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Survey for Carbapenem Resistant *Klebsiella Pneumoniae* among Patients Attending Aminu Kano Teaching Hospital, Kano, Nigeria

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Abstract

Carbapenem resistant Klebsiella pneumoniae is becoming a more significant pathogen because of the grave clinical risk it poses which affects patient's treatment outcome. This study aimed to survey for carbapenem resistant Klebsiella pneumoniae (CRKP) among patients attending Aminu KanoTeaching Hospital, Kano State, Nigeria. A total of 600 clinical samples including urine, sputum and swabs were collected and screened. Klebsiella pneumoniae were isolated and confirmed using culture, microscopy and biochemical test according to standard microbiological techniques. Phenotypic carbapenem resistant and carbapenemase production were detected using meropenem discs diffusion method and modified Hodge test respectively. Genotypic carbapenemase productions were confirmed by detecting the presence of blaKPC genes using PCR and Gel electrophoresis. Antibiotics susceptibility profiles of the CRKP isolates were evaluated using disc diffusion method and interpreted using CLSI protocols. The study revealed the incidence of Klebsiella pneumoniae infection as 14% with the highest occurrence in sputum (18%) and the lowest in swab (10%). Of the total number of isolated K. pneumoniae, 14.3% were found to be carbapenem resistant. Up to 75% of CRKP isolates were found to produce carbapenamase and harbor blaKPC genes. Antibiotic susceptibility profile of these isolates revealed colistin and tigecycline as the most active antibiotics in vitro (92%).The isolates were found to be generally resistant to cefepime, ceftriaxone, cefuroxime, cotrimoxazole and ciproflaxin (100%).Up to 8% of the isolates were sensitive to Gentamicin and Nalidixic acid, 25% to Nitrofurantoin, 50% to Minocycline and 17% to Amikacin. This study verifies the existence of carbapenem resistant Klebsiella pneumoniae isolates that are highly resistant to other antibiotics in patients attending AKTH Kano which is an indication of increase in drug resistance. This requires the need for newer tactics in infection control to prevent the spread of carbapenem resistant isolates.

Key words: AKTH,Carbapenem, Klebsiella pneumoniae, Resistance

INTRODUCTION

Klebsiella penumoniae is a rod shaped, encapsulated, Gram negative, non-motile member of Enterobacteriaceae family(Sydney, 2004). Approximately one third of Gram negative infections are caused by *Klebsiella* species including urinary tract, respiratory and wound infections, cystitis, endocarditis and septicemia (Sydney, 2004).Isolate of *K. pneumoniae* have been shown to be resistant to practically all classes of antibiotics by accumulating genes from mobile plasmids and integrons and by gradually altering chromosomally encoded genes (Ssekatawa *et al.*, 2017, Jinrong *et al.*, 2017)

Carbapenem resistant *Klebsiella pnuemoniae (*CRKP) are bacterial strains resistant to carbapenems, an antibiotic that is only used as last resort to treat infections caused by Enterobacteriaceae that are multidrug resistant (Jinrong *et al.*, 2017). Carbapenems are structurally related to betalactam antibiotics but possess widest range of activity and highest effectiveness against bacteria than all other betalactams (Penicillin, cephalosphorins and monobactams). Thus they are frequently saved for very serious infection or as last resorts treatments (Jinrong *et al.*, 2017). Carbapenems act by inhibiting bacterial enzyme transpeptidase, thereby preventing synthesis of peptidoglycan leading to cells lysis (Dahab *et al.*, 2017).

CRKP emergence is an important challenge in the health care setting as it is resistant to practically all commonly prescribed antibiotics. Infection with these organisms have caused elevated death and morbidity rates among immune compromised individuals, persons with extended hospital stay, critically ill patients and those exposed to invasive devices (Jinrong *et al.*, 2017).

The primary issue is that, when treating resistant bacterial strains such as Gram negative multidrug resistant and extended spectrum beta lactamase (ESBLs) producers, carbapenem is frequently suggested as last resort antibiotic. This has significantly limits the treatment options especially for life threatening infections (Kerbauy *et al.*, 2016).

The synthesis of carbapenamase (carbapenem hydrolyzing enzyme) and the loss or reduction of outer membrane protein expression are the usual mechanisms behind carbapenem resistance in *K. pneumoniae* (Jinrong *et al.*, 2017; Azimi *et al.*, 2013).Transferable plasmids contain the genes encoding carbapenamase, which makes it easier for these genes to spread to various strains and species throughout the world. This transfer is one of the hardest challenges in the field of infection control (Jinrong *et al.*, 2017).

Several part of the world are already endemic for carbapenamase encoding genes(*bla*KPC).In USA 11% of *K. penumoaniae* infections were due to carbapenem resistant strains, 23.2% in Kenya and 10.3% in Uganda(Ssekatawa *et al.*,2018). In India 13% of *Escherichia coli* infections and 57% *K. pneumonia* infections were caused by carbapenemase producing strains (Ssekatawa *et al.*, 2018). Aminu and Lawal *et al.*, (2019) reported the incidence of carbapenem resistant enterobacteriaceae(CRE) of 8.46% and 10% carbapenem resistant *K. pneumoniae* from wound infection in Kano. Dahab *et al.*,(2017) also reported the incidence of CRE of 13% in Khartoum. It is therefore important to have updated information on the situation in different part of the world. Active surveillance for the presence of CRKP and carbapenamase encoding genes are of utmost importance for application of measures to prevent transmission and infection considering high risk patients, clinical areas and extent of spread.

Survey for infection caused by CRKP could help in optimizing patient's treatments, infection control and limit the spread of the resistant agents. This study therefore aimed to survey for CRKP in patient Attending Aminu Kano Teaching Hospital with view to identify common clinical source of the organisms, its susceptibility to antimicrobials and possibly a resistance mechanism.

MATERIALS AND METHODS

The study was conducted in Aminu Kano teaching hospital fromJanuary, 2020 to January, 2022.The hospital is the largest tertiary medical facility in Kano state, and it is located at 11°57¹45˝N8°33¹07¹¹E/11.9626°N8.5519°E coordinates. The study protocol was approved by ethical committee of the hospital with reference number NHREC/21/08/2008/AKTH/EC/2482.

Minimum Sample size for the study was estimated using Open Epi version 2.3 statistical software. Previous prevalence of 10% reported by Aminu and Lawal (2019) in Kano was used.

A total of 600 clinical samples including urine (400), sputum (100) and swab (100) were collected using aseptic microbiological techniques (Cheesbrough, 2010). Samples were inoculated onto MacConkey agar medium using streak plate technique and incubated at 37°C overnight. Discrete colonies were morphologically identified, Gram stained and observe microscopically. Gram negative rods/bacilli isolates were further characterized using different standard biochemical reaction including oxidase, triple sugar iron agar, citrate utilization, urease production, indole production and motility tests(Cheesbrough, 2010., Arora and Arora, 2016).

Antibiotic Susceptibility Testing

Pure isolates of *Klebsiella pneumoniae* were subjected to antibiotic susceptibility testing. Resistance to carbapenem antibiotics was detected using disc diffusion method according to CLSI (2017)guide lines. Suspension matching 0.5 McFarland standard of overnight pure culture of the organisms was made in nutrient broth. Using sterile cotton swab the standardized inoculums was inoculated on Mueller Hinton agar and allowed to dry. The antibiotics discs 10µg each of Imipenem, Meropenem and Ertepenem (Oxoid) were gently and firmly placed on the surface of inoculated media using sterile forceps. The plates were then incubated at 37°C for 24 hours. Zone of inhibition was measured and the reading was compared with CLSI (2017) cut off. Diameter of > 19mm is considered highly sensitive < 13mm as resistant and 15 – 18mm as intermediate.

Carbapenem resistant and intermediate isolates were further screened for susceptibility to other antibiotics using Agar disc diffusion technique. One milliliter of standardized overnight culture of the isolates was flooded over the surface of Mueller Hinton Agar (MHA) plates. The plates were allowed to dry and the standard antibiotics discs (oxoid) including cefuroxime, Ceftriaxone gentamicin, cefepime, cotrimoxaxole, amikacin, gentamicin, ciprofloxacin, tigecycline, colistin, nalidixic acid and nitrofurantoin were gently and firmly place using sterile forceps. The inoculated plates were incubated at 37°C for 24hours. The diameter of the zone of inhibition produced by each antibiotic was measured, recorded and interpreted according to CLSI (2017) guidelines.

Phenotypic detection of carbapenamase production

Phenotypic detection of cabapenamase production from CRKP were achieved using modified Hodge test (MHT) based on CLSI (2017) instruction. Briefly suspension of 0.5 McFarland

standard of *E. coli* ATCC 25922 was prepared in normal saline to 1:10 dilution. The suspension was then inoculated on Mueller – Hinton Agar medium using sterile cotton swab. A 10µg meropenem disc was placed on the centre of the agar plate and the tested bacteria was streaked in a straight line from the edge of the plate to the edge of the disc. The plates were incubated at 37ºC overnight. Strains with the appearance of inhibition zone with clover leaf shape around meropenem disc were considered positive for *Klebsiella pneumoniae* carbapenamase production (KPC) (Plate I).

Detection of KPC genes

Molecular analysis was conducted at Centre for biotechnology research Bayero University, Kano. Carbapenem resistant strains of *Klebsiella pneumoniae* and some susceptible isolates (negative control) were subsequently analyzed by PCR method to detect *bla*KPC genes using below Primers (Bradford *et al*., 2004).

KPC – forward 5^1-ATGTCACTGTATCGCCGTCT-3^1

KPC– reverse 5^1-TTTTCAGAGCCTTACTGCCC-3^1

DNA extraction was carried out using norgen Biotek DNA extraction Kit according to manufacturer's procedure. PCR was carried out in 0.2ml PCR tubes containing a cocktail mix of 10µl master mix (promega), 6µl water, 1µl forward primer, 1µl reverse primer and 2µl DNA template to form 20 µl PCR cocktail. The PCR conditions were initial denaturation at 94ºC for 5 minutes followed by 94ºC for 30 seconds, annealing 51ºC for 30 seconds, extension at 72ºC for 1 minute and final extension at 72ºC for five minutes in 35 cycles.

The amplified PCR products were loaded on the agarose gel and run on biorad electrophoretic machine at 120 V for 60 minutes. The gel bands were viewed using Gel – Doc (Bio- rad)(Plate II).

Statistical Analysis

Data generated from the study was analyzed using SPSS version 26 statistical software. The results was summarized using mean and percentages and interpreted using Chi- square, level of significance was fixed at 5%.

RESULTS

Out of 600 Samples Screened 14% (50/400) urine, 18% (18/100) Sputum and 10% (10/100) swabs yielded growth of *Klebsiella pneumoniae* as presented in Table 1. Sputum samples had significantly highest infection rate(X^2 = 183.4, P< 0.05) compared to swabs and urine samples. The rate of occurrence of carbapenem resistant and carbapenamase producing *K. Pneumoniae* (phenotypic and genotypic detection) from different clinical samples is presented in Table 2. Of the total number of *K. pneumoniae* isolates Screened, 12 out of 84 (14.3%) were found to be carbapenem resistant .The rate of occurrence of CRKP was found to be highest in sputum (22%), followed by swab (14%) and the least occurrence was found in urine (11%) (P<0.05).

Up to 75% of CRKP were found to produce carbapenamase using phenotypic detection (MHT) method (Table 3). All carbapenamase producers isolated in this study (100%) harbor *bla*KPC genes (Table 4). The rate of occurrence of CRKP based on patient demographic information revealed no significant difference between male and females (14.3% each) Table 5. Based on the age group of patients with clinical infection, highest rate of infection with CRKP was found to be more common among age groups 50 – 59years (75%) followed by age group > 60 years (50%) (Table 6). Infection rate with CRKP was also found to be higher among hospitalized or inpatients (20%) compared to outpatients (10%) (P < 0.05) Table 7.

Antimicrobial susceptibility profile of the isolated CRKP is presented in Table 8. The isolates were found to be generally resistant to cefepime, ceftriaxone, cefuroxime, cotrimoxazole and ciproflaxin (100%). Up to 8% of the isolates were sensitive to Gentamicin and Nalidixic acid, 25% to Nitrofurantoin, 50% to Minocycline and 17% to Amikacin, The Isolates were more sensitive to Tigecycline and Colistin (92% each)

Table 1: Distribution of *Klebsiella pnuemoniae* Isolates Based on Clinical Samples

Clinical sample	Number of samples screened	Number of *K. pneumoniae* positive samples	Percentage (%)
Urine	400	56	14
Sputum	100	18	18*
Swab	100	10	10
Total	600	84	14

P= 0.001 * significant difference at 5%

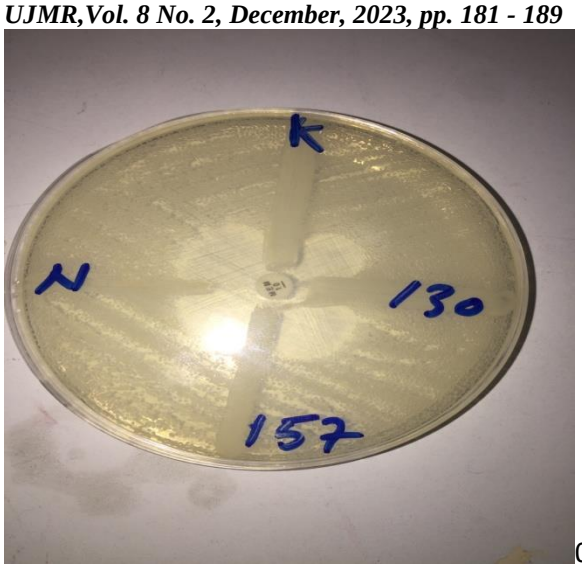

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Plate I: Modified Hodge Test Showing Carbapenamase production

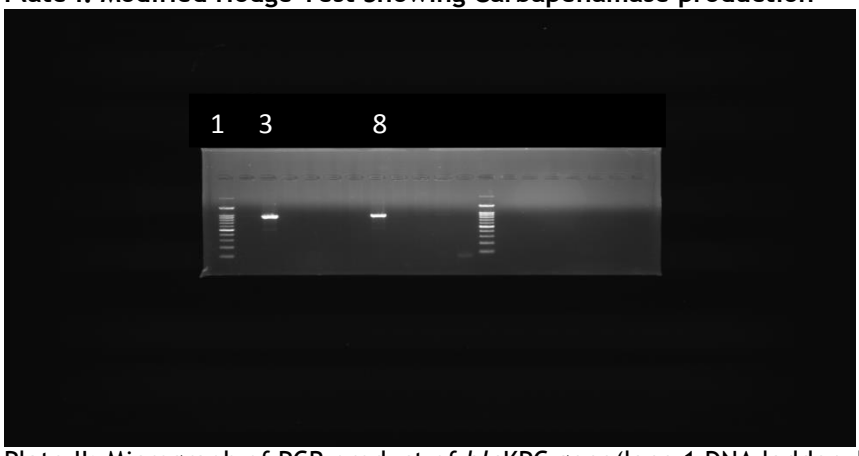

Plate II: Micrograph of PCR product of *bla*KPC gene(lane 1 DNA ladder, lane 3 and 8positive bands with amplicon size of approximately 893 bp..

Table 2: Occurrence of Carbapenem Resistant *K. pnuemoniae* from Different Clinical Samples

Sample	Number of *K. pnuemoniae*	Number of *CRKP* (%)
Urine	56	6 (11)
Sputum	18	4(22)*
Swab	10	2(20)
Total	84	12(14.3)

CRKP- Carbapenem resistant *K. pneumoniae* P=0.001 * significant difference at 5%

Table 3: Occurrence of Carbapenamase Producing *K. pnuemoniae* among Carbapenem Resistant Isolates

Sample	Number of *CRKP* (%)	KPC positive isolates (MHT Positive)	Percentage (%)
Urine	6	4	67
Sputum	4	3	75
Swab	2	2	100
Total	12	9	75

KPC- *K. pneumoniae* carbapenamase* significant difference at 5%, CRKP- Carbapenem resistant *K. pneumoniae*

Table 4: Occurrence of *BlaKPC* Gene among Carbapenamase Producing *K. pnuemoniae* Isolates

Sample	Number of Carbababenamase Producing isolates (MHT positive)	*bla*KPC Positive isolates	Percentage (%)
Urine	4	4	100
Sputum	3	3	100
Swab	2	2	100
Total	9	9	100

Table 5: Distribution of Carbepenem Resistant *K. pnuemoniae* Isolates Based on Patients Gender

Gender	**Number Screened**	**No. positive for KP**	**No. positive for CRKP**
Male	300	35	05(14.3%)
Female	300	49	07 (14.3%)

KP = *Klebsiell*a *pneumoniae* P=0.009
CRKP = Carbapenem resistant *Klebsiella pneumoniae*

Table 6: Distribution of Carbapenem Resistant *Klebsiella pnuemoniae* based on Age of the Infected Subjects

Age range (years)	Number of subject screened	Number of subjects positive for *K. pnuemoniae*(%)	Number of*CRKP*(%)	Number of *CSKP*(%)
0-9	116	18(15.5)	01 (5.6)	17(94.4)
10 – 19	58	08(13.8)	00(00)	08(100)
20 – 29	184	24(13)	00(00)	24(100)
30 – 39	120	14(11.7)	01 (7.1)	13 (92.9)
40 – 49	64	06(9.4)	02 (33.3)*	04(66.7)
50 – 59	24	06(25)	04 (66.7)*	02 (33.3)
>60	34	08(23.5)	04 (50)*	04 (50)
Total	600	84(14)	12(14.3)	72(85.7)

CSKP- Carbapenem sensitive *K. pneumoniae* P=0.001 * significant difference at 5%
CRKP- Carbapenem resistant *K. pneumoniae*

Table 7: Distribution of Carbapenem Resistant *K. pnuemoniae* from Inpatient and Outpatient

	Number screened	Number positive for KP	Number of CRKP
Inpatient	200	40(20%)*	08(20%)*
Outpatient	400	42(11%)	04(10%)

P=0.001 * significant difference at 5%

Table 8: Antibiogram of Carbapenem Resistant *Klebsiella pnuemoniae* Isolates

Antibiotics (µg)	Number of susceptible isolates	Number of resistant isolates
Cefepime (30)	0 (0.0)	12(100)
Ceftriaxone (30)	0(0.0)	12(100)
Cefuroxime (30)	0(0.0)	12(100)
Cotrimoxaxole (1.25/23.75)	0(0.0)	12(100)
Ciprofloxacin (5)	0(0.0)	12(100)
Minocycline (30)	6(50)	6(50)
Amikacin (30)	2(16.7)	10(83.3)
Gentamicin (10)	1(8.3)	11(91.7)
Tigecycline(10)	11(91.7)	04(8.3)
Nitrofurantoin(30)	03(25.0)	08(75.0)
Colistin (10)	11(91.7)	1(8.3)
Nalidixic acid (30)	01(8.3)	11(91.7)

DISCUSSION

Klebsiella Pneumoniae is now considered as clinically important organism due to its tendency to develop resistance to many antibiotics and consequently resulted in fatal treatment outcomes (Nordmann and Carrer,2011). This study revealed 14.0% occurrence of *K. pnemoniae* infection in patients attending Aminu Kano Teaching Hospital Kano. This prevalence is similar to 14% obtained in Brazil by Alexander *et al.*(2006). Infection rate with *K. Pneumoniae* was higher in Bangladesh (24%) and Egypt (68.99%) as reported by Chakraborty *et al.* (2016) and Khalifa *et al.* (2017) respectively. Aminu and Lawal (2019) also reported higher prevalence of 36% in some hospitals in Kano.

In this study *Klebsiella pneumoniae* was mostly isolated in sputum samples with occurrence rate of 18% when compared to urine and swab samples with percentage occurrence of 14 and 10% respectively. Similar study by Aya *et al.* (2022) in Egypt revealed higher number of *K. Pneumoniae* from Sputum Culture (41.4%). Kumar *et al.*(2019) also reported sputum specimens as the most frequent source of *K. pneumoniae* pathogens. Wang *et al.* (2019) stated that the most typical location of *K. pneumoniae* infections was the respiratory tract in the republic of Ghana. Khalifa *et al.* (2017) recorded higher isolation from blood samples (39%) while Ali and Ismael (2017) reported higher recovery from urine sample. Similarly Rabie and Abdallah (2019) reported greater urine sample recovery of *K. pneumoniae* in Zagazig university hospital Egypt. This variation could be due to the differences in the inclusion criteria of the subjects for the studies and the climatic condition of the study area. The higher recovery of *K. pneumoniae* in sputum sample could be associated to the fact that the organism colonizes human mucosal surfaces in the gastrointestinal tract and oropharynx and is therefore thought to be the primary cause of hospital acquired pneumonia. This could be justified by the fact that most of the subjects with respiratory tract infections included in this study were hospitalized.

Detection of drug resistant pathogens such as *Carbapenem resistant Klebsiella pneumoniae* is an important step for successful infection control. When treating resistant bacterial strains such as Gram negative multidrug resistant and ESBLs, carbapenem is frequently suggested as last resort antibiotic. This study revealed that 14.3% of *K. pneumoniae* isolated from patients attending AKTH were carbapenem resistant. This incidence is higher than 10% reported by Aminu and Lawal (2019) in patients attending Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase general hospital Kano. Aya *et al* (2022) reported the incidence of CRKP as 25.4% in tertiary care hospital Egypt. The higher incidences and rapid spread of carbapenem resistant *Klebsiella pneumoniae* could be related to some human factors such as excessive or improper usage of antibiotics due to uncontrolled public access to antibiotics and lack of proper infection control measures such as stewardship policies in health care facilities. Using sub therapeutic doses of antibiotics in Agricultural Sectors can also contributes to the development of resistance (Jinrong *et al.*, 2017, Podschun and Ullman, 1998).

Carbapenamase production have been identified as the primary mechanism of resistance to carbapenem in *Klebsiella pneumoniae*. Of the total number of CRKP Screened using MHT 9 of 12 isolates (75%) were found to be carbapenamase producers. Similar study by Mosca *et al.* (2013) in Italy revealed carbapenamase production in 84% of carbapenem resistant isolates evaluated using MHT. Jeya *et al.* (2013) reported 82.4% among CRKP using MHT. Up to 25% of CRKP Isolates in this study were MHT Negative or were Negative for carbapenamase enzymes Production. Other mechanism of resistance such as over production of ESBL or AmpC enzymes and the loss or reduction of outer membrane protein expression could be the mechanism of resistance in these isolates (25%) (Jinrong *et al.*, 2017).

*bla*KPC gene harbouring bacteria such as *K. Pneumoniae* are known to produce carbapenamase enzyme that can hydrolyse a broad range of B-lactamase antibiotics such as Penicillins, Cephalosporins and Carbapenems. These strains are major cause of concern for health care system around the world.Due to the presence of *bla*KPC gene on transferable plasmids, resistant genes can more easily spread to various strains and species worldwide (Jinrong *et al.*, 2017).

This study revealed that *bla*KPC gene was detected in all the *K. pneumoniae* isolates that phenotyopically produce carbapenamase (100%). Similar study by Mosca *et al* (2013) showed that 100% MHT Isolates harbor *bla*KPC genes. This study showed *bla*KPC genes prevalence of 75% among CRKP and 100% among MHT positive Isolates. Jeya *et al.* (2013) reported that 67.4% of carbapenem resistant enterobacteriaceae have the *bla*KPC gene in India. Deshpande *et al* (2006) reports *bla*KPC gene positivity rate of 86% (44/51) among carbapenem resistant isolates in United state medical Centre.

This study revealed that CRKP were found to be more common among older age groups. This is similar to the findings of Aya *et al.* (2022) who also reported higher incidence of CRKP among elderly subjects. Higher incidence of CRKP among elderly subjects could be related to their lower immunity which could contribute to poor drug response and consequently increase the risk of developing resistance (Karen *et al.*, 2008). Most of these age groups possess a number of comorbidities and have undergone invasive procedures that can predisposed them to nosocomial pathogens that are usually resistant to antibiotics.

Prevalence of *K. pneumoniae* infection and CRKP were found to be higher among in patients/hospitalized patients (20%) compared to outpatient (10%). This finding is in agreement with previous study which also found that antibiotic resistance level is higher in isolates from hospitalized patients than outpatient (Siraj *et al.*, 2014). A number of studies have identified persistent reservoirs of frequently resistant *K. peumoniae* in air conditioning units, sinks, drains and wards on an assortment of general and surgical equipment (Aumeran, 2010). They also reported an outbreak of *K. pneumoniae* infection among 16 patients who had undergone the same surgical procedure. The infection was linked to the endoscope used in the procedure (Aumeran *et al.*, 2010). Macrae (2001) also reported an outbreak of multiple strains of antimicrobial resistant *K. pneumoniae* in neonate unit which was linked to an already discharged patients necessitating the closure of the ward.

Antibiogram of CRKP showed high degree of antibiotic résistance to the tested drugs except colistin and tigecycline. Aya *et al.*(2022) also reported high level of resistance among CRKP isolates from Egypt. Ramsamy *et al.* (2020) reported that all CRKP isolated from their study were extensively drug resistant (XDR).The high incidence and spread of clinically extensively drug resistant strains endangers public health by increasing the risk of spreading diseases, severe illness and death (Zhao*et al.*, 2015). Up to 92% of the isolates were found to be sensitive to colistin and tigecycline. A similar study by Makharita *et al.* (2020) reported high sensitivity of CRKP isolates to colistin (86.5%). Several studies reported colistin as a last resort treatment option for carbapenem resistant bacteria isolates (Makharita *et al.*, 2020).The emergence of colistin resistance is therefore of great concern and can be of public health threat as it can lead to treatment failure and consequently death. One isolate (8%) of CRKP was found to resist all the tested antibiotics including colistin and tigecycline. Makharita *et al* (2020) also reported 13.5% carbapenem resistant isolates as colistin resistant. Similarly Fattouh *et al* (2015) reported 22.4% of KPC producers as colistin resistant.

CONCLUSION AND RECOMMENDATION

The study revealed that up to 14.2% *Klebsiella Pneumoniae* isolated from patients attending AKTH, Kano are Carbapenem resistant. The infection with these organisms was found to be more common among patients with respiratory tract infections, older age groups and admitted or hospitalized patients. Carbapenem resistance is commonly associated with carbapenamase enzyme production and *bla*KPC resistance genes. Carbapenem resistance *K. pneumoniae* isolated in this study demonstrated a strong degree of resistance to all classes of antimicrobial tested except tigecycline and colistin. These can therefore be considered as the best treatment option for CRKP. The high incidence of multidrug resistant CRKP in AKTH, Kano necessitated urgent intervention to curtail the spread of this résistance. This can be achieved through active surveillance, rapid and accurate detection of the organism, strict infection control practices and treatment guidelines.

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