, A.A. (2008) Medical Laboratory Science. Theory and Practice. TataMacCraw-Hill Publishing Companies, New Delhi, 681-686.
- [3] Castillo, R.Y.F., Flores, M.C.A., Gonazalez, A.J.F., Diaz, M.F., Harel, J. and Barrera, G.L.A. (2018) An Evaluation of Multi-Drug-Resistant *Escherichia coli* Isolates in Urinary Tract Infections from Aguascalientes, Mexico: Cross Sectional Study. *Annals of Clinical Microbiology and Antimicrobials*, **17**, Article No. 34. <https://doi.org/10.1186/s12941-018-0286-5>
- [4] Pakbin, B., Bruck, W.M. and Rossen, J.W.A. (2021) Virulence Factors of Enteric Pathogenic *Escherichia coli*: Review. *International Journal of Molecular Sciences*, **22**, Article 9922. <https://doi.org/10.3390/ijms22189922>
- [5] Jawetz, E., Melnik, J.L. and Adelberg, E.A. (2007) Medical Microbiology. 24th Edition, Lange Medical, Los Angeles, 252-259.
- [6] Timothy, K. (2017) The Pathogenesis of *Escherichia coli* Urinary Tract Infection. *Journal of Intech Open*. <https://doi.org/10.5772/intechopen.69030>
- [7] Moreno, E., Andreu, A., Perz, T., Sabat, M., Johnson, J.R. and Parats, G. (2006) Relationship Between *Escherichia coli* Strains Causing Urinary Tract Infection in Women and the Dominant Faecal Flora of the Same Hosts. *Epidemiology and Infection*, **134**, 1015-1023. <https://doi.org/10.1017/S0950268806005917>
- [8] Maria, E.T., Giorgio, G.G. and Massimo, E.M. (2017) Uropathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-Antibiotic Antimicrobial strategies. *Frontiers in Microbiology*, **8**, Article 280574. <https://doi.org/10.3389/fmicb.2017.01566>
- [9] Peterson, L.M. and Reed, R.S. (2019) Hematuria. *Primary Care. Clinics in Office Practice*, **46**, 265-273. <https://doi.org/10.1016/j.pop.2019.02.008>
- [10] Barocas, D.A., Boorjian, S.A. and Alvarez, R.D. (2020) Microhematuria: AUA/SUFU

- Guideline. *Journal of Urology*, **204**, 778-786.
<https://doi.org/10.1097/JU.0000000000001297>
- [11] Cheesbrough, M. (2000) *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press, New York, 602-615.
- [12] Charan, J. and Biswas, T. (2013) How to Calculate Sample Size for Different Study Designs in Medical Research. *Indian Journal of Psychological Medicine*, **35**, 121-126.
<https://doi.org/10.4103/0253-7176.116232>
- [13] Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd Edition, Cold Spring Harbor Laboratory Press, New York, 45-56.
- [14] Tamura, K. and Nei, M. (1993) Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and Chimpanzees. *Molecular Biology and Evolution*, **10**, 512-526.
- [15] Muhari-Stark, E. and Burcart, G.J. (2018) Glomerular Filtration Rate Estimation Formula for Paediatric and Neonatal Use. *Journal of Pediatric Pharmacology & Therapeutics*, **23**, 424-431. <https://doi.org/10.5863/1551-6776-23.6.424>
- [16] Menyfah, Q.A., Fulwash, Y.A. and Fadilash, Q.A. (2018) An Evaluation of *Escherichia coli* in Urinary Tract Infection in Emergency Department of KAMC in Riyadh, Saudi Arabia: Retrospective Study. *Annals of Clinical Microbiology and Antimicrobial*, **17**, Article No. 3. <https://doi.org/10.1186/s12941-018-0255-z>
- [17] Baudron, C.R., Panhard, X., Clermont, O., Mentre, B., Denamur, E. and Lefort, A. (2014) *Escherichia coli* Bacteremia in Adult: Age-Related Differences in Clinical Bacteriological Characteristics, and Outcome. *Epidemiology and Infection*, **142**, 2672-2683.
<https://doi.org/10.1017/S0950268814000211>
- [18] Nicolle, L.E. (2000) Urinary Tract Infections in Long-Term-Care Facilities. *Infection Control and Hospital Epidemiology*, **22**, 167-175. <https://doi.org/10.1086/501886>
- [19] Marik, P.E. and Zaloga, G.P. (2001) The Effect of Aging on Circulating Levels of Proinflammatory Cytokines during Septic Shock. *Journal of American Geriatric Society*, **49**, 5-9. <https://doi.org/10.1046/j.1532-5415.2001.49003.x>
- [20] Opal, S.M., Girard, T.D. and Ely, E.W. (2005) The Immunopathogenesis of Sepsis in Elderly Patients. *Clinical Infectious Diseases*, **41**, S504-S512.
<https://doi.org/10.1086/432007>
- [21] Nikhil, V., Ashish, K., Rajan, V. and Andrew, C.T. (2013) Haematuria Secondary to Benign Prostatic Hyperplasia: Retrospective Analysis of 116 Men Identified in a Single One Stop Haematuria Clinic. *Journal of Current Urology*, **6**, 146-149.
<https://doi.org/10.1159/000343529>