

ANTIBIOTIC RESISTANCE OF *KLEBSIELLA PNEUMONIAE* ISOLATED FROM RECTAL SWABS OF NEONATES FROM SOME HOSPITALS WITHIN KADUNA METROPOLIS

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ABSTRACT

Klebsiella pneumoniae belong to the family *Enterobacteriaceae*, and have been associated with a wide range of diseases and antibiotic resistance which has contributed to the high mortality rate of neonates. This study was carried out to determine the trend in antibiotic resistance of *Klebsiella pneumoniae* on neonates from some hospitals in Kaduna metropolis. Three hundred and eighty (380) neonatal rectal swab samples were collected from five hospitals using sterile swab sticks and cultured on CLED Di (bevis) and MacConkey. Bacteria isolates were characterized using cultural methods and using Analytical Profile Index test kit and the apiweb. Phenotypic detection of Extended Spectrum Beta Lactamase (ESBL) was carried out using double disc diffusion method; presence of carbapenemase was tested using modified Carbapenem inactivation method (mCIM) Out of the 380 samples, 16 (4%) were *Klebsiella pneumoniae* isolates. API confirmed the isolates to be *Klebsiella pneumoniae* sp *pneumoniae*. The antibiogram showed all isolates were resistant to augmentine at 30µg (16, 100%) and cefuroxime (16, 100%), ceftazidime (12, 75%). Antibiotics *K. pneumoniae* exhibited low resistance are meropenem (0, 0%), nitrofurantoin (1, 6%) and ofloxacin (1, 6%) while Carbapenem resistant *Klebsiella pneumoniae* (CRKp) was found in 1 (6%) of the isolates resistant to imipenem. 5 (31%) isolates produced ESBL and 6 (38%) isolates were multi drug resistant (MDR). *Klebsiella pneumoniae* sp *pneumoniae* isolated from neonatal rectal swab was completely resistant to augmentine and cefuroxime, MDR, ESBL and CRKp was observed in the isolates. Presence of ESBL genes and carbapenem resistant *K. pneumoniae* (CR-Kp) poses a great concern to the health of neonates.

Keywords: Antibiotic resistance, Neonate, Extended spectrum beta lactamase, Multidrug resistance, *K. pneumoniae*, Rectal swab.

INTRODUCTION

The neonatal period is the first twenty- eight (28) days of life (Wardlaw *et al.*, 2014). According to Dreyer and Liebl (2018), gastrointestinal tract bacterial diversity of babies is mainly as a result of developmental diet (breast milk or formula), method of birth (vaginal or Caesarean section), and early-life antibiotic use. Neonates are prone to disease conditions such as diarrhoea (United Nations International Children and Education Fund-UNICEF, 2012) and blood stream infections (Stoll *et al.*, 2002). Bacteria of the family *Enterobacteriaceae* have been documented as the major causes of childhood diseases leading to morbidity and

mortality (Singh *et al.*, 2002; Kent *et al.*, 2016). *Klebsiella pneumoniae* is a common environmental human- and animal-associated Gram-negative bacterium that has become a major cause of nosocomial infections worldwide (Jones, 2010; Moradigaravand *et al.*, 2017). Classical *K. pneumoniae* infections affect people with compromised immune systems, such as patients undergoing radiation therapy, neonates, and the elderly (Patro and Rathinavelan, 2019). Some hypervirulent *K. pneumoniae* serotypes with elevated production of capsule polysaccharide are able to infect healthy persons prior to infection and cause life-threatening community-acquired infections, such as: meningitis, severe pneumonia, necrotizing fasciitis, endophthalmitis and pyogenic liver abscess (Li *et al.*, 2014) *K. pneumoniae* also causes urinary tract infections and soft tissue infections (Patro and Rathinavelan, 2019).

In order for *Klebsiella pneumoniae* to cause infection, it must overcome the host innate immune defense mechanical barriers (e.g. epithelia of the skin, mucociliary clearance, the low-pH environment of the genitourinary tract or gastrointestinal tract); circumvent the humoral defence (i.e. complement system) and cellular innate defenses (Patro and Rathinavelan, 2019). Multi-drug resistance (MDR) defined as non-susceptibility to at least one agent in three or more antimicrobial categories Ndzime *et al.*, (2021) have been observed in *K. pneumoniae* (Chakraborty *et al.*, 2016; Ndzime *et al.*, 2021). The presence of efflux pumps serve as a means of multi-drug resistance in microorganism as it is not specific and it can pump any drugs (Moradigaravand *et al.*, 2017). Extended-spectrum beta-lactamase (ESBL) are β-lactamase that are a rapidly evolving group of beta lactamases which are able to hydrolyse third generation cephalosporins and aztreonam yet are inhibited by clavulanic acid (Paterson and Robert, 2005) Beta lactamases have been identified in *K. pneumoniae* (Odjana *et al.*, 2014; Deshiri *et al.*, 2018). Carbapenem resistant enterobacteriales (CRE) are enterobacteriales which have been confirmed to be resistant to at least a carbapenem antibiotic or produce carbapenemase (CDC, 2019). When it produces an enzyme that inactivates a carbapenem it is said to be a carbapenem producing CRE (CDC, 2019). According to Sriram *et al.* (2021) in Nigeria, there have been 42.86% positive isolates of CRE of *Klebsiella* origin. *K. pneumoniae* carbapenemase causes high morbidity and mortality (Nordmann *et al.*, 2009; CDC, 2019).

MATERIALS AND METHODS

Ethical Approval

The Ministry of Health in Kaduna state gave ethical approval (for four hospitals) and another specifically for Barau Dikko Specialist Hospital Kaduna.

Study Design

This study was carried out in Kaduna metropolis. Kaduna state coordinates are 10.3764° North and 7.7095° East. Kaduna metropolis comprises of Kaduna North, Kaduna South, Igabi and Chikun Local Government Areas (Aliyu and Suleiman, 2016). It was approved by Kaduna state ministry of health and Barau Dikko Specialist Hospital.

Study Population

The study population for this research included all neonates who were on admission in the neonatal intensive care unit and those who received Bacillus Calmette- Guerin (BCG) and oral polio vaccine. It included preterm babies who were receiving their first immunization and excluded babies who were more than four (4) weeks old. A total of 380 samples was collected of which seventy-six (76) rectal swab samples was collected from each of the five hospitals.

Sample Size

A total of 380 samples of rectal swabs were collected of which seventy-six (76) rectal swab samples were collected from each of the five hospitals. The formula (Cochran, 1977; Pourhoseingholi *et al.*, 2013) was used to calculate the population size:

$$N = \frac{Z^2 * P * (1-P)}{d^2}$$

Where N= Number of samples

Z= Statistics for 95% confidence interval

P= Prevalence rate

d= Allowed error

The prevalence rate of 0.494 was obtained from Kigbu *et al.* (2016) who used standard culture methods to observe the colonization of intestinal bacteria in neonates from birth to 14 days after birth having a confidence interval of 95% at 1.96 and error of 0.05

Isolation of Bacteria from Rectal Swab of Neonates

Rectal swab was streak plated on CLED Di (bevis) agar incubated at 37°C for 24 h (Cheesbrough, 2006). Large pink mucoid lactose fermenters on CLED Di (bevis) were sub cultured on MacConkey agar. Isolated colonies were gram stained and viewed using oil immersion magnification. Analytical Profile index API 20 E™ by BioMerieux SA France was used to identify the isolates.

Antibiotic Resistance of *K. pneumoniae*

Antibiogram of the bacteria isolates was carried out using the following antibiotics: penicillin (augmentine, 30µg), cephalosporins (ceftazidime- 30µg, cefuroxime- 30µg and cefixime- 5µg), fluoroquinolone (ciprofloxacin- 5µg and ofloxacin- 5 µg), an aminoglycoside (gentamicin- 10µg), a nitrofurantoin- 300µg) and a carbapenem (imipenem- 10µg and meropenem 10 µg).

Phenotypic Confirmation of Extended Spectrum Beta Lactamase- Producers

The 0.5 McFarland turbidity standard suspensions were made from the isolates of *Klebsiella pneumoniae*. Using this inoculum, lawn

was made on Muller Hinton Agar (MHA) plate. Discs of Ceftazidime and Ceftazidime + Clavulanic acid (30 mcg/10 mcg) were placed aseptically on the surface of MHA. The distance of 15 mm was kept between the discs and incubated overnight at 37°C. An increase of ≥ 5 mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the ESBL production by the organisms (CLSI, 2017).

The Modified Carbapenem Inactivation Method

To perform the mCIM test, a loopful of bacterial isolate was emulsified in 2mL trypticase soy broth then, a susceptibility-testing disk containing 10 µg of meropenem was immersed into the suspension of the test organism and incubated for four (4) h at 35°C.

Afterwards, the disk was removed from the suspension using an inoculation loop, placed on Mueller-Hinton agar plate already inoculated with a suspension of susceptible *E. coli* indicator strain (ATCC 29522) and subsequently incubated at 35°C.

The bacterial isolate produced carbapenemase when the meropenem susceptibility disk was hydrolysed allowing uninhibited growth of the susceptible indicator strain. Disks incubated in suspensions that do not contain carbapenemases yielded a clear inhibition zones that corresponded to the measured standard of CLSI, 2017.

RESULTS

A total of 16 (4%) isolates of *K. pneumoniae* was isolated and identified as *K. pneumoniae* sp *pneumoniae* using API 20E (Table 1).

Table 1 Distribution of *K. pneumoniae* from Hospitals within Kaduna Metropolis

Sample location	Sample No.	<i>K. pneumoniae</i>
BH	76	8(11%)
GH	76	1(1%)
KH	76	1(1%)
SH	76	5(7%)
YH	76	1(1%)
Total	380	14(4%)

Key: BH, GH, KH, SH, YH= hospital codes No. = Number

Table 2 shows the antimicrobial resistance profile of the *K. pneumoniae*. The isolates had

100% resistance to both augmentine and cefuroxime and 75% resistance to ceftazidime. There was very low resistance to ofloxacin 6% and nitrofurans (6%) but no resistance (0%) to meropenem.

Table 2: Antibiotic Resistance Trends of the *Klebsiella pneumoniae* Isolates (n= 16)

Antibiotic	Class	R (%)	I (%)	S (%)
Cefuroxime	Cephems	16(100)	0(0)	0(0)
Cefixime	Cephems	5(31)	4(25)	7(44)
Ceftazidime	Cephems	12(75)	4(25)	0(0)
Augmentin	Penicillin	16(100)	0(0)	0(0)
Ciprofloxacin	Quinolones	2(13)	1(6)	13(81)
Ofloxacin	Quinolones	1(6)	1(6)	14(88)
Gentamicin	Aminoglycoside	3(19)	0(0)	13(81)
Nitrofurantoin	Nitrofurans	1(6)	3(19)	13(81)
Imipenem	Carbapenems	1(6)	7(44)	9(56)
Meropenem	Carbapenems	0(0)	3(19)	13(81)

KEY: I- Intermediate S- Susceptible R- Resistant n= number of isolates

Six (38%) *K. pneumoniae* isolates were multi drug resistant (Figure 1) while the ten (62%) were not MDR

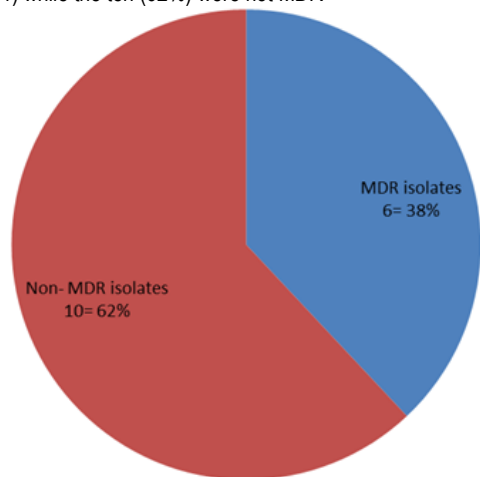


Figure 1: *Klebsiella pneumoniae* MDR isolates from Antibiogram

The result of combined disc diffusion test (Table 3) showed that five (5; 31%) of the *K. pneumoniae* isolates were ESBL producers.

Table 3: Confirmation Test for Extended Spectrum Beta Lactamase (ESBL) Production using Combined Disc Diffusion Method

Sample codes	CAZ+CAL	CAZ	Difference (mm)	ESBL
B13	20.5	20.5	0	Absent
B14	22	15.5	7	Present
B27	25.5	22.5	3	Absent
B32	22	21	1	Absent
B39	24.5	16.5	8	Present
B55	24.5	23.5	1	Absent
B61	20	19	1	Absent
B70	19.5	17	3	Absent
G47	19	17	2	Absent
K55	26	15	11	Present
S40	19	17.5	2	Absent
S43	25	23	2	Absent
S47	24	23	1	Absent
S51	19.5	17.5	2	Absent
S53	24	13	11	Present
Y30	30.5	19	12	Present

Key: CAZ+CAL= ceftazidime and clavulanic acid CAZ= ceftazidime

Modified carbapenem inactivation method (mCIM) result (Table 4) showed that 3 isolates had inconclusive result for carbapenemase production while thirteen (13) of the samples indicated that there was no carbapenemase production.

Table 4: Modified Carbapenem Inactivation Method (mCIM) using Meropenem Antibiotic in millimeters

Sample	Indeterminate Positive	Negative	Interpretation
B13		19	Carbapenemase not detected
B14		19	..
B27	18		Testing inconclusive
B32		19	Carbapenemase not detected
B39		19	..
B55		19.5	..
B61		20	..
B70		19	..
G47		19	..
K55		22.5	..
S40		19	..
S43	18		Testing inconclusive
S47	18		..
S51		19	Carbapenemase not detected
S53		19	..
Y30		19	..
Control		26.5	..

Key: B, G, K, S and Y= Codes representing the hospitals from which the samples were taken and the respective sample numbers

DISCUSSION

A total of 16 (4%) *K. pneumoniae* was isolated. In the study of intestinal bacterial colonization of neonates in the first two weeks of life, Ibadan south west Nigeria, Kigbu *et al.* (2016) observed a

proliferation of *K. pneumoniae* from 0% day one to a 7.1% day three and a consistent 20% on the 9th and 14th days.

Analytical Profile Index (API) in Plate I identify and apiweb confirms the 16 rectal swab isolates as *Klebsiella pneumoniae* sp *pneumoniae*.

The resistance effect of *K. pneumoniae* was very high for both augmentine and cefuroxime (100%) while ceftazidime (75%). Arowosegbe *et al.* (2017) also observed 100% resistance of *Klebsiella* sp to cefuroxime, 83% resistance to ampicillin and 67% resistance to ceftazidime in a study on the sensitivity pattern of *Klebsiella* sp isolated from neonatal septic patients in Abeokuta-Nigeria. A 100% resistance to augmentine was also observed by Ojdana *et al.*, (2014) from ESBL clinical isolates in Poland as well as Bouamri *et al.* (2014) in Morocco. The fact that only the beta lactam ring- has to be overcome in penicillins and cephalosporins (Benjamin, 2015), mutations brought about in the plasmid of *K. pneumoniae* (Li, *et al.*, 2014) can overcome the beta lactam barrier leading to the high rate of resistance to augmentine (amoxicillin clavulanate) and the cephalosporins. The presence of ESBL genes can also reduce the limit the effect of the antibiotics (Nordmann *et al.*, 2012).

There was 13% resistance to ciprofloxacin and 6% resistance to ofloxacin. Ojdana *et al.* (2014) observed 16.7% resistance to ciprofloxacin in a study on the occurrence of ESBL genes in Poland. Arowosegbe *et al.* (2017) reports 33% resistance to ciprofloxacin and 17% resistance to ofloxacin from neonatal sepsis in Nigeria. Resistance to fluoroquinolones is caused by mutations in the genes encoding the targets of DNA-topoisomerases (Shaikh *et al.*, 2015). The low resistance to the fluoroquinolones could be attributed to the efficacy of the antibiotics. Fluoroquinolones are given in severe cases of infection; one could assume that it is not frequently prescribed and abused.

In this study, *K. pneumoniae* resistance to Nitrofurantoin was 6%. A low resistance to nitrofurantoin was observed by Ndzime *et al.* (2021) on the study of uropathogenic clinical strains in Franceville, Gabon while, Kothari and Sagar (2008) recorded a 34% resistance. Omeregje *et al.* (2012) noted resistance (16.7%) to nitrofurantoin among *Klebsiella species* in a neonatal UTI study in Benin, Nigeria. The low resistance noted by each of these studies can be attributed to its mechanism of action against microorganisms. Nitrofurantoin has multiple bactericidal properties which act in concert to kill invading bacteria through DNA and RNA damage, protein damage, as well as inhibition of the [citric acid](#) cycle (Benjamin, 2015). Therefore, a resistant bacterium has to undergo mutations that confer resistance to at least all four mechanisms of nitrofurantoin defense to be able to resist it (Benjamin, 2015). The differences may be due to the site from which the organism was isolated. A UTI infection will require the triggering of virulent factors in *K. pneumoniae* such as synthesis of capsule polysaccharide, production of Type 1 fimbriae (essential for establishing UTI), production of enterobactin and expressing efflux pumps (Li *et al.*, 2014) leading to more antibiotic resistance. In effect this indicates the efficacy of the antibiotic.

A MDR isolate which is resistance to three or more classes of antibiotics had Carbapenem Resistant *K. pneumoniae* (CRKp) resistant to imipenem 1(6%). Deshiri *et al.* (2018) recorded low resistance to imipenem 2 (1.6%). Both recorded very low rates though the difference in the percentage compared to the number

isolated can be due to the sample size in each study. Carbapenem resistant enterobacteriaceae has been associated with MDR (Hirsch and Tam, 2010; CDC, 2019) which is in agreement with this study. There is a gradual resistance of *K. pneumoniae* to carbapenems.

There was no (0%) resistance to meropenem in this study. Similar resistance was observed by Ojdana *et al.* (2014) to meropenem in the study of the occurrence of ESBL genes in Poland and by Kothari *et al.* (2008) childhood UTI Janshedpur, India. A 56.5% resistance to meropenem was observed from a neonatal sepsis study in Cairo, Egypt by [Ghaith et al.](#) (2020). The low resistance to the carbapenems could be attributed to its resistance to be hydrolyzed by the organisms. The difference observed in [Ghaith et al.](#) (2020) could be attributed to the fact that *K. pneumoniae* isolates had multiple Carbapenem genes giving them the ability to resist meropenem where as in this study the genes identified were ESBL genes as well as in most of the other studies that had zero resistance to carbapenem which are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing Enterobacteriaceae (Shaikh *et al.*, 2015). Generally, it's an indication that meropenem is very effective when used to treat infections caused by ESBL producing *K. pneumoniae*.

Six (38%) *K. pneumoniae* isolates were MDR predominantly to augmentine (amoxicillin- clavulanic acid), cefuroxime and gentamicine. Ndzime *et al.* (2021) obtained 57% MDR in amoxicillin- clavulanic acid, cefotaxime and trimethoprim-sulfamethoxazole predominantly from infants' uropathogenic strains in Franceville, Gabon and Chakraborty *et al.* (2016) obtained 56% MDR to Ampicillin, Amoxicillin, Ceftriaxone and Cotrimoxazole from clinical specimens of different age groups in Sylhet, Bangladesh. The sample size influenced the isolation of MDR isolates obtained in this study compared to the clinical samples sizes of the other studies. Also, the colonization of *K. pneumoniae* was the focus whereas in the other studies cause of infection was the focus. This goes to show that both first and second generation cephalosporins are becoming ineffective as attributed by Abrar *et al.* (2019) as well as use of augmentine (Feglo and Sakodie, 2016). Presence of MDR is indication that many antibiotics are becoming less effective in the treatment of *K. pneumoniae* infections and MDR normal flora in the gut of a neonate is an infection ready to happen where hygiene is poor.

Five (31%) *K. pneumoniae* ESBL- producers were obtained in this study. This is half the result obtained by Desta *et al.* (2016) who obtained a 68% prevalence colonization rate in neonatal gastrointestinal tract of hospitalized patients in Ethiopia while Sa'adu *et al.* (2019) recorded 50% prevalence in a neonatal sepsis study in Ilorin, Nigeria and Oduro- Mensah *et al.* (2016) in Ghana had 42.3% prevalence. The studies indicate the increase in ESBL production in *K. pneumoniae* to beta lactam antibiotics which should be a cause of concern for developing countries. The geographical location could be an influence to the ESBL production variation.

Carbapenemase production using mCIM had 3(19%) *K. pneumoniae* isolates test inconclusive (18mm zone of inhibition). Both CLSI (2017) and Katsikas *et al.* (2017) have alluded to the high specificity and sensitivity of mCIM for carbapenemase detection. This indicates that no carbapenemase was produced; nonetheless there is a possibility that the isolates could be resistant to other carbapenems apart from meropenem.

Conclusion

A total of *Klebsiella pneumoniae* spp *pneumoniae* was isolated and identified using cultural methods and Analytic Profile Index. There were *K. pneumoniae* 16(4%). The *K. pneumoniae* isolates were all resistant to cefuroxime (100%) and augmentine (100%); there was none (0%) resistant to meropenem. There was carbapenem resistant *K. pneumoniae* 1(6%). Five (31%) isolates produced ESBL and 6 (38%) were MDR. Presence of ESBL genes and carbapenem resistant *K. pneumoniae* (CR-Kp) poses a great concern to the health of neonates. If this supposed normal flora becomes invasive replacing sensitive commensals with resistant strains, when such neonate becomes ill, the likely drugs will be ineffective due to the resistant strains already present. Also, in case of emergencies due to septicemia mortality may be very swift. It is therefore important for medical personnel to carry out sensitivity test and document the drug resistance profiles of pregnant women so as to know what to expect or prescribe when their child or children are ill due to certain diseases.

It is wrong to administer antibiotics without knowledge of the antimicrobial sensitivity of the individual- this is the major cause of the proliferation of resistance organisms. The major limitation of this study was that medical record of the mothers such as antibiotic use, mode of delivery and personal use of antibiotics as well as antibiotic resistance profile was not obtained. This would have helped in determining the probable cause of resistance observed in this study.

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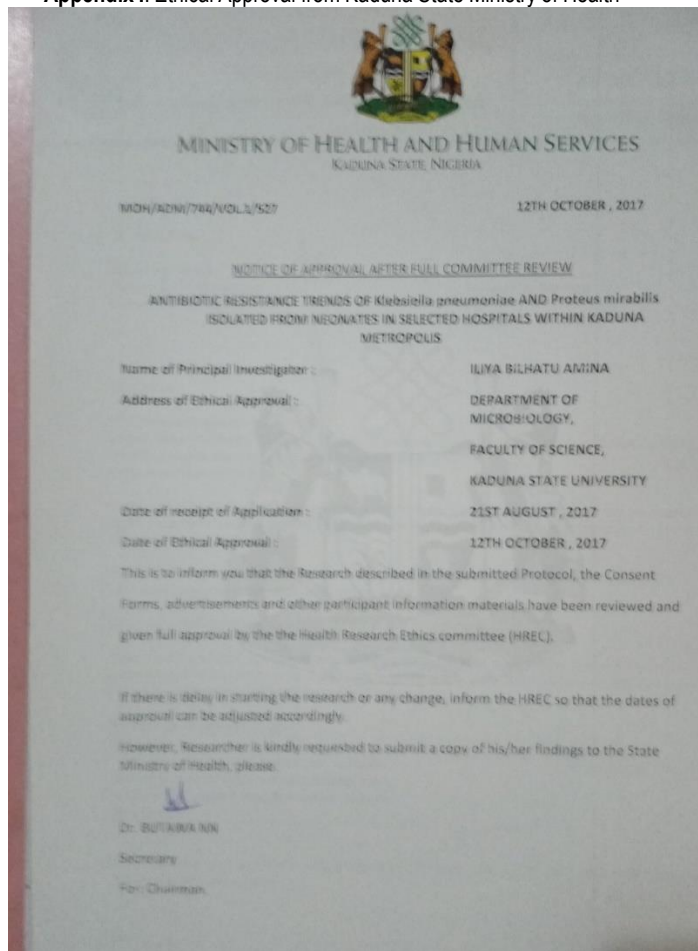
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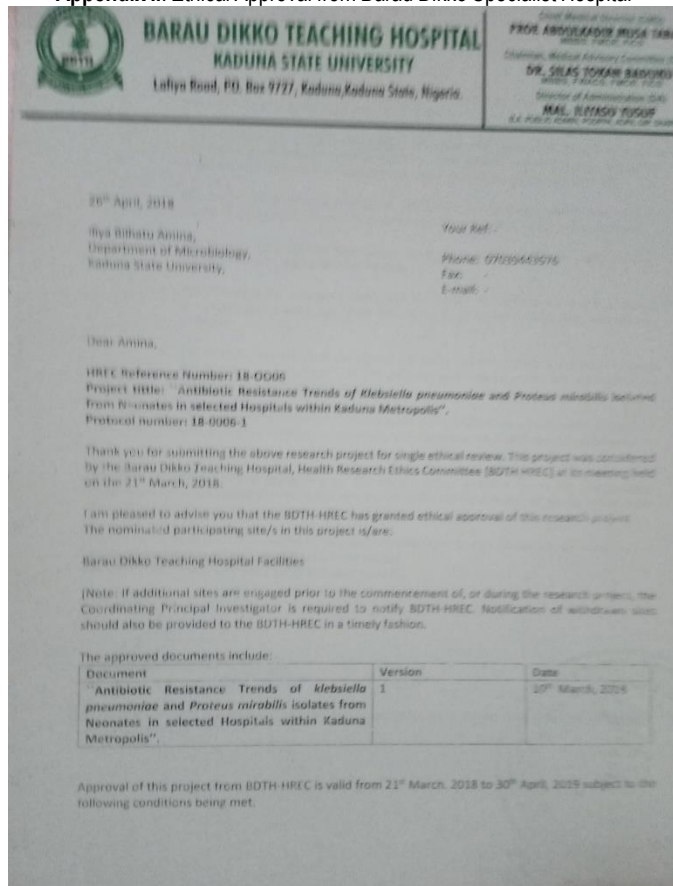
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Appendix I: Ethical Approval from Kaduna State Ministry of Health



Appendix II: Ethical Approval from Barau Dikko Specialist Hospital



Appendix III: Breakpoints for Determination of Antibiotic Resistance in Millimeters (CLSI, 2017)

Antibiotics	Quantity	S	I	R
Cefuroxime	30µg	≥18	15- 17	≤14
Cefixime	5µg	≥19	16- 18	≤15
Ceftazidime	30µg	≥21	18- 20	≤17
Augmentin	30µg	≥18	14- 17	≤13
Ciprofloxacin	5µg	≥21	16- 20	≤15
Ofloxacin	5µg	≥16	13- 15	≤12
Gentamicin	10µg	≥15	13- 14	≤12
Nitrofurantoin	300µg	≥17	15- 16	≤14
Imipenem	10µg	≥23	20- 22	≤19
Meropenem	10µg	≥23	20- 22	≤19

Key:
 S- Sensitive
 µg- micro gram
 ≥ - Greater than or equal to
 ≤ - Less than or equal to
 I- Intermediate
 R- Resistant
 CLSI- Clinical Laboratory Standard Institute