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Surveillance for Antibiogram Pattern of Nosocomial Bacteria from two Selected Hospitals in Kano State, Nigeria

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Abstract
In a bid to determine the level of antimicrobial resistance among bacterial pathogens feared to be nosocomially transmitted in some hospitals, Firstly, patients of HCAI was identified and followed-up accordingly with standardized questionnaire in the hospitals. Sample were collected for bacteriological assay using Culture and biochemical methods for the investigation of bacteria associated with nosocomial, Antibiogram pattern was assessed by Disk diffusion as to mark the resistant ones and presence of gene encoding for mecA and blaZ with the aid of PCR. At AKTH, A total of 63% of isolates were Gram-positive while 37% were Gram-negative bacteria. *S. aureus* were the most common isolates with frequency of 37%. Most of all the Gram-positive isolates were resistant to cefuroxime (30µg) and Amoxicillin (10µg) while they shown high sensitivity to other antibiotics tested against them. Only 18% of *S. aureus* are suspected MRSA. Most of Gram-negative isolates were found to be resistant to amoxicillin (10µg) 100%, gentamicin (10µg) 71% and chloramphenicol (30µg) 57%. At MMSH, 86% of the isolates were Gram-positive while 14% were Gram-negative bacteria. *S. aureus*, 42% were the most common isolates. All the isolates were resistance to cefuroxime and Amoxicillin (10µg) while they showed sensitivity to other tested antibiotics. while most shows sensitivity to other tested antibiotics. Only 24% of *S. aureus* were MRSA. 63% of the tested MRSA were mecA positive while 100% were blaZ positive. The high rates of resistome of isolates represent a substantial threat to the public where well-structured and continuous surveillance system for antimicrobial resistance is needed to encourage the monitoring of antimicrobial therapy.
Keywords: *S. aureus*, Risk-factor, Surveillance, Resistance, Antimicrobial

INTRODUCTION

Healthcare-acquired infections (HCAIs), also known as nosocomial infections are infections that patients get while receiving treatment for medical or surgical conditions (Alkali *et al.*, 2019). A nosocomial infection (NI) is a localized or a systemic infection resulting from an adverse reaction to infection agents or its toxins that develops in 48 hours or more after admission and was not incubating on admission (Kouchak and Askarian, 2012). Nosocomial infections remain a major cause of mortality and morbidity worldwide. Despite the highly specialized interventions and policies, the rate of infection is still high due to the emergence of antimicrobial-resistance bacteria (Moti *et al.*, 2018).

Staphylococcus aureus (*S. aureus*) is perhaps the greatest concern of human pathogens because of its intrinsic virulence because of its ability to cause a diverse array of life-threatening infections and its capacity to adapt to different environmental conditions (Poumajaf *et al.*, 2014). Nowadays, this organism is the leading overall cause of healthcare associated infections globally and, as more patients are treated outside the hospital settings, is an increasing concern in the community (NNIS, 2011; Stenhem *et al.*, 2010).

Resistance of microbial strains to antibiotics is a major public health concern that requires multidisciplinary approach towards curbing it out (Anderson and Houghes, 2010). Resistance of pathogenic bacteria to drugs has now become increasingly commonplace (Sevillano *et al.*, 2006). There is consensus that patients with old age problems admitted to hospitals for one or the other ailment generally are multi-morbid and have reduced immune competence and eventually become increasingly compromised by antibiotics resistant bacteria (Sahu *et al.*, 2012).

According to Okesola and Oni (2009), most Nigerian microbial isolates indicated high resistance in both Gram-positive and Gram-negative organisms through examination of antimicrobial susceptibility assay. Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Staphylococcus aureus* (VRSA) and *Enterococci*, Multidrug resistant gram negative bacteria such as those with Extended Spectrum Beta-Lactamase (ESBL) resistance and carbapenem resistant Enterobacteriaceae, multi-drug and extensive-drug resistant *Mycobacterium tuberculosis* (MDR-TB and XDR-TB) (Anzaku *et al.*, 2018). Antimicrobial drug resistance is usually not monitored in under resourced countries because they lack surveillance networks, and appropriate diagnostics. This acceleration problem accounts for substantial number of excess deaths, especially among infant (Vernet *et al.*, 2014). Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have become widespread even in under-resourced countries (Vernet *et al.*, 2014).

MATERIAL AND METHODS

Study Population and Hospitals

Two hospitals were selected for the study and these are; Aminu Kano Teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital (MMSH). The Hospitals are strategically located for access to both urban and rural populations from the 44 Local Government Areas of Kano State.

The study population comprises of both male and female patients that developed any illness that cannot be explain by an underlying illness 48hrs or more after admission.

Ethical Approval

The study was reviewed and ethical clearance was obtained from both Aminu Kano Teaching Hospital ethical committee and Ministry of Health Kano state. Each of the participants or parent/caretaker was informed and voluntary, written and signed consent/assent was obtained from the participant (Appendix I and II).

Data and Sample Collection

A total number of 150 non duplicated Urine, wound and blood samples were collected from the entire two study area (AKTH=75 and MMSH=75). Demographic data was obtained from patients using Questionnaire in order to assess the Risk factors

Bacterial Isolation

In vitro characterization of bacteria was carried out using standard culture and biochemical tests. Each specimen was streaked on to differential and selective culture media (Oxoid, Ltd, UK) including CLED, Chocolate (Oxoid, UK), Mannitol salt agar and MacConkey agar (Tm, Media) for investigation of bacteria.

Biochemical tests

All the isolated organisms were Gram-stained and Biochemical test was carried on the bases of their Gram reaction standard biochemical reactions, including catalase, coagulase (Poumajaf *et al.*, 2014), indole, citrate and oxidase, urease and motility. Reading of the culture was done after overnight incubation at 37^0C.

Antimicrobial sensitivity testing

This test was carried out using disk diffusion method according to the guideline of the Clinical Laboratory Standard Institute (CLSI, 2014). In brief, a bacterial suspension adjusted to 0.5 MacFarland on to Muller- Hinton agar was evenly strict on a plate to ensure even distribution of inoculum in confluents lawn of growth (Hudzick, 2009). Antibiotics impregnated Oxoid disc of Cefoxitin (30µg), Gentamicin (10µg), Cefuroxime (30µg), chloramphenicol (30µg), Erythromycin (15µg) and Amoxicillin (10µg) for Gram positive isolates while Imipenem (10µg), Chloramphenicol (30µg), Amoxicillin (10µg), Cefoxitin (30µg), Gentamicin (10µg) and Tetracycline (30µg) (Gram negative) were placed on the surface of the agar with the aid of forceps. After the disc were placed, the lid of the plate was replaced, inverted and was incubated at 35°C inside Air incubator for 18hours While Staphylococcus against Cefoxitin plate were incubated for a full 24hrs before reading. Results of other disc were taken after 18hrs except *Staphylococcus* against Cefoxitin for phenotypic detection of MRSA which was re-incubated for a total of 24hrs before reading (Hudzicki, 2009). Following incubation, Zone sizes of inhibition were measured to the nearest millimeters using a vernier caliper. The zone size was recorded on a recording sheet which was interpreted using CLSI, 2021 guidelines to determine the susceptibility or resistance of the organism to each drug tested indicated as either susceptible (S), Intermediate (I) or resistant (R) (Magiorakos *et al.*, 2012; Tolera *et al.*, 2018)

Data Analysis

Descriptive statistics were used to report prevalence rate and cumulative prevalence of HCAIs.

RESULTS

Table 1 present a total number of 150 nosocomial patient that were identified in Aminu Kano teaching hospital and Murtala Muhammad specialist hospital, Kano, Nigeria.

Out of the identified patients included, 44.7% were male while 55.3% were female; Age range between 1-14 and 50 and above tends to have highest percentage of identified patient with 31.3 and 39.4% respectively; Surgical ward had the highest number of confirmed patient (46%); length of stay between 10days and above have the most number of patient (53.3%) and lastly 66.75 of the identified patient were married while the remaining 33.3% were single. Total number of patient under catheters were 85 (56.7%) while 65 (43.3%) are without catheters. 59 (39.3%) were immunodeficiency patient while 91 (60.7%) are not And Lastly 32 (21.3%) patient are receiving antimicrobial while 118 (78.7%) were not.

The distribution of bacteria isolates from identified nosocomial patients at both study area base on their site of infection is shown in Table 2. Surgical site infection accounts to 53.7% and is the most frequent site of infection follows by Urinary tract and blood stream with 30 (31.6%) and 14 (14.7%) respectively. Out of the 95 positive isolate obtained in this study, *S. aureus* 38 (40%) is the most predominant isolates followed by CoNS, *E. coli*, *Kleb sp.*, *P. aeruginosa* and *Strep sp* with 28(29%), 9(10%), 9(10%), 6(6%) and 5 (5%) respectively. Table 3 and 4 shows the susceptibility patterns of the isolates against selected antimicrobial agents as all the Gram positive isolate were resistance to Cefuroxime (30µg) while most of them were also resistance to Amoxicillin (10 µg) except *Streptococcus sp.* which was sensitive to the drug at both study area. Imipinem (10µg) was active against all Gram negative isolates while they were resistant to Amoxicillin (10µg). *E. coli* and *Kleb sp.* were resistance to Gentamicin (10µg) at AKTH while *P. aeruginosa* was sensitive to it. At AKTH, 37% were isolates positive for *S. aureus* , 18% were MRSA isolates, 33.3% were mecA positive and 100% were blaZ positive while at MMSH, 43% were isolates positive for *S. aureus,* 24% were MRSA isolates, 80% were mecA positive and 100% were blaZ positive.

Table 1: Some Demography and Predisposing Factors for the Possible Nosocomial Infections in Patients in both AKTH and MMSH, Kano (2020).

Characteristics	AKTH n (%). N=75	MMSH n (%). N=75	Total n (%). N=150
Sex			
Male	31(41.0)	36(48.0)	67(44.7)
Female	44(59.0)	39(52.0)	83(55.3)
Age (in years)			
1-14	20(27.0)	27(36.0)	47(31.3)
15-35	10(13.0)	5(7.0)	15(10.0)
36-49	19(25.0)	10(13.0)	29(19.3)
50 and Above	26(35.0)	33(44.0)	59(39.4)
Admission wards			
Medical	29(39.0)	23(31.0)	52(34.7)
Surgical	30(40.0)	39(52.0)	69(46.0)
Pediatric	16(21.0)	13(17.0)	29(19.3)
Length of stay			
‹ 3days	0(0.0)	0(0.0)	0(0.0)
3-5days	10(13.0)	5(7.0)	15(10.0)
6-9days	26(35.0)	29(39.0)	55(36.7)
10 and above	39(52.0)	41(54.0)	80(53.3)
Marital status			
Single	20(27.0)	30(40.0)	50(33.3)
Married	55(73.0)	45(60.0)	100(66.7)
Use of Catheter			
Yes	46(61.0)	39(52.0)	85(56.7)
No	29(39.0)	36(48.0)	65(43.3)
Immunodeficiency			
Yes	30(40.0)	29(39.0)	59(39.3)
No	45(60.0)	46(61.0)	91(60.7)
Patient received Antimicrobial			
Yes	19(25.0)	13(17.0)	32(21.3)
No	56(75.0)	62(83.0)	118(78.7)

Table 2: Distribution of Bacterial Isolates from patients with NIs in the two Hospitals, Kano State

Bacteria	Surgical Site 51(53.7)		Urinary Tract 30(31.6)		Blood Stream 14(14.7)		Total n(%)
	AKTH n(%)	MMSH n(%)	AKTH n(%)	MMSH n(%)	AKTH n(%)	MMSH n(%)	
S. aureus	14(44)	10(53)	1(10)	7(35)	2(50)	4(40)	38(40)
Strep. Sp.	2(6)	0(0)	0(0)	0(0)	0(0)	3(30)	5(5)
CoNS	7(22)	9(47)	2(20)	7(35)	1(25)	2(20)	28(29)
E. coli	1(3)	0(0)	4(40)	3(15)	0(0)	1(10)	9(10)
P. aeruginosa	3(9)	0(0)	2(20)	0(0)	1(25)	0(0)	6(6)
Kleb sp.	5(16)	0(0)	1(10)	3(15)	0(0)	0(0)	9(10)
Total	32(33.7)	19(20)	10(10.5)	20(21.1)	4(4.2)	10(10.5)	95(100)

Key; CoNS; Coagulase Negative Staphylococci
AKTH: Amino Kano Teaching Hospital; MMSH: Murtala Muhammed Specialist Hospital

Table 3: Antimicrobial Susceptibilities of Gram positive Bacteria Associated with Nosocomial Infections in Patients at AKTH and MMSH, Kano (2020)

Antibiotic (µg) ATCC25923	*S. aureus*		*CoNS*		*Strep. Sp.*		
	AKTH N=17	MMSH N=21	AKTH N=10	MMSH N=18	AKTH N=2	MMSH N=3	AKTH N=1
GN (10)	21.6(S)	26.5(S)	25.5(S)	19.5(S)	19.2(S)	18.6(S)	30.0(S)
FOX (30)	26.5 (S)	32.1(S)	32.6(S)	36.2(S)	36.5 (S)	29.0(S)	33.0(S)
E(15)	27.5 (S)	35.0 (S)	15.3(I)	24.5(S)	13.2(R)	23.2(S)	33.0(S)
C (30)	30.6 (S)	19.5(S)	18.2(S)	22.6 (S)	22.5(S)	33.6 (S)	31.0(S)
AML(10)	5.4 (R)	9.2(R)	6.2(R)	12.6 (R)	20.1 (S)	16.2 (S)	10.0 (R)
CXM (30)	0 (R)	0 (R)	0 (R)	0 (R)	0(R)	0(R)	10.0(R)

GN: Gentamicin; FOX: Cefoxitin; E: Erythromycin; C: Chloramphenicol; AML: Amoxicilin; CXM: Cefuroxime, CoNS: Coagulase Negative Staphylococcus; S: Sensitive; I: Intermediate; R:Resistant.
N: Number of Isolates

Table 4: Antimicrobial Susceptibilities of Gram-negative Bacteria Associated with Nosocomial Infections in Patients at AKTH and MMSH (2020).

Antibiotics	*E. coli*		*Kleb Sp.*		*P. aeruginosa*
	AKTH N=5	MMSH N=4	AKTH N=6	MMSH N=3	AKTH N=6
IMP 10µg	29.6 (S)	25.6(S)	32.6(S)	29.2(S)	24.2(S)
C 30µg	20.6(S)	19.5(S)	15.4(I)	13.2(I)	11.5(R)
AML 10µg	6.2(R)	8.0(R)	6.0(R)	14.2(R)	8.5(R)
FOX 30µg	15.2(R)	20.5(S)	24.6(S)	23.6(S)	19.6(S)
GN 10µg	6.0(R)	14.5(S)	9.8(R)	16.0(S)	15.5(S)
TE 30µg	14.5(S)	16.8(S)	11.8(I)	16.8(S)	17.2(S)

IMP: Imipenem; C: Chloramphenicol; AML: Amoxicillin; FOX: Cefoxitin; GN: Gentamicin; TE: Tetracycline; S: Sensitive; I: Intermediate; R: Resistant. N: Number of Isolates.
The Zone of inhibition was interpreted as Sensitive, Intermediate and Resistant according to CLSI, 2021.

Table 5: Distribution of *S. aureus* and MRSA Isolates based on mecA and blaZ gene presence

Sampling sites	No. (%) of samples Positive for *S. aureus*	No. (%) of MRSA Isolates	No. (%) mecA positive	No. (%) of blaZ positive
AKTH	17(37)	03(18)	01(33.3)	03(100)
MMSH	21(43)	05(24)	04(80)	05(100)

AKTH: Amino Kano Teaching Hospital; MMSH: Murtala Muhammed Specialist Hospital
x^2 =0.224, P<0.05 df= 1, There is no significant difference

The result of PCR products of mecA and blaZ on 1.5% agarose gel were shown below in Fig 4.1. The size of the amplicon for mecA and blaZ gene corresponds to control which is 336bp and 400bp respectively. From lane 1-3 is a test isolate of AKTH while from 4-8 is for the test isolates of MMSH while lane 9 serves as control. The result that shows a band that's correspond to the ladder base pair 336 around each lane were termed mecA positive isolate while the band that correspond to 400bp were termed blaZ positive.

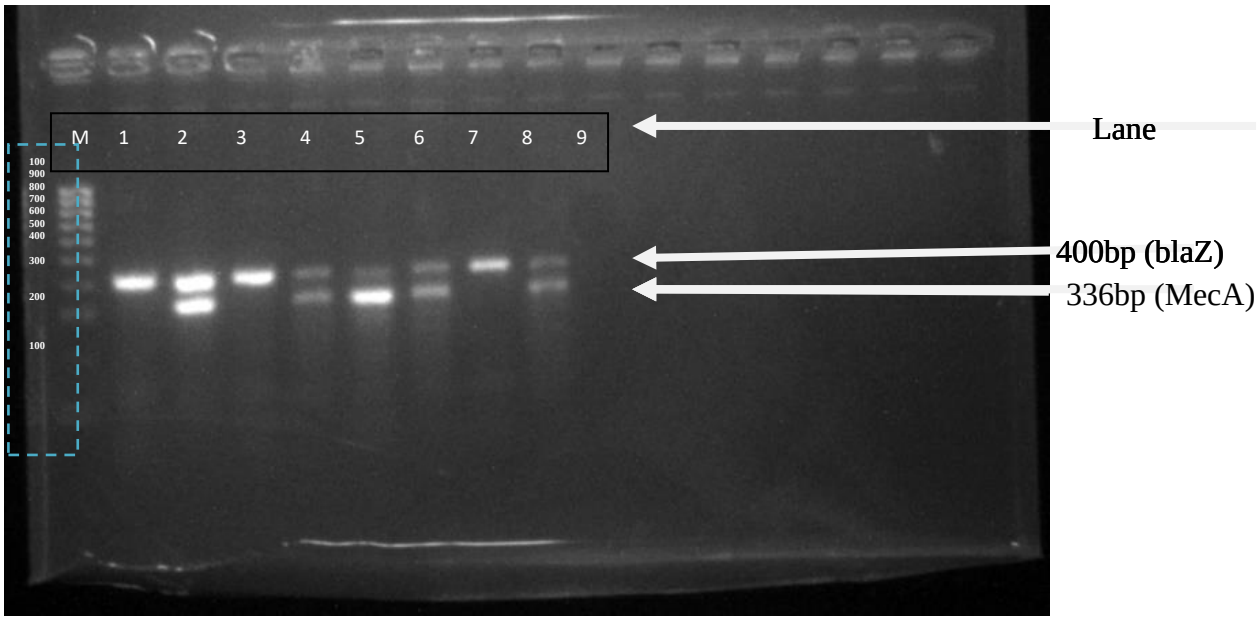

Figure 4.1: Gel-electrophorogram for mecA and blaZ gene in *S. aureus* isolates
Lane 1-3 = AKTH
Lane 4-8 = MMSH
Lane 9 = Control
This figure shows the polymerase chain reaction (PCR) result for the detection of both mecA and blaZ gene from suspected MRSA isolates.

DISCUSSION

Nosocomial infections are one of the major public health problems around the world, however it vary from one country to the other because of the differences in surveillance approach. It can result to high morbidity and mortality (Tolera *et al.*, 2018). In this study, 150 non duplicated clinical samples were analyzed of which participant of age group 50years and above have the highest prevalence of 59 (39.4%) followed by age group 1-14yrs 47 (31.3%), the least age group observed are 15-35years and 36-49years with 15 (10%) and 29 (19.3%) respectively. Ninety five (63.3%) samples were showed to be positive from all the sample process of which MMSH have highest isolated organisms of 49(51.6%) and AKTH with 46(48.4%). Surgical ward has highest confirmed patients with 69(46%) followed by Medical and then pediatric with 52 (34.7%) and 29(19.3%) respectively. Tolera *et al.* (2018) report in their study that most of their identified patients is from gynecology 103 (26.1%) followed by medical 102 (25.9%) and surgical ward 99 (25.1%). Morgan and Johns (2016) report in their study that the risk of developing a health-care associated infection (HCAIs) increases linearly by age; a 2011 prevalence study reported a 11.5% HCAIs prevalence rate in patients over the age of 85, which decrease significantly with younger age 11.27% in 75-84 group, 10.64% in 65-74 and 7.37% in patients under the age of 65 (Cairns *et al.*, 2011).

The overall most frequent site of infection is surgical 51(53.7%) followed by Urinary tract 30(31.6%) then bloodstream 14(14.7%). the highest positivity was found in surgical site 32(33.7%) in AKTH, 20 (21.1%) from urinary tract in MMSH and 19(20%) from surgical site also in MMSH, the least positivity 4(4.2%) and 10(10.5%) was found in bloodstream at both AKTH and MMSH respectively. While surgical site infections account for the largest proportion of HCAIs in the age group of under 65, HCAIs in the elderly are primarily attributed to urinary tract infections (Smith *et al.*, 2008).

From a total number of 95 isolates obtained in this study, Gram-positive isolates were the commonest 71(74.7%) and Gram-negative 24(25.3%) of the total number of isolates. Gram-positive cocci 55.6% have been reported as the most commonly associated organisms with hospital acquired infections (Tolera *et al.*, 2018). Alkali *et al.* (2019) reported that Gram negative isolates 77.5% is the most commonly organism associated with HCAIs.

In this study, the high percentages of resistance of the isolates were observed in Cefuroxime (30µg), Amoxicilin (10µg) and Cholramphenicol (30µg) with least resistant of the isolates to Imipenem (10µg) and cefoxitin (30µg).

This finding corresponds with what was obtained by Alkali *et al.*,(2019) where Amoxicillin was less active to almost all the isolates, 84.6% and 75.0% resistant to *E. coli* and *P. aeruginosa* respectively while 7.7% and 6.9% of *E. coli* and *Strep. sp*. respectively shows least resistant activity to imipenem at MMSH. In Uganda, Peter *et al.,* (2017) reported 97% of the isolates resistant to Amoxicilin (10µg).

This study also revealed that 18% and 24% prevalence of MRSA in both AKTH and MMSH respectively. Okon *et al*. (2013) reported the detection of 12.5% MRSA from clinical specimens from six tertiary hospitals in North eastern Nigeria and Olowe *et al*. (2013) reported a higher prevalence of 19.2% MRSA from clinical isolates in medical microbiology laboratory of university teaching Hospitals, Ado-Ekiti. This finding is similar to that of this study.

CONCLUSION

This study has identified *S. aureus* as the common pathogen associated with nosocomial infections where surgical site tends to be the most frequent site of infection. While antimicrobial stewardship was addressed through susceptibilities assay, majority of the Gram-positive were resistant to cefuroxime while majority of the isolates shows high degree of sensitivity to Cefoxitin and Imipenem. The prevalence of MRSA among the *S. aureus* isolates were 18% and 24% at both AKTH and MMSH respectively. 100% of all the tested isolates were blaZ positive while 62.5% were mecA positive.

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Appendix I

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NHREC/28/01/2020/AKTH/EC/2898

AKTH/MAC/SUB/12A/P-3/VI/2998 **5th October, 2020**

Issa Shariffdeen Bale
Department of Microbiology
BUK, Kano.

Ufs:

The Head of Department
Microbiology
BUK, Kano.

ETHICS APPROVAL

Further to your application in respect of your research proposal titled "Surveillance for Antimicrobial Resistant Bacteria among Nosocomial Isolates in Two Hospital in Kano, Nigeria". The Committee reviewed the proposal and noted same as a prospective study.

In view of the above, Ethics approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Research Ethics Committee.

Regards,

Abubakar S. Mahmud
Secretary, Research Ethics Committee
For: Chairman

Appendix II

KANO STATE OF NIGERIA
MINISTRY OF HEALTH
2nd & 3rd Floor, Post Office Road,
P.M.B. 3066, Kano.

Commissioner: 08023337417
Permanent Secretary: 09096619985
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MOH/Off/797/T.I/1948 24th February, 2020

*Ref:*_____ *Date:*_____

Sheriffdeen Issa Bale,
Department of Microbiology,
Faculty of Science,
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RE: APPLICATION FOR ETHICAL APPROVAL

Reference to your letter dated 17th February, 2020 on the above request addressed to the Chairman Health Research Ethics Committee of the Ministry requesting for ethical approval to conduct a Research work at Aminu Kano Teaching Hospital and Murtala Muhammad Specialist Hospital, Kano State.

2. The research entitled ***"Surveillance for Antimicrobial Resistance Bacteria among Nosocomial Isolates from Two Hospitals in Kano State, Nigeria"*** is for the Award of Masters Degree in Microbiology (Medical).

3. In view of the foregoing, I wish to convey the Ministry's approval for you to conduct the research at the above mentioned hospitals.

4. You are also requested to share your findings with the Ministry of Health, Kano State.

5. Best Regards

Abubakar Ahmad
Ag. DPRS
Secretary (HREC)
For: Honourable Commissioner