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Prevalence of Antibiotic Susceptibility Pattern of *Proteus Mirabilis* Isolated from Urine Samples of Patients Attending Select Hospitals in Kaduna Metropolis, Nigeria

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Abstract
Proteus mirabilis belongs to the order of Enterobacteriaceae family of morganellaceae, a Gram-negative rod-shape bacterium and a normal bacterial flora of intestinal tract of humans and animals. *P. mirabilis* constitute to high morbidity and mortality. The aim of this study was to isolate and determine prevalence of antibiotic susceptibility profile of *P. mirabilis* in urine samples of patients. A total of 200 urine samples collected from four hospitals, were cultured on CLED Di (Bevis), nutrient and blood agar plates. Ten (10) isolates were isolated, 7 were *Bacillius spp*. 3 were positive to *P. mirabilis*, designated as; TH_1, CH_2 and TH_5. Prevalence of 3(1.5%) *P. mirabilis* were obtained. The isolates were susceptible to septrin 3(100%), ciprofloxacin 3(100%), pefloxacin 3(100%), chloramphenicol 3(100%), tarivid 3(100%), streptomycin 3(100%) and gentamycin 2(66.7%). More hospitals in Kaduna should be screened to curb the low prevalence. Following the high susceptibility profile, the antibiotics should be recommended for the treatment of infections associated with *P. mirabilis*

Keywords: Prevalence, Susceptibility, Antibiotic, Urine, Bacteria, Patients

Introduction
Proteus mirabilis is classified under the Enterobacteriaceae order and the Morganellaceae family; it is a Gram-negative, rod-shaped bacterium that is typically found as part of the normal bacterial flora in the intestinal tract of both humans and animals. This bacterium has the potential to lead to symptomatic urinary tract infections, such as cystitis and pyelonephritis and is also identified in cases of asymptomatic bacteriuria, especially among the elderly and individuals with type 2 diabetes (Mathew & Lancaster, 2019). *P. mirabilis* is the most commonly isolated species from clinical samples, with 90% being from urinary tract infections (UTIs), but also from extra-intestinal infections such as respiratory, eye, ear, nose, skin, burn, meningoencephalitis, osteomyelitis and wound infections (Schaffer & Pearson, 2020), As noted by Rather (2021), the swarming ability of *Proteus mirabilis* is significant because it plays a role in the bacteria's pathogenesis, with this swarming ability linked to the expression of virulence factors. *Proteus mirabilis* exhibits a distinctive bulls-eye pattern on agar plates resulting from the uniform periodic

transitions between its vegetative and swarming states (Morgenstein *et al* 2023). This organism shows heightened resistance to a variety of antimicrobial agents, leading to alterations in treatment methodologies and contributing to unfavorable outcomes, which have ultimately increased the mortality rates among hospitalised patients (Lockhart *et al* 2022). Being one of the prevalent causes of urinary tract infections, along with various other Gram-negative Enterobacteriaceae, ongoing monitoring of the antimicrobial susceptibility of this clinical isolate is crucial to maintain an up-to-date understanding of its susceptibility patterns (Pokhrel *et al* 2020). Consequently, the objective of this study was to isolate and determine the prevalence of the antibiotic susceptibility profile of *P. mirabilis* in urine samples from patients visiting selected hospitals in the Kaduna metropolis.

Materials and Methods

Kaduna is one of the states in Nigeria. It comprises twenty-three local government areas. The study areas lie between latitudes 10º14´42″N and 10º48´29″N and longitude 7º1´16″E and 7º47´10″E with an area of 3156 Km.^{2} The hospitals are presented in the map (Fig. 1), which are Barau Dikko teaching hospital, General hospital Rigasa, Gwanna-Awan General Hospital Kakuri, and General Hospital Sabon-Tasha respectively.

The sample size of the study was determined by using Cochran's formula

- N = $\frac{t^2 x\ P(1-P)}{m^2}$ (Cochran's, 1997 & Suwaiba *et al* 2020)

Where:

- N = number of samples
- m = margin of error = 0.05
- p= percentage of existing prevalence 15% (Hassan *et al* 2019)
- t = t-value at 95% coincidence interval = 1.96
- imputation of the figures into the sample size formula
- n = $\frac{(1.96)^2 x\ 0.150(1-0.150)}{(0.05)^2}$
- n = $\frac{3.8416\ x\ 0.150\ x\ (0.850)}{0.0025}$
- n = $\frac{0.489804}{0.0025}$
- n = 195.9
- 200 clinical samples were collected.

Sterile universal containers were used to gather clean catch urine samples following the methodology outlined by Solberg *et al* (2021) & Kraychick *et al* (2024). A total of 200 midstream urine (MSU) samples were collected from male and female patients across all age groups inside sterile disposable bottles. Patients received instructions on how to collect the samples and the importance of quickly delivering them to the medical microbiology laboratory at Barau Dikko Teaching Hospital (BDTH), Gwamna Awan General Hospital Kakuri (GGHK), General Hospital Sabon-Tasha (GHST) and General Hospital Rigasa (GHR). The samples were labeled and transported

to the Department of Microbiology at Kaduna State University, Kaduna, in an ice pack, and were analysed within 30 minutes to 1 hour after collection.

Two hundred (200) urine samples were collected and subjected to centrifugation at a speed of 1500 rpm for five minutes. The supernatant was discarded, and the remaining residues (along with the collected urinary catheters) were collected using non-pyrogenic swabs that had been moistened with physiological saline, then aseptically inoculated onto the surface of Cystine Lactose Electrolyte Deficient (CLED) agar, followed by incubation at 37°C for 24 hours in aerobic conditions. After the overnight incubation period, the culture plates were examined for signs of microbial growth. Selected cultured colonies were then picked and sub-cultured, followed by another incubation at 37°C for 24 hours to achieve pure cultures (Baker 1971 & Umar *et al* 2020).

The Gram staining method was utilised, followed by microscopic examination and biochemical assays based on the works of Oyeleke & Manga (2008), Cheesbrough (2010), Oyekele *et al* (2011), & Hamza *et al* (2020). The features of the isolates were evaluated in comparison with Bergey's Manual for Determinative Bacteriology (Cheesbrough 2010; Oyeleke *et al* 2011; Ajenifuja & Oni 2022).

The susceptibility and resistance of bacteria to antibiotics were evaluated using the disk-diffusion method. Bacterial colonies from Mueller-Hinton (MH) agar plates were transferred into McCartney bottles containing sterile normal saline to achieve a bacterial density of 3×10^8 organisms per milliliter, as indicated by the McFarland standard scale number 1. Freshly cultured colonies were evenly streaked onto freshly prepared Mueller-Hinton (MH) agar plates utilising disposable sterile swabs. The plates were allowed to dry for a short period, after which discs of selected antimicrobial agents (ertapenem 10ug, meropenem 10ug, septrin 30ug, chloramphenicol 30ug, sparfloxacin 10ug, ciprofloxacin 30ug, amoxicillin 30ug, augmentin 10ug, gentamicin 30ug, pefloxacin 30ug, tarivid 10ug, and streptomycin 30ug) were placed on the surface of the inoculated agar. The plates were incubated at 37°C for 24 hours. After the overnight incubation period, the culture plates were examined for growth inhibition and resistance. A ruler was utilised to measure the inhibition zones (Cheesbrough 2010; Aslem *et al* 2020 & Hamza *et al* 2020). The isolates were classified as sensitive or resistant to the respective antibiotics by comparing the measured values with the recommended standard charts (CLSI 2021 & CLSI 2022). Isolates that were resistant to the selected antimicrobials underwent further molecular testing to identify resistant genes.

Results

Table 1 presents the colonial characteristics of ten (10) isolates from urine samples of 200 patients, who visited the four hospitals in Kaduna metropolis. Seven (7) of the isolates appear to belong to the *Bacillus spp*. and are identified by sample codes RgGH002, BDTH3, KCH2, BDTH7, RgGH840, BDTH2, and BDH4. These isolates exhibit yellowish creamy, mucoid colonies on CLED agar, light yellow creamy, opaque colonies on chocolate agar and rough, opaque, fussy colonies on nutrient agar. They showed a

positive (+) rod shape reaction when subjected to Gram staining, indicating they are likely *Bacillus spp*. Conversely, three (3) isolates from the ten (10) were identified as *Proteus mirabilis*, with sample codes TH1, CH2, and TH5. These isolates demonstrated a negative (-ve) rod shape reaction in Gram staining and are probably *Proteus mirabilis*. Their characteristic appearance on CLED agar is blue translucent, with swarming and irregular edges, while on chocolate agar, they present dull creamy swarming, and on nutrient agar, they exhibit a glistening, irregular edge with swarming; additionally, they showed a negative (-ve) rod shape in Gram reaction, indicating they are likely *Proteus mirabilis*.

The biochemical identification results for the isolates are summarised in table 2, where isolates TH5, TH1, and CH2 exhibited Methyl Red (+), Citrate (+), Sucrose (-), Lactose (-), Voges-Proskauer (-), Gas (+), catalase (+), Hydrogen Sulphide (+), Indole (-), and Glucose (+), suggesting they represent *Proteus mirabilis*.

Table 3 shows the prevalence of *Proteus mirabilis* across the four (4) hospitals where urine samples were collected, identified as BDTH, GHR, GHST, and GGHK. From the fifty (50) urine samples collected from each hospital, the prevalence of *P. mirabilis* was found to be 2(4.0%) in BDTH, 0(0%) in GHST, 0(0%) in GHR, and 1(2.0%) in GGHK, resulting in an overall prevalence of 1.5% across the hospitals. The antibiotic susceptibility profile of the isolates is presented in table 4. The antibiotic profile demonstrates high susceptibility to septrin 3(100%), ciprofloxacin 3(100%), pefloxacin 3(100%), chloramphenicol 3(100%), tarivid 3(100%), streptomycin 3(100%) and gentamycin 2(66.7%).

Discussion

The overall prevalence of *Proteus mirabilis* found in the urine samples of patients across four different hospitals is 1.5%. The low prevalence noted in this study may be a result of earlier reports highlighting the scarcity of *Proteus mirabilis* isolates from clinical specimens. This observation aligns with the 1.0% prevalence reported by Khodair & Al-Asady (2021), although, it is slightly higher than what was found in their research. Conversely, Suhartono *et al* (2022) & Jabur *et al* (2021) documented a somewhat elevated prevalence of 2.0%. Additionally, Senthamarai *et al* (2021) reported a prevalence of 5.2%, Feglo *et al* (2022) indicated 7.0%, Serry *et al* (2024) found 11.7%, while El-Sokkary *et al* (2019) & Ahmed (2020) reported figures of 12.4% and 13.2%, respectively. Nonetheless, the limited distribution of *P. mirabilis* isolates in the four hospitals, alongside negative findings from certain facilities, may be due to the failure to screen other hospitals or because patients did not follow the previously provided guidelines for collecting urine samples using sterile containers. This disregard occurred despite the orientation given to them, which aimed to prevent contamination of urine samples with unwanted organisms or because patients were undergoing antibiotic treatment during the sampling period. This is consistent with the findings of Ike *et al* (2020), who stated that approximately 32% of patients or their guardians did not receive sufficient instructions regarding the proper collection, storage (refrigeration), and prompt transportation of samples to the laboratory within one hour. Semwal *et al* (2020) reported

similar conclusions in a study assessing the effects of instruction on urine collection and storage practices by patients and their attendants.

The antibiotics resistance pattern observed in this study as indicated in Table 3, is in accordance with previous findings that states; *P. mirabilis* isolates were susceptible to streptomycin 100%, ciprofloxacin 75%, pefloxacin 70%, gentamycin 50% (Tula & lyoha 2014; Umar *et al* 2020; & Akujobi *et al* 2022). Consequently, since it has been established those antibiotics used in this study demonstrated a high percentage of effectiveness against UTI caused by *P. mirabilis*, it is recommended that these antibiotics be suggested for treating UTIs and other infections associated with *P. mirabilis*, aligning with previous recommendations from Umar *et al* (2020) & Akujobi *et al* (2022).

Conclusion

The overall prevalence rate of *P. mirabilis* isolated across the four (4) different facilities within Kaduna metropolis was 1.5%. All the isolates of *Proteus mirabilis* isolated from urine samples were susceptible to septrin 3(100%), ciprofloxacin 3(100%), pefloxacin 3(100%), chloramphenicol 3(100%), tarivid 3(100%), streptomycin 3(100%) and gentamycin 2(66.7%). More hospitals in Kaduna should be screened to curb the low prevalence of *P. mirabilis.* Septrin, ciprofloxacin, pefloxacin, chloramphenicol, tarivid, streptomycin and gentamycin should be use for treatments of UTI and other infectious diseases associated with *P. mirabilis* following the high susceptibility profile of the isolates.

Table 1: Colonial morphology of the isolates on growth media

Samples Codes	CLED Di (Bevis) Agar	Chocolate Agar	Nutrient Agar	Probable Bacteria
$RGH00_2$	Yellowish creamy, Mucoid colonies	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*
TH_3	Yellowish creamy, Mucoid colonies	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*
CH_1	Yellowish creamy, Mucoid colonies	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*
TH_7	Yellowish creamy, Mucoid colonies	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*
RGH_{840}	Yellowish creamy, Mucoid colonies	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*
TH_2	Yellowish creamy, Mucoid	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*

	colonies			
TH_4	Yellowish creamy, Mucoid colonies	Light yellow creamy, Opaque	Rough, opaque, Fuzzy white	*Bacillus spp*
TH_1	Blue Translucent Swarming characteristics	Irregular edge dull creamy Swarming characteristics	Glistening, Irregular Edge, swarming characteristics	*Proteus mirabilis*
CH_2	Blue Translucent Swarming characteristics	Irregular edge dull creamy Swarming characteristics	Glistening, Irregular Edge, swarming characteristics	*Proteus mirabilis*
TH_5	Blue Translucent Swarming characteristics	Irregular edge dull creamy Swarming characteristics	Glistening, Irregular Edge, swarming characteristics	*Proteus mirabilis*

Keys:
RGH_{002}- Rigasa General Hospital Isolate (002), **TH_3**- Barau Dikko Teaching Hospital Isolate (3), **TH_7**- Barau Dikko Teaching Hospital (7), **CH_1**- Gwamna Awan General HospitalKakuri Isolate (1), **RGH_{840}**- Rigasa General Hospital Isolate (840), **TH_2**- Barau Dikko Teaching Hospital Isolate (2), **TH_4**- Barau Dikko Teaching Hospital Isolate (4), **TH_1**- Barau Dikko Teaching Hospital Isolate (1), **CH_2**- Gwamna Awan General HospitalKakuri Isolate (2), **TH_5**- Barau Dikko Teaching Hospital Isolate (5).

Table 2: Biochemical characteristics of isolates from urine samples of patients

Isolates With Codes	Gram Reaction	Cat.	Ind.	Citra.	MR.	VP	GL	H_2S	Lac	Suc	G	Probable Bacteria
RGH_{002}	+ve rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
TH_2	+ve rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
CH_1	+ve rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
TH_7	+ve rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
RGH_{840}	+ve rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
TH_2	+ve rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
TH_5	-ve Rod-shape in clusters	+	-	+	+	-	+	+	-	-	+	*Proteus mirabilis*
TH_I	-ve Rodshape in clusters	+	-	+	+	-	+	+	-	-	+	*Proteus mirabilis*
TH_4	+ve Rod shape	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
CH_2	-ve Rodshape in clusters	+	-	+	+	-	+	+	-	-	+	*Proteus mirabilis*

Keys:
+ve- Positive MR - Methyl Red + - Positive Cir – Citrate Suc - Sucrose
-ve -Negative Lac- Lactose VP -Vogas Proskauer G- Gas
Cat -catalase H2S - Hydrogen Sulphide - -NegativeInd-Indole GL -Glucose

Table 3: Prevalence of *Proteus mirabilis* in Urine Samples from Different Hospitals (n=3)

Sample Location	Sample No	TH_5	CH_2	TH_1	Total (%)
BDTH	50	1(2.0)	0(0)	1(2.0)	2(4.0)
GHR	50	0(0)	0(0)	0(0)	0(0)
GHST	50	0(0)	0(0)	0(0)	0(0)
GGHK	50	0(0)	1(2.0)	0(0)	1(2.0)
Total	200	1(2.0)	1(2.0)	1(2.0)	3(1.5)

Key:
BDTH- Barau Dikko Teaching Hospital; **GHR**- General Hospital Rigasa; **GHST**- General Hospital Sabon-Tasha; **GGHK**- Gwanna Awam General Hospital Kakuri; **n** - Number of the isolate; **%** -Percentage

TH_5
CH_2 } Positive isolates of *Proteus mirabilis*
TH_1

Table 4: Antibiotic Susceptibility Pattern of *Proteus mirabilis* isolates (n =3)

	Antibiotic Names	Disc Conc	Standard Breaking Point	S (%)	R (%)
(SxT)	Septrin	30ug	16≥	3(100)	0(0.0)
(CH)	Chloramphenicol	30ug	18≥	3(100)	0(0.0)
(CPx)	Ciprofloxacin	10ug	21≥	3(100)	0(0.0)
(Am)	Amoxacillin	30ug	18≥	0(0.0)	3(100)
(Au)	Augmentin	30ug	18≥	1(33.3)	2 (66.7)
(CN)	Gentamycin	30ug	17≥	2(66.7)	1(33.3)
(PEF)	Pefloxacin	30ug	16≥	3(100)	0(0.0)
(OFX)	Tarivid	10ug	16≥	3(100)	0(0.0)
(S)	Streptomycin	30ug	15≥	3(100)	0(0.0)
(ErT)	Ertapenem	30ug	20≥	0(0.0)	3(100)
(Mem)	Meropenem	30ug	18≥	1(33.3)	2(66.7)

Key: n=number of +ve Isolate; **Conc** = Concentration; **S**=Susceptibility; R=Resistance; ≥=Greater; ≤=Less than; () – Bracket; Standard break point: CLSI 2021.

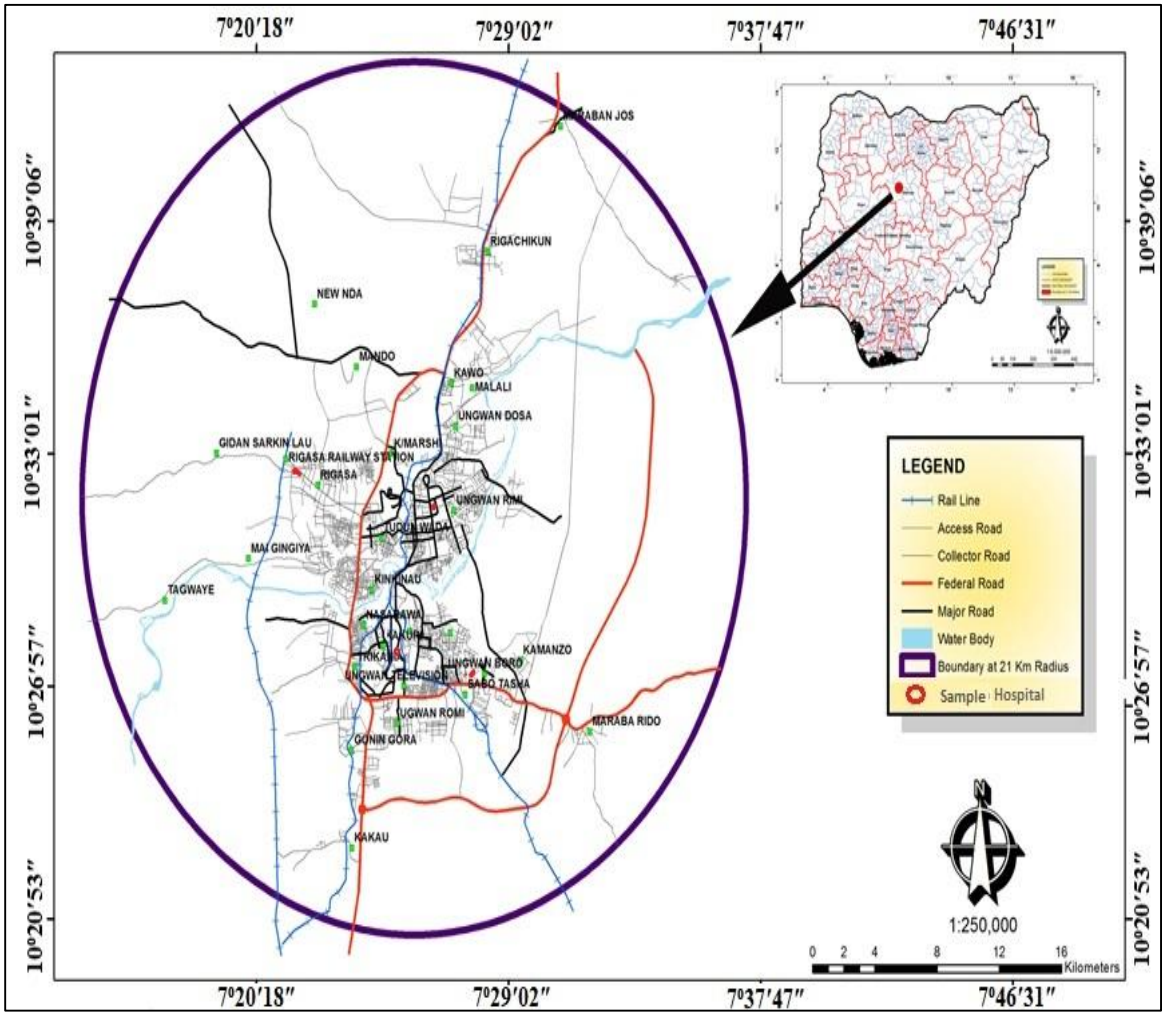

Fig 1: Map of Kaduna Metropolis (KASU GIS LAB, 2021)

MINISTRY OF HEALTH
KADUNA STATE NIGERIA

MOH/ADM/744/VOL.1/938 **10TH NOVEMBER, 2020**

NOTICE OF EXPEDITED REVIEW AND APPROVAL

PREVALENCE OF *PROTEUS MIRABILIS* IN URINE SAMPLES OF PATIENTS AND DETECTION OF SOME ANTIBIOTIC RESISTANCE GENES

Name of Principal Investigator:	**CHINEDU BLESSING CHIDOZIE**
Address of Principal Investigator:	**DEPARTMENT OF MICROBIOLOGY KADUNA STATE UNIVERSITY, KADUNA**
Date of receipt of Application:	**21ST OCTOBER, 2020**
Date of Ethical Approval:	**10TH NOVEMBER, 2020**
Date of Expiry of Approval:	**10TH NOVEMBER, 2021**

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other particular information have been reviewed and given Expediated approval by the Health Research Ethics Committee (HREC) of Kaduna State Ministry of Health.

If there is delay in starting the research or any need for correction, inform the HREC Secretariat so that the dates of approval or other corrections can be effected accordingly.

However, Research is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. SUNDAY JOSEPH
For: Chairman

SMOH Resource Centre Kaduna Date 8/12/2020

Independence Way, P.M.B 2014, Kaduna,
Kaduna State-Nigeria
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Fig 2: Ethical Approval from Kaduna State Ministry of Health

BARAU DIKKO TEACHING HOSPITAL
KADUNA STATE UNIVERSITY
Lafiya Road P.O Box 9727 Kaduna, Kaduna State, Nigeria

Chief Medical Director (CMD)
PROF. ABDULKADIR MUSA TABARI
MBBS, FMCR, FINCS
Chairman, Medical Advisory Committee (CMAC)
DR. SILAS TOKAN BADUKU
MBBS, FWACS, FMCR, FICS
Director of Administration (DA)
MAL. ILIYASU YUSUF
B.A PUBLIC ADMIN, PGDPPA, DIP. SHARIA LAW

14th Jnuary, 2020

Your Ref: MSC/MCB/SCS/00049/2016
Phone:08064695390

Chinedu Blessing Chidozie,
Dept. of Microbiology,
Kaduna State University,
Kaduna.

Dear Chinedu,

HREC Reference Number: 20-0014
Project Title: "Prevalence of *Proteus mirabilis* in Urine Samples of Patients And Detection of Some Antibiotic Resistance Genes"
Protocol Number 20-0014-1

Thank you for submitting the aboev research project for single ethical review. This project was considered by the Barau Dikko Teaching Hospital, Health Research Ethics Committee [BDTH HREC] at its meeting held on the 8th January, 2020.

I am pleased to advise you that the BDTH-HREC has granted ethical approval of this research project.
The nominated participating site/s in this project is/are:

Barau Dikko Teaching Hospital Facilities

[Note: If additional sites are engaged prior to the commencement of, or during the research project, the Coordinating Principal investigator is required to notify BDTH-HREC. Notification of withdrawal sites should be provided to the BDTH-HREC in a tmely fashion.
The approved documents include:

Documents	Versions	Date
"Prevalence of Proteus mirabilis **in Urine Samples of Patients And Detection of Some Antibiotic Resistance Genes"**	1	12th December, 2019

Approval of this project from BDTH-HREC is valid from 8th January, 2020 to 7th January, 2021 subject to the following conditions being met:

The Coordinating Principal Investigator will immediately report anything that might warrant review of ethical approval of the project.

- The Coordinating Principal Investigator will notify the BDTH-HREC of any event that requires a modification to the protocol or other project documents and submit any required amendments in accordance with the instructions provided by the HREC.
- The Coordinating Principal Investigator will submit any necessary reports related to the safety of research participants in accordance with BDTH-HREC policy and procedures.
- The Coordinating Principal Investigator will report the BDTH-HREC and notify the HREC when the project is completed at all sites.
- The Coordinating Principal Investigator will notify the BDTH-HREC if the project is discontinued at a participating site before the expected completion date, with reasons provided.
- The Coordinating Principal Investigator will notify the BDTH-HREC of any plan to extend the duration of the project past the approval period listed above and will submit any associated required documentation.
- The Coordinating Investigator will notify the BDTH-HREC of his or her inability to continue as Coordinating Principal Investigator including the name of and contact information for a replacement.

This letter constitutes ethical approval only. This project cannot proceed at any site until separate research governance authorization has been obtained from the CEO or delegate of the institution under whose auspices the research will be conducted at that site.

Should you have any queries about the BDTH-HREC's consideration of your project please contact Admin officer, Barau Dikko Teaching Hospital, Lafia Road Kaduna.
The BDTH HREC wishes you every success in your research.

Yours faithfully,

Dr. J G Makama
Chairman

BDTH HRECThis HREC is constituted and operates in accordance with the National Health Research Ethics Committee (NHREC)

Fig 3: Ethical Approval from Barau Dikko Teaching Hospital (BDTH)

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