



Prevalence and Antibiotic Susceptibility Pattern of *Escherichia Coli* Isolated from Urine Samples of Urinary Tract Infection Patients

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Abstract: The study was aimed to determine the prevalence and antibiotic Susceptibility pattern of *Escherichia coli* isolated from urinary tract infection patients in Kano, Nigeria. A total of 50 (21 males and 29 females) urine samples were collected from UTI patients attending urology clinic of Aminu Kano Teaching Hospital Kano for period of 6 month from December 2016 to May, 2017. Ten (10) ml of each urine sample was centrifuged at 2000 revolution per minute (rpm) for 5 minutes, the supernatant was discarded and the sediment was inoculated onto Nutrient agar for isolation of *E. coli*. The isolates were characterized using conventional microbiological methods. The bacteria isolates were subjected to antibiotic susceptibility testing using the agar diffusion method. The Prevalence of *E. coli* in the urine sample of UTI patients showed 44 out of 50 samples were positive for *E. coli* which accounted for 88%. Higher incidence of *E. coli* was found among subjects of age between 41 – 70 years. The result showed high sensitivity of isolates to gentamicin (82%), ciprofloxacin (75%), streptomycin (70%) and chloramphenicol (65%). On the other hand, high degree of resistivity rates to ampicillin (85%), amoxicillin (85%), septrin (90%), erythromycin (75%) and tetracycline (75%) were detected. *Escherichia coli* are one of the leading causes of urinary tract infections in humans.

Keywords: Antibiotics, *Escherichia coli*, prevalence, resistance, susceptibility

1. INTRODUCTION

Many human diseases are as result of infections caused by pathogenic bacteria, either internal or external of the human host. One of such bacterial infection is urinary tract infection (UTI), involving the presence of bacteria in the urinary tract (UT) which is naturally sterile [1]. Urinary tract infection (UTI) describes microbial colonization or inflammation of the bladder (cystitis), urethra (urethritis), or renal pelvis and kidneys (pyelonephritis). It is caused by the presence and growth of microorganisms in the urinary tract, which are the single commonest bacterial infections of all age groups [2].

Bacteria are the primary organisms that cause UTI. The urinary tract infection is commonly caused by gram negative bacilli bacteria in the family Enterobacteriaceae and usually belongs to genera *Escherichia*, *Proteus*, *Klebsiella*, *Enterobacter* and *Pseudomonas* [3]. Urinary

tract infection is mainly caused by gram-negative organisms that include *E. coli* 60-70%, *Klebsiella* 10%, *Proteus* 5–10%, and *Pseudomonas* 2–5% and gram-positive bacteria, group B *Streptococcus* and *Staphylococcus species* [4]. According to Ronaldo [5], the common etiologic agents of UTI include *Escherichia coli*, *Klebsiella spp*, *Staphylococcus aureus*, *Pseudomonas spp* and *Streptococcus*. Bacteria colonization of the urinary tract is predominantly caused by Gram-negative species, such as *Escherichia coli*, *Klebsiella*, *Proteus* and *Pseudomonas* and rarely, by Gram-positive organisms such as haemolytic *Streptococci* and *Staphylococcus saprophyticus*. Gram positive bacteria cause 15-20% while negative bacteria cause 80-85% [6]. Among gram negative *Escherichia coli* is the most frequent pathogen [7] but in complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella*, *Proteus*, *Serratia*, *Enterobacter* and *Pseudomonas*.

Among gram positives *S. saprophyticus*, *E. faecalis*, *S. pyrogenes*, and *S. aureus* are usually prevalent and are resistant to variety of antibiotics [8].

Distribution of urinary pathogens and their susceptibility to antibiotics varies regionally so it becomes necessary to have knowledge of distribution of these pathogens and their susceptibility to antibiotics in a particular setting [9]. Most commonly isolated organisms although a variety of culture can cause UTIs, most are due to *E. coli*, bacteria that are common in the digestive tract and routinely found in stool other bacteria that may cause UTIs include species of *Klebsiella*, *Enterococcus* and *Staphylococcus*. Bacteria known as *E. coli* cause the majority of lower urinary tract infections. *Escherichia coli* are the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections [10]. *Escherichia coli* are gram-negative, non-spore forming bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae [11]. Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, and translucent and are rose-pink in colour. Some strains grown on Blood agar, result in colonies being surrounded by zones of haemolysis. Colonies are smooth, circular, 1-1.5mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on Cystine-lactose-electrolyte deficient (CLED) medium [12]. This microorganism is usually susceptible to a variety of antibiotics, such as Trimethoprim, Sulphamethoxazole, ciprofloxacin and Nitrofurantoin. The study was aimed to determine the prevalence and antibiotic susceptibility pattern of *Escherichia coli* isolated from urinary tract infection patients in Kano, Nigeria.

2. MATERIAL AND METHODS

2.1. Study Area

The study was conducted at Urology clinic of Aminu Kano Teaching Hospital Kano (AKTH). Kano state is located in the North-west Nigeria with coordinates 11° 30' N 8° 30' E. It shares borders with Kaduna state to the south-west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the West and Niger republic to the North. It has a total area of 20,131km² (7,777sqm) and population of 11,058,300 [13].

2.2. Ethical Clearance

An approval for the study was obtained from Research and Ethic committee of Aminu Kano Teaching Hospital Kano. The aim of the study was explained clearly to the clients and informed consent obtained before proceeding to the study.

2.3. Study Population

A total of 50 (21 males and 29 females) urine samples were collected from UTI patients attending urology clinic of Aminu Kano Teaching Hospital Kano for period of 6 month from December 2016 to May, 2017.

2.4. Samples Collection

Early morning mid-stream urine samples of about ten (10) ml were collected using clean and sterilized plastic bottles with air-tight screw cap tops. Each urine sample bottle was labeled 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. Ten (10) ml of each urine sample was centrifuged at 2000 revolution per minute (rpm) for 5 minutes, the supernatant was discarded and the sediment was used for microbial culture.

2.5. Isolation of Escherichia coli

Isolation of *E. coli* was conducted according to the method describe by Prescott et al. [14]. During the process, a sterile wire loop was deep into the sediments of urine sample of the patients and streaked onto the surface of Nutrient agar (Life save Biotech, USA). The procedure was repeated for all the sample and the plates were incubated 37° C for 24 hours. The presumptive colony of *E. coli* from each plate was further sub-cultured to obtained pure culture. The pure isolates of *E. coli* were preserved in peptone water for further use.

2.6. Identification of Escherichia coli

The preserved isolates were confirmed as *E. coli* using conventional microbiological methods which include Gram staining, lactose fermentation, cystine-lactose-electrolyte deficient (CLED) medium growth and motility test as well as biochemical (Indole, methyl orange, voges proskauer, nitrate reduction and citrate utilization) tests according to the methods described by Holt *et al.* [15].

2.7. Gram Staining

Gram staining was done according to method described by Cheesbrough [6]. A thin smear was made by emulsifying an overnight culture of the

isolate in normal saline on a well labeled clean glass slide. The smear was air dried and fixed by heat. This is followed by flooding the slide with crystal violet as primary stain for 30 seconds and then rinsed the slide with distilled water. The smear was flooded with Lugol's iodine as a mordant to fix the primary stain and then rinsed with distilled water after 60 seconds. The slide was decolorized using acetone and rinsed immediately. Counter stain with safranin followed and left for 30 before being rinsed off. The stain smear was air dried and observed under microscope.

2.8. Biochemical test

2.8.1. Indole Test

The tested organism was inoculated into peptone water and incubated at 37^o C for overnight, the kovač's reagent was added. If there is pink ring the result was indicated as positive. If there is no pink ring in the surface the result was indicated as negative.

2.8.2. Citrate Utilization Test

In this test organism has ability to use citrate as only source of carbon. By straight loop apart of tested colonies was emulsified in koser's citrate media and incubated at 37^oc for 24 hours. A blue color with growth indicated as positive, no change in color indicated the negative result.

2.8.3. Methyl Red and Voges Proskauer Test

A pure culture of the test organism was inoculated in the MR/VP broth and incubated at 35^o C for 24 hours. Five Drops of MR reagent was added to the broth. A pure culture of the test organism was inoculated into MR/VP broth and incubated for 24 hours at 37^o C. An aliquot of 1 ml of the broth was added into a sterile test tube and add 0.6ml of VP reagent was added and shaken gently, then allowed it to expose into the atmosphere for 15 minutes.

2.8.4. Nitrate Reduction Test

Nitrate broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37^oC for 24 hours. A dropper full of sulfanilic acid and that of α naphthalamine were added to the broth.

2.8.5. Motility Test

The isolate was inoculated into motility medium by a fine stab with a sterile needle to a depth of 2 cm length of the bottom of the tube. It was then incubated at 37^o C for 24 hours. Line of inoculation was defined and the medium was cleared for non motile organism while the line of inoculation was not defined for motile

organism and the rest of the medium was somewhat cloudy for the motile organisms [6].

2.8.6. Antibiotic Susceptibility Testing

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar diffusion method as described by Bauer *et al.* [16]. Mueller Hinton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37^oC for 24 hours. Sensitivity pattern of the isolates to Ampicillin (30 µg), Streptomycin (30 µg), Augmentin (10 µg), Erythromycin (30 µg), Amoxicillin (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Tetracycline (30 µg), Ciprofloxacin (10 µg) and Seprin (30 µg) produced by Abtek pharmaceutical limited, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediate susceptible and resistant according to the European committee on antimicrobial susceptibility testing (EUCAST) breakpoint for interpretation of MICs and zone diameters [17].

3. RESULTS

3.1. Demographic Distribution of the Subjects

Table1. Demographic Distribution of the Subjects with Percentage Prevalence

Parameters	Number	Prevalence (%)
Age (years)		
Less than 18	01	2
18 – 40	16	32
41 – 70	28	56
71 – above	05	10
Sex		
Male	21	42
Female	29	58
Marital status		
Single	08	16
Married	42	84
Types of resident		
Urban	19	38
Rural	31	62

The Demographic distribution of the subjects is presented in Table 1. A total of 50 subjects (21 males and 29 females) participated in the study, all of which are patients diagnosed with urinary tract infection. The age category, sex, marital status and type of resident were considered for demographic distribution of the subjects.

3.2. Identification of Escherichia coli

The result for identification of *E. coli* is presented in Table 2 below. The isolate was

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identified based on Gram staining, cultural characteristics and biochemical test. The result showed that the isolate is Gram negative bacteria, positive for MR and motility test but negative for indole, VP and citrate utilization test. The isolate cannot ferment lactose and produce transparent colourless colony in both MacConkey and CLED agar

Table2. Morphological and Biochemical Characterization of *E. coli*

S/N	Test	Inference
1	Gram staining	-
2	Indole	+
3	Methyl-red	+
4	Voges Proskauer	-
5	Citrate utilization	-
6	Nitrate reduction	+
7	Motility	+
8	Lactose fermentation	+
9	CLED medium growth	Opaque yellow colony

3.3. Prevalence of *Escherichia coli*

The Prevalence of *E. coli* in the urine sample of UTI patients is presented in Table 2. The result showed 44 out of 50 samples were positive for

Table4. Antibiotic Sensitivity testing of the *E. coli* against some antibiotics

Antibiotics	Conc. (µg)	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Ampicillin	30 µg	0 (0)	7 (15)	37 (85)
Streptomycin	30 µg	31 (70)	4 (10)	9 (20)
Augmentin	10 µg	12 (27)	10 (23)	22 (50)
Erythromycin	30 µg	7 (15)	4 (10)	33 (75)
Amoxicillin	30 µg	0 (0)	7 (15)	37 (85)
Chloramphenicol	30 µg	29 (65)	2 (5)	13 (30)
Gentamicin	10 µg	36 (82)	6 (13)	2 (5)
Tetracycline	30 µg	4 (10)	7 (15)	33 (75)
Ciprofloxacin	10 µg	33 (75)	4 (9)	7 (16)
Seprin	30 µg	0 (0)	4 (10)	40 (90)

Key: n = frequency, % = percentage

4. DISCUSSION

Urinary Tract Infections are caused by microbial invasion and subsequent multiplication in urinary tract. The results of this study on prevalence of urinary tract infection among the subjects showed that prevalence was high in individuals between the ages of 41 - 70 years with about 56% prevalence consisting of 28 out of 50 subjects. This difference in prevalence could be based on differences in sanitary conditions and observed personal hygiene. More female were recorded in the study than male. This is in accordance with previous study conducted by Shah et al. [18]. Higher female patient is associated with combination of urinary and genital system as well as other factors such as pregnancy. The finding of the presence study

E. coli which accounted for 88%. Higher incidence of *E. coli* was found among subjects of age between 41–70 years.

Table3. Prevalence of *E. coli* in the urine sample of UTI patients

Age (year)	Frequency (n)	<i>E. coli</i> + (n)	Prevalence (%)
Less than 18	01	01	02
18 – 40	16	15	30
41 – 70	28	23	46
70 – above	05	05	10
Total	50	44	88

3.4. Antibiotic Sensitivity Testing

The sensitivity and resistivity of *E. coli* against the antibiotics used is presented below (Table 4). Some of the antibiotics were active against the isolates while resistance to others. The result showed high sensitivity of isolates to gentamicin (82%), ciprofloxacin (75%), streptomycin (70%) and chloramphenicol (65%). On the other hand, high degree of resistivity rates to ampicillin (85%), amoxicillin (85%), seprin (90%), erythromycin (75%) and tetracycline (75%) were detected.

revealed higher prevalence (88%) of *E. coli* in the urine samples of UTI patients. *E. coli* was observed as the most common etiologic agent of UTI, which is also in accordance with previous studies [19,20]. Rate of *E. coli* isolation was highest in elderly patients (41 – 70 years). This finding was in conformity with that of Iqbal [21]. The pathogenic ability of *E. coli* is largely affordable by the flexible gene pool through the gain and loss of materials [22, 23]. Antibiotic resistant isolates of *E. coli*, especially those that are fluoroquinolones resistant and those producing extended spectrum β-lactamases have increased significantly during the 2000's and in certain areas while many nosocomial and community-acquired *E. coli* are now resistant to several important antimicrobials [24].

In this study, the overall resistance of *E. coli* to antimicrobials was high. The result showed high sensitivity of isolates to gentamicin (82%), ciprofloxacin (75%), streptomycin (70%) and chloramphenicol (65%). On the other hand, high degree of resistivity rates to ampicillin (85%), amoxicillin (85%), septrin (90%), erythromycin (75%) and tetracycline (75%) were detected. The result is consistent with the findings of previous studies. The resistance rate in this study was in conformity with that of Khan et al. [25]. High resistance rate of *E. coli* to ampicillin and amoxicillin in this study justify the study conducted by Sabir et al. [26] who reported 100% resistant of *E. coli* to beta lactam drugs. Resistance to tetracycline and erythromycin in this study was in line with the study conducted by Andargachew et al. [27] and Petkovsjeek et al. [28] in Sudan and Slovenia respectively. One of the major reasons for this high resistance can be co-expressed resistance mechanisms in the species. Increasing rates of antimicrobial resistance among *E. coli* is a growing concern worldwide. Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries. The higher resistance against the above antimicrobials could be as a result of repeated or prolonged use or exposure of uropathogens to antibiotics repeated use of antibiotics can damage urethral flora, allowing uropathogens to colonize and subsequently to infect the urinary tract, leaving clinicians with very few choices of drugs for the treatment of UTI [28,29]. Moreover, this condition enables bacteria to exchange their genetic material through horizontal gene transfer resulting in resistant gene that confer resistance to particular antibiotics [30]. In this study, *E. coli* isolates were sensitive to gentamicin, ciprofloxacin, streptomycin and chloramphenicol. Similar studies conducted by Okonko et al. [31] in Nigeria have reported comparable susceptibility rates. High sensitivity to ciprofloxacin and gentamicin was also recorded from previous studies conducted in India [32].

5. CONCLUSION

Escherichia coli are one of the leading causes of urinary tract infections in humans. The finding of this study revealed that *E. coli* was observed as the most common etiologic agent of UTI. Its prevalence accounted for 88% of the urine samples examined. The result showed high sensitivity of the isolates to gentamicin (82%),

ciprofloxacin (75%), streptomycin (70%) and chloramphenicol (65%). On the other hand, high degree of resistivity rates to ampicillin (85%), amoxicillin (85%), septrin (90%), erythromycin (75%) and tetracycline (75%) were detected. The judicious use of antibiotic is recommended which will help to limit the increasing rate of drug resistance in the pathogens.

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