



PREVALENCE AND MOLECULAR DETECTION OF *ESCHERICHIA COLI* FROM PATIENTS WITH URINARY TRACT

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Abstracts

The emergence of Escherichia coli associated with urinary tract infections (UTIs) is a serious public healthcare problem globally, causing frequent morbidity and even death due to limited therapeutic options. This study investigated the prevalence and molecular detection of Escherichia coli from patients with Urinary Tract Infection in State Specialist Hospital, Maiduguri, Borno State Nigeria. The urine samples of 400 patients were obtained from State Specialist Hospital Maiduguri and were cultured for significant bacteriuria. The E. coli isolates were identified and confirmed using standard microbiological procedures, Polymerase chain reaction (PCR) analysis, respectively. This study identified 7.5% prevalence of E. coli isolates in urinary tract infections patients with higher proportion among the females and 7 (23.3%) of the isolates showed the presence of Beta heamolysin production. The findings in this study further emphasizes the need for antimicrobial stewardship with one health approach which will provide timely data for effective antibiotic treatment policy, necessary to preserve the efficacy of antibiotics that are commonly used for the treatment of patients with urinary tract infections.

Key words: Prevalence, *E. coli*, urinary tract infections, Urine, Polymerase chain reaction, Heamolysin

Introduction

Urinary tract infection (UTI) is the colonization of the urinary tract by pathogenic microorganisms. Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting about 150 million people globally, annually (McCann *et al.*, 2020). Urinary tract

infections (UTIs), often referred to as cystitis or pyelonephritis, commonly present with symptoms like frequent urination, a strong urge to urinate, and a burning feeling during urination (Li and Leslie, 2003). Additionally, untreated or incorrectly treated urinary tract infections can lead to the persistence and

dissemination of uropathogens, potentially leading to systemic infection known as urosepsis (Ortega, 2020). UTIs are often recurrent, frequently difficult to treat and can cause parenchymal damage to the kidney, leading to renal insufficiency and further complications (Prakash *et al.*, 2009). UTIs impose a substantial burden on society and the healthcare system in relation to diagnosis, management, loss of productivity, morbidity and sometimes death (Kudinha, 2017). UTI has been implicated to occur more frequently in women than in men because of their shorter urethra which has closer proximity to the opening of the intestinal tract (Abbo and Hooton, 2014).

Clinically, UTIs are categorized as either uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities (Hooton, 2012). These infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Hooton, 2012). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility (Foxman, 2014). Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices (Levison and Kaye, 2013).

UTIs are mainly caused by bacteria however, fungi and some viruses have also been implicated (Kudinha, 2017). The most common etiologic pathogens associated with UTIs include Gram-negative *Enterobacteriaceae* families such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Proteus* species (Chakraborty *et al.*, 2017). *E. coli* is the dominant causative agent in all patient age groups, causing 80–90% of all UTIs (Mlugu *et al.*, 2023). A better understanding of UTI pathogenesis is necessary especially for the most common etiologic agent of urinary tract infections *E. coli* is crucial for the treatment and prevention of UTIs. *E. coli* is a prominent member of the normal intestinal microbiota of humans and animals (Zhang and Foxman, 2003). The distinctive *E. coli* strain that causes most UTIs has been designated as uropathogenic *E. coli* (UPEC) which possess diverse virulence-associated factors (VFs) that assist them in attaching to, invading and injuring the host and this include adhesins, toxins, siderophores, protective polysaccharide coatings, invasins and serum resistance-associated proteins, the presence and numbers of such VFs predict *in vivo* virulence (Johnson and Russo, 2005).

The high volume of antibiotic prescriptions witnessed in Africa associated with UTIs treatment has led to the increasing emergence of antibiotic resistance in the uropathogens (Tansarli *et al.*, 2013). Furthermore, the misuse and overuse of the common antibiotics in the treatment of UTIs has also compounded the emergence of antibiotics resistance, the increasing resistance to therapeutically important antimicrobial agents and the recent emergence of the

virulent and multidrug-resistant *E. coli* have made UTI management challenging (Ikram *et al.*, 2015).

Urinary tract infections (UTIs) is one of the most common bacterial infections worldwide, affecting millions of people each year. They are often recurrent, difficult to manage, and can lead to kidney damage and other complications. The burden of UTIs extends beyond patients, placing significant pressure on healthcare systems through repeated diagnoses, treatments, and hospital visits. Among the causative agents, *Escherichia coli* is the major etiologic pathogen with increasing prevalence and antimicrobial resistance, which serves as a growing public health concern.

There is limited data on the prevalence and molecular characteristics of *E. coli* strains causing UTIs in Maiduguri, Borno State, Nigeria. Understanding the local epidemiology and genetic makeup of these strains is crucial for guiding effective treatment and prevention strategies. Hence this study investigated the prevalence and molecular detection of *Escherichia coli* among patients with UTIs attending the State Specialist Hospital, Maiduguri.

MATERIALS AND METHODS

Materials

Laboratory and PCR Consumable Materials; Hand gloves, sterile cotton swab sticks, Savlon[®] disinfectant, liquid soap, permanent marker, masking tape, water, Primers (Eurofins, USA), DNA Template (Eurofins, USA), Nucleotides and Taq polymerase. **Glass Wares;** Measuring

cylinders (ASTM E-1272), Wide mouthed bottles, Beakers (ASTM International), Test tubes (Halomedical Borosilicate), Bijou bottles and McCartney bottles (IndiaMARTS). **Plastic Wares;** Pipette tips and sterile Petri dishes (JRZ Plastilab, Lebanon) 2mL, 5 mL, 10 mL and 20 mL syringes, PCR Tube, (Eurofins, USA). **Equipment;** Incubator (Memmert[®], Germany), triple beam weighing balance, autoclave, hot air oven (Gentlab, UK), refrigerator and dryer (Gentlab ltd, UK) gel cassette, electrode chamber, comb, tank and UVitetransilluminator (Avebury, Cambridge U.K) master mix, thermal cycler, (Eurofins, USA). **Culture Media;** Nutrient agar (NA), nutrient broth (NB), Mueller Hinton agar (MHA), chrom agar, cysteine lactose electrolyte deficient agar (CLED) and eosin methylene blue agar (EMB), blood agar all from Oxoid Limited, England, were used. **Chemical Reagents;** The following reagents and chemicals were used for this study: Sodium chloride (Fisher Scientific Company New Jersey, USA), normal saline pH 7 (Sigma Chemicals Company St. Louis M.O. USA), 96 % ethanol (BDH Chemical Ltd., England), wash solution (Fermentas, UK), elution buffer (Fermentas, UK), tris-acetate EDTA, sodium dodecyl sulphate, glycerol from (Sigma chemical Ltd., England), ethidium bromide dye (Sigma chemical Ltd., England), 10mM Tris-HCl (Sigma chemical Ltd., England), re-suspension solution (Fermentas, UK) and lysis solution (Fermentas, UK).

Methods

Study Area

The sample collection site was the State Specialist Hospital Maiduguri, the hospital is a secondary health care facility that is license by Nigeria Ministry of health with facility code of 08/21/1/1/0024. The hospital has twenty (20) wards with 500 beds capacity and the hospital is located in the heart of Maiduguri along Shehu Laminu way a few kilometers from the general post office in Zango Hausari Maiduguri. It serves as the main service and referral hospital for Maiduguri Metropolis and for the entire local governments of Borno State.

This study was conducted in Maiduguri, the capital of Borno State and it's the largest of the six States in North-Eastern Nigeria. It lies on latitude 115°N and longitude 135°E, and occupies an area of 50,778 square kilometers. Borno State is bordered by the Republic of Niger to the North, Chad to the North-East, Cameroon to the East and Yobe State to the North-West, Adamawa State to the South-East. The estimated population of Borno State according to 2006 population census report is 4,098,391. The major languages are Kanuri, Shuwa, Marghi, Mandara and Hausa. Agriculture is the main pre-occupation of the people of Borno, as over 80% of its population engaged in crop cultivation, animal husbandry and fishing (Wathanafa *et al.*, 2022).

Sample Size Determination

The sample size was determined based on estimated confidence level ($Z\alpha$) of 95 % and maximum tolerable error (e) of 5 %. Using the prevalence rate of 80 % of all UTIs (Mlugu *et al.*, 2023). The required sample size for this study was determined using the

formula described by Jaykaran *et al.* (2011), as showed below:

$$n = Z_{1-\alpha/2}^2 * P (1 - p) / d^2$$

$$n = 1.96^2 * 0.8 (1 - 0.8) / 0.05^2$$

$$n = 245.824$$

Where,

$Z_{1-\alpha/2}$ = standard normal variate at 5% type 1 error which is 1.96

P = expected proportion in population based on previous studies or pilot studies.

d = Absolute error or precision. The samples 245.824 were rounded up to 400.

Study Design and Samples Collection

Convenient sample selection method were used to select the samples used in this study and descriptive statistics was used to interpreted the result. A cross sectional study of the patients suspected with UTIs in State Specialist Hospital Maiduguri, Borno State were investigated. Four hundred (400) samples of urine were collected from patients suspected to have UTIs, who deposited their urine samples at the medical laboratory of the hospital according to physician instructions. A liquor quantity of each urine samples deposited at the medical laboratory was collected and transported in an ice pack to the Pharmaceutical Microbiology Laboratory within 2 hours of collection for culture and future investigations. The samples collection commenced after the ethical approval was received.

Isolation of *E. coli*

Each urine sample was aseptically inoculated on sterile nutrient agar plate using a sterile loop of 4.0 mm diameter designed to deliver 10 µL and streaked for discrete colonies before it was incubated at 37 °C for 24 hours. The sample growth that gave bacterial count of $\geq 10^5$ colonies per mL (after multiplying the plate count by 100) was considered as significant while those that gave $< 10^5$ colonies per mL were considered as insignificant (Cheesbrough, 2006).

Each of the discrete colonies that emanated from the urine sample were placed on chrom agar, cysteine lactose electron deficient (CLED) agar and eosin methylene blue (EMB) agar for identification, the isolates that gave dark pink on chrom agar, yellow on CLED, and metallic green on EMB were presumptively identified as *E. coli* and the isolates were then probed for *16S rRNA E. coli* gene using polymerase chain reaction (PCR) analysis for confirmation.

Deoxyribonucleic acid (DNA) Extraction of *E. coli* Isolates

A loopful of colonies were picked from each of the overnight culture of *E. coli* isolates and suspended in 50 µL of sterile distilled water with 1X Tris-EDTA buffer in an Eppendorf tube to extract the DNA of the isolates. The suspension was boiled at 100 °C for 10 mins, the boilate was transferred immediately to the freezer (-20 °C) for 10 minutes and maintained at room temperature before centrifuging at 10,000 rpm for 10 mins. The resulting supernatant of each isolate was collected, stored at 4 °C and used as DNA template in PCR analyses (Odetoyin *et al.*, 2022).

Genotypic Detection of *E. coli*

All organisms suspected to be *E. coli* by their phenotypic characteristics were confirmed genotypically as *E. coli* by amplifying their *16S rRNA* gene using Polymerase chain reaction (PCR), the primers used were ECO-1 GACCTCGGTTTAGTTACAGA and ECO-2 CACACGCTGACGCTGACCA at 585 base pair (Mamun *et al.*, 2016). *E. coli* strain 25922 was used as the positive control while water was used as the negative control. A 25 µL reaction mixture contained 12.5µL of 2X master mix, 10 pmol each of the primers (Eurofins, USA), 2.4 µL of the DNA template and made up with nuclease free water. Amplification conditions were as follows: Initial denaturation at 95 °C for 5 mins; 35 cycles of denaturation at 94 °C for 45 s, annealing at 45 °C for 45 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 mins. Each PCR product (10 µl) was electrophoresed on a 1.5 % (w/v) agarose gel in 1X TAE. Gels containing 5 uL of 10 µg/ml of ethidium bromide were visualized under ultraviolet (UV) light using an UVitec transilluminator (Avebury, Cambridge UK) (Odetoyin *et al.*, 2022).

Screening of *E. coli* Isolates for Haemolysin Production

All the confirmed *E. coli* isolates were screened for haemolysin by punching the isolates on freshly prepared sterile 5 %v/v blood agar (consisting of 5 mL of human blood in 100 mL of nutrient agar) plates using a straight wire loop and then the plate was incubated at 37 °C for 24 hours. Thereafter, the plates were observed for green to black colouration (partial lysis of red blood cells –

alpha haemolysis) and clear zones (complete lysis of red blood cells - beta haemolysis) around the punched isolates, indicate the production of haemolysin (Onanuga and Selekere, 2016).

Ethical Approval

Ethical approval with reference number SSH/GEN/641/Vol.1 was obtained from the Hospital Ethical Committee of the State Specialist Hospital Maiduguri, Borno State on the 3rd March 2021

RESULTS

Study Population

A total of 400 patients suspected to have UTIs comprising of 153 (38.3%) males and 247 (61.8%) females of ages ranging between 1 and 84 years were involved in this study. The age and gender distribution of this population is shown in the Table 1. revealing age group 20-29 as the group with the highest samples and group 80-89 as the group with the lowest samples.

Table 1: Age and Gender Distribution of UTIs Samples

Age Group (years)	Number of Samples	Gender (%)	
		Male	Female
0-9	20	12 (3)	8 (2)
10-19	66	24 (6)	42 (10.5)
20-29	112	33 (8.3)	79 (19.8)
30-39	92	36 (9)	56 (14)
40-49	52	14 (3.5)	38 (9.5)
50-59	35	17 (4.3)	18 (4.5)
60-69	16	11 (2.8)	5 (1.3)
70-79	5	4 (1)	1(0.3)
80-89	2	2 (0.5)	0
Total	400	153 (38.3)	247 (61.8)

Frequency of Bacteriuria in the Samples

A total of 207 (51.8%) of the 400 samples collected yielded growth but only 50 (24.2%) samples showed significant growth $\geq 10^5$ CFU/mL among which 18 (36%) were males and 32 (64%) were females as shown in the Table 2

Table 2: Age and Gender Distribution of Significant Bacteriuria

Age Group (Years)	Number of Samples	Number of Significant Bacteriuria (%)	Gender	
			Male	Female
0-9	20	2 (4)	2	0
10-19	66	9 (18)	3	6
20-29	112	15 (30)	4	11
30-39	92	8 (16)	4	4
40-49	52	9 (18)	1	8
50-59	35	5 (5)	2	3
60-69	16	1 (2)	1	0
70-79	5	1 (2)	1	0
80-89	2	0	0	0
Total	400	50 (100)	18 (36)	32 (64)

Phenotypic and Genotypic Detection of *E. coli* Isolates

A total of 50 isolates yielded significant bacteriuria, out of which 30 (60%) isolates

were identified as *E. coli* phenotypically and the 30 isolates were also confirmed to possess the *E. coli* 16S rRNA gene using PCR analysis as showed in Plate 1.

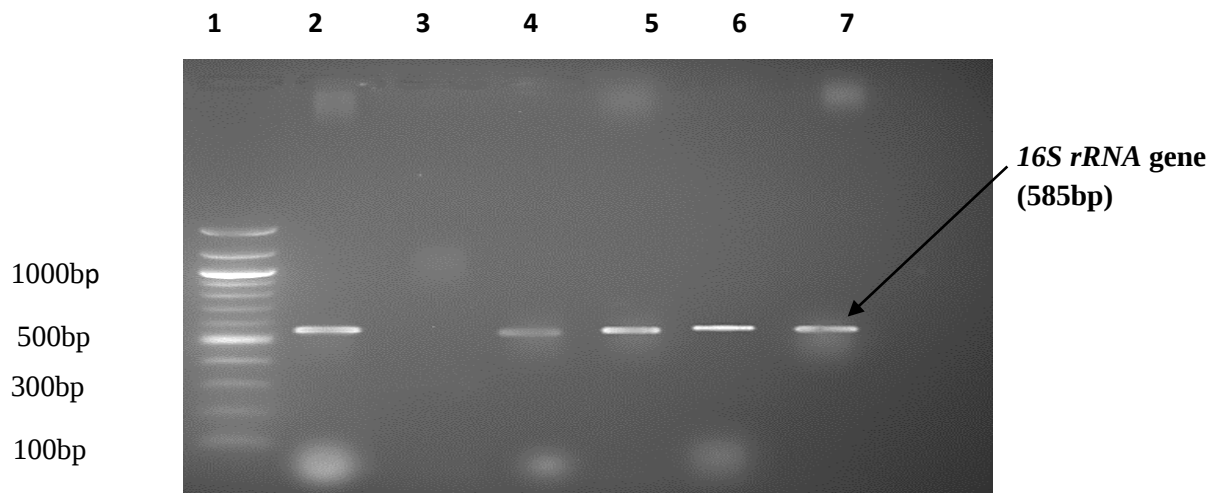


Plate 1: A gel picture showing the bands of the amplified *Eco* gene of *Escherichia coli* isolates. Lane 1: 100bp ladder; Lane 2 (Positive control - *E. coli* 25922); Lane 3 (Negative control); Lanes 4-7 (Positive isolates-PE1-PE4)

Table 3: Age and Gender Distribution of *E. coli* Isolates

Age Group (Years)	Number of Samples	Number of <i>E. coli</i> Isolate (%)	Gender (%)	
			Male	Female
0 – 9	20	1 (3.3)	1 (3.3)	0
10-19	66	5 (16.6)	2 (6.7)	3 (10)
20-29	112	9 (30)	3 (10)	6 (20)
30-39	92	7 (23.3)	3 (10)	4 (13.3)
40-49	52	3 (10)	0	3 (10)
50-59	35	3 (10)	1 (3.3)	2 (6.7)
60-69	16	1 (3.3)	1 (3.3)	0
70-79	5	1 (3.3)	1 (3.3)	0
80-89	2	0	0	0
Total	400	30 (100)	12 (40)	18 (60)

Phenotypic Screening of *E. coli* Isolates for Haemolysin production

All the *E. coli* isolates were screened for possible haemolysin production and the

results revealed that only 7 (23.3%) of the *E. coli* isolates phenotypically expressed Beta-haemolysin production as shown in Table 4

Table 4: Phenotypic Haemolysin Production among *E. coli* Isolates

Type of Haemolysis	<i>E. coli</i> Isolates (%)
Alpha	0
Beta	7 (23)
Non-Haemolytic	23 (77)
Total	30 (100)

DISCUSSION

Urine samples of 400 patients were obtained from the State Specialist Hospital, in this study with 153 (38.3%) males and 247 (61.8%) females with ages ranging between 1 and 84 years. This distribution showed that there are high number of female patients with UTIs compared to their male counterpart, and agrees with the previous study conducted by Abbo and Hooton (2014). who reported that females are more liable to UTIs due to shorter urethra that is at closer proximity to the opening of their intestinal tract (anus). Also,

the age group with the highest samples was 20 – 29 years implying that the sexually active ages are more vulnerable to UTIs and this tallies with the findings of Aiyegoro *et al.* (2007) and Abdu *et al.* (2018).

The studied urine samples indicated that 50 (24.2%) yielded significant bacteriuria which was distributed among 18 (36%) males and 32 (64%) females, this may be because of their shorter urethra that is at closer proximity to the opening of their intestinal tract (anus)

and the female gender tend to visit the hospital more often for medical services compare to their male counterpart and this agrees with the findings of Abdu *et al.* (2018) in Maiduguri, Nigeria and Odoki *et al.* (2019) in Uganda.

The prevalence of *E. coli* isolates in UTIs patients was 30/400 (7.5%) which is lower than the 39.2% reported by Mohammed *et al.* (2016) in Maiduguri, Nigeria and the 49 % obtained by Orrett (2001) in Trinidad, which may be due to the difference in time, health facilities and vehicular sources of *E. coli*. This study also showed that, females had a higher prevalence of *E. coli* isolates in 18 (60%) than their male counterpart with 12 (40%) and this is in agreement with an earlier study carried out by Abdu *et al.* (2018) in Maiduguri, Nigeria, Odongo *et al.* (2020) in Uganda and Oladeinde *et al.* (2011) in Okada, Edo State Nigeria. The highest prevalence of *E. coli* isolates (30%) was recorded in the age group between 20 and 29 and this finding conforms with Abdu *et al.* (2018) in Maiduguri and Okafor and Nweze (2020) in Nsukka, Nigeria.

A total of 30 isolates of *E. coli* were screened for haemolysin production phenotypically in this study, out of which 7 (23.3%) showed the presence of Beta haemolysin production, this indicates the potential pathogenicity of the *E. coli* isolates from patients with significant bacteriurea. The low frequency of haemolytic

E. coli isolates observed in this study indicates the need for genotypic virulence test, as it is the final test that can pick the expressed and dormant virulence genes present. This finding was similar to that of Onanuga and Selekere (2016) who observed that approximately, 13% of antibiotic resistant bacteria produced haemolysins.

CONCLUSIONS

The emergence of *Escherichia coli* associated with urinary tract infections (UTIs) is a serious public healthcare problem globally, causing frequent morbidity and even death due to limited therapeutic options. This study identified 7.5% prevalence of *E. coli* isolates in urinary tract infections patients with higher proportion among the females and 7 (23.3%) of the isolates showed the presence of Beta haemolysin production. The findings in this study further emphasizes the need for antimicrobial stewardship with one health approach which will provide timely data for effective antibiotic treatment policy, necessary to preserve the efficacy of antibiotics that are commonly used for the treatment of patients with urinary tract infections.

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Ethical Clearance

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Our Ref: SSH/GEN/641/Vol. 1 Your Ref: Date: 01st March, 2021

Moh'd Halilu Dahiru,
PGA/18/08/03/088

Department of Pharmaceutical &
Pharmaceutical Microbiology,
Faculty of Pharmacy,
University of Maiduguri,
Borno State.

Dear Sir/Madam,

RE: ETHICAL CLEARANCE/APPROVAL

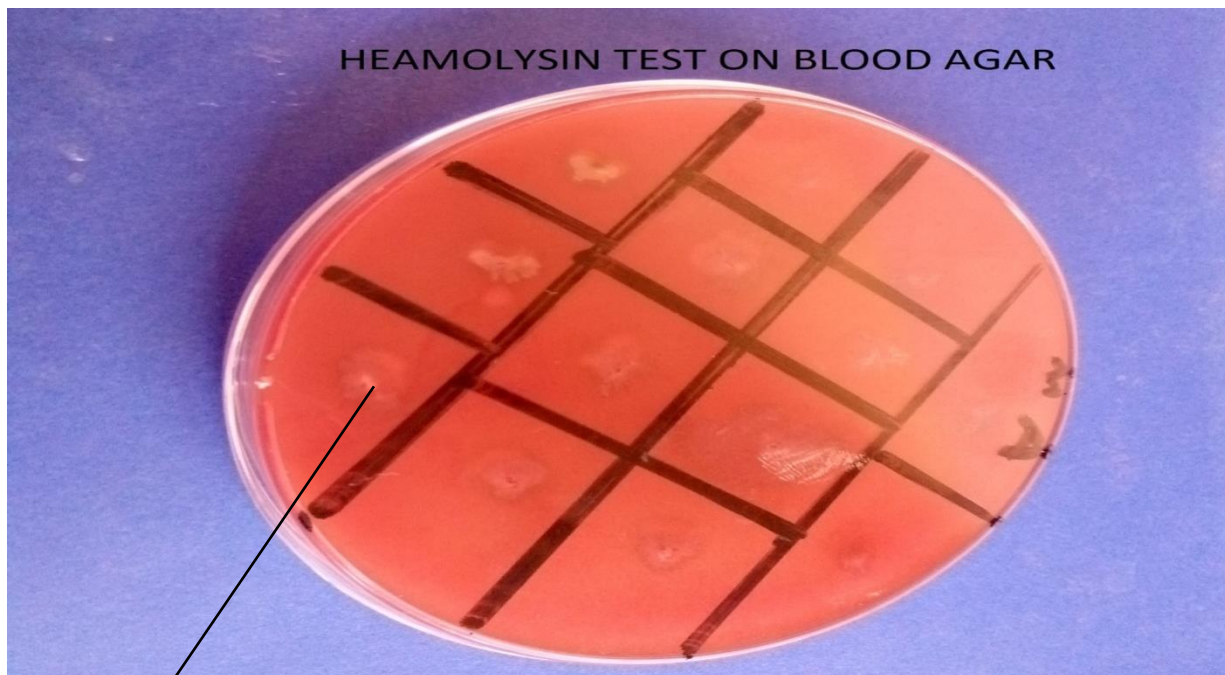
With reference to your letter dated 29th January, 2021, I am directed to write and inform you that after careful review of your proposal; the Ethical Committee has approved your study on *Molecular Characteristics of Antibiotic Resistant Escherichia Coli Isolates From Patient with Urinary Tract Infection In State Specialist Hospital Maiduguri, Borno State*. In view of the above, you are to maintain utmost confidentiality of the information provided and strictly use of the purpose mentioned in the application. You are to submit a copy of the project to the Ethical Committee.

The Ethical Committee wishes you a happy study time.

Yakubu M. Ali Kirawa
Secretary, Ethical & Research Comm.
For: Chairman

Cc.
H.O.D Health Information Management, Medical Lab Services.

Picture of Haemolysin Production on Blood agar



Beta Haemolysis