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Incidence of carbapenemase production among antibiotic resistant *Klebsiella* isolates in Zaria, Nigeria

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Abstract

Carbapenemases are diverse group of Beta lactamases produce by Gram negative rods. These groups of enzymes are indicated in resistance to Beta lactam antibiotics that used to be drug of last resort in the control of infectious diseases. Incidence of Gram negative organisms producing these groups of enzymes may be a threat to management of infectious diseases in a developing country like Nigeria. In this study, incidence of Carbanemase production among antibiotic resistant *Klebsiella* species isolated from clinical samples of four hospitals in Zaria, Kaduna state, Nigeria, were analyzed. Conventionally identified *Klebsiella* isolates from the Hospitals were collected and characterized by standard microbiological methods to different species. Antibiotics susceptibility testing using Kirby Bauer agar diffusion method was conducted on the isolates. Isolates found resistant to Meropenem and/or Imipenem were tested for carbapenemase production using modified Hodges test (MHT). A total of 164 clinical isolates of suspected *Klebsiella species* were collected from the four hospitals under the study. The isolates generally came from urine (35.4%), sputum (26.2%), ear swab (17.1%), wound swab (13.4%) and vaginal swab (7.9%). Based on morphological and biochemical characterization, 130 of the isolates were identified as *Klebsiella species*. Further characterization and identification using Microgen identification kit, confirmed *Klebsiella oxytoca* (14.0%), *Klebsiella pneumoniae* (12.8%), *Klebsiella ozaenae* (1.2%), while 72% were other members of enterobacteriaceae. Antibiotic susceptibility testing of *Klebsiella* confirmed isolates showed resistance to Ceftriaxone (89.1%), Cefixime (82.6%), Amoxicillin/clavulanic acid (50%), Meropenem (37.0%), Cefoxitin (34.8%), Gentamicin (24.1%), Ofloxacin (23.9%) and Imipenem (10.9%).Out of 17(37.0%) isolates resistant to Meropenem, 14 (30.4%) were *Klebsiella pneumoniae*, while 2(4.3%) were *Klebsiella oxytoca*. Eleven (11) (23.9%) *Klebsiella pneumoniae* produced Carbapenemases. The findings showed that there is incidence of Carbapenemase production among *Klebsiellai* solates from some hospitals in Zaria, Nigeria.

Keywords: Carbapenemases, Antibiotics, *Klebsiella* isolates**,** susceptibility

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Introduction

Klebsiella is recognized as a top pathogen in nosocomial infections, particularly *Klebsiella pneumoniae*; most of its isolates are multidrug resistant (Nordmann et al., 2011). *Klebsiella pneumoniae* is the common cause of community-acquired bacterial pneumonia, which has high fatality rate if untreated, particularly in chronic

alcoholics (Tu et al., 2009). *Klebsiella species* were found to cause 34.3% of all nosocomial bacterial infections in Nigeria tertiary healthcare facility (Nejad, et al., 2011). Urinary tract infections and surgical sites infections were reported as the most prevalent nosocomial infections caused by *Klebsiella species* (Ige et al., 2011). Also, in pediatric wards, *Klebsiella species* are often found pathogens involved in neonatal sepsis (Zhanel et al., 2008).

Second generation cephalosporins such as; Cefuroxime show high activity against Klebsiella, this makes them potential alternatives for treating *Klebsiella* related urinary tracts infections (Tumah, 2005). Aminoglycosides and quinolones possess a broad spectrum of activity against both Gram positive and Gram negative organisms including *Klebsiella* (David, 2012). Carbapenems are members of Beta lactams with the broadest spectrum of activity and greatest potency against Gram positive and Gram negative bacteria. Carbapenems are often used as antibiotics of last resort when patients with infections are suspected of harboring resistant bacteria (Paterson, 2004). This is the more reason why resistance to Carbapenems may pose a great challenge to infection control system globally.

The major forms of resistance in *Klebsiella species* are attributed to their ability to acquire resistance genes which are either borne on plasmids or carried on chromosomes (Haris et al., 2007). Some of resistance genes reported among *Klebsiella species* may include: extended spectrum beta-lactamases (ESBLs) and plasmid mediated Amp C beta-lactamases (PMABLs). Most of the organisms carrying these genes have been reported to be susceptible to carbapenems, resistant to carbapenems that might have been the antibiotic of last resort have been reported to be encoded by Carbapenemase genes (Cao et al., 2000). Another mechanism of resistance to Carbapenem antibiotics is via mutation by combination of porin loss and the presence of plasmid-mediated beta lactamases (Cao et al., 2000). Determination of Gram negative producing these enzymes become important has it constitutes a great threat to healthcare delivery. This study therefore aimed at investigating the incidence of Carbapenemase production among *Klebsiella* isolates collected from some hospitals in Zaria, Nigeria.

Materials and Methods

Study Area and Sample Size Determination

The study was carried out at Zaria using random selection of Hospitals at different geographical distribution of Zaria, Nigeria. The following Hospitals were selected for isolates collection from December, 2015 to March, 2016: Ahmadu Bello University Teaching Hospital, Shika, Hajia Gambo Sawaba General Hospital Kofar Gayan, Major Ibrahim Abdullahi Memorial Hospital Sabon Gari and St Lukes Anglican Hospital, Wusasa.

Ethical Considerations

Ethical approval with number MOH/ADM/7444/VOL./342 was obtained from Ministry of Health and Human Services, Kaduna State, Nigeria.

Sample Collections and Processing

A total of 164 isolates identified as *Klebsiella species* were collected from the Microbiology unit of the four selected hospitals within a period of four months. The identified isolates were inoculated on Nutrient agar slants and transported in an ice pack to Pharmaceutical Microbiology laboratory of Ahmadu Bello University, Zaria. The isolates were incubated at 37^0Cfor 24 to 48 hours before storage in a refrigerator at 4^0C pending biochemical analysis.

Biochemical Identifications and Characterization

The stored isolates were inoculated into nutrient broth to resuscitate the organisms and incubated at 37^0C for 24 hours. The overnight cultures were streaked on MacConkey agar plate; the plates were inverted and incubated at 37^0C for 24 hours. Pure pinkish mucoid colonies from the MacConkey plates were subjected to conventional biochemical test for identification of *Klebsiella species*. Isolates confirmed as *Klebsiella specie* were subjected to Microgen identification kits for enterobacteriaceae, to identify them to species level.

Antibiotics

A total of eight (8) antibiotics, which represent the most commonly prescribed antibiotics for treatments of *Klebsiella* related infections in Zaria were used for the study. Antibiotics used were Imipenem (IMP, 30ug), Meropenem (MEM, 30ug), Ofloxacin (OFX, 5ug), Cefoxitin (FOX, 30ug), Cefriaxone (CRO, 30ug), Cefixine, Amoxycillin Clavulanic acid (AMC, 30ug) and Gentamicin (CN, 30ug). They were all produced by Oxoid.

Antibiotic susceptibility Testing

This was carried out using Kirby-Bauer modified disc agar diffusion techniques (Cheesbrough, 2006). Suspension of overnight growth of each isolates on nutrient agar plates was standardize and compared with 0.5

MacFarland standard turbidity, the suspension was inoculated on Mueller-Hinton agar plates using sterile swab. The surface of the agar was allowed to dry and the antibiotics discs were placed on the surface of the agar using a sterile forceps. After 30 minutes of applying the discs, plates was inverted and incubated at 35°C for 16-18 hours. The plates were then examined and the diameter of each zone of inhibition around the discs was measured in mm, using a ruler. The organisms were classified as sensitive or resistant based on the CLSI (2014) interpretative chart as shown below:

Table 1: Clinical and Laboratory Standards Institute interpretative chart

Antibiotics	Disc potency (Mcg)	Diameter of zones of inhibition (mm)	
		Resistant	Susceptible
Imipenem	10	≤19	≥23
Meropenem	10	≤19	≥23
Amoxicillin clavulanic acid	30	≤19	≥19
Ceftriaxone	30	≤20	>23
Cefoxitin	30	≤14	≥18
Cefixime	5	≤15	≥19
Ofloxacin	5	≤20	≥31
Gentamicin	30	≤14	≥17
Imipenem	10	≤19	≥23
Meropenem	10	≤19	≥23

Determination of Multiple Antibiotics Resistant Index (MARI)

Multiple antibiotics resistance index (MARI) was determined for each isolate by dividing the number of antibiotics to which the organisms was resistant by the total number of antibiotics tested (Paul et al, 1997).

Determination of Carbapenemase Production

Isolates showing resistance to Meropenem were subjected to confirmatory test for carbapenemase production using the modified Hodge Test (MHT) (Walsh et al, 2011). Suspension of overnight growth of *E.coli* ATCC 25922 was standardized and compared with 0.5 MacFarland standard turbidity, the suspension was evenly inoculated on surface of Mueller Hinton agar plates. Meropenem (10ug) disc was placed on the surface at the center of the Mueller Hinton agar plate. In a straight line, the test organism was streaked from the edge of the Meropenem disc to the edge of the culture plate. The plates were incubated at 37C for 24hours, after which the plates were examined for flattening and indentation at point of intersect of the organism and *E.coli* ATCC 25922 within the zone of inhibition of the Meropenem. Four organisms were tested on the same Mueller Hinton agar plate.

Statistical Analysis

Data were processed using Microsoft Excel 2010 and were represented using tables, charts and percentage.

Results

A total of 164 isolates identified as *Klebsiella* isolates were collected from four sampled hospitals. Significant percentage of the isolates [28% (46)] were validated to belong to Klebsiella species, 50% (23) were *Klebsiella oxytoca*, 45.7% (21) were *Klebsiella pneumoniae*, 4.3% (2) were *Klebsiella ozaenae* while others were Gram negative organisms from different Enterobacteriaceae. The distribution of the isolates from their sources are shown in Table 1, while Table 2 shows the biochemical reaction of the *Klebsiella species* and table 3 shows how many of the *Klebsiella species* were isolated from different hospitals.

Table 2: Distribution of Isolates

Hospitals Evaluated	Isolate source				
	Urine (n=58)	Blood (n=28)	HVS (n=13)	Wound (n=22)	Sputum (n=43)
Ahmadu Bello University Teaching Hospital, Shika	21	11	6	7	10
Hajia Gambo Sawaba, General, Hospital, Kofar Gaya, Zaria	12	5	3	5	12
Major Ibrahim Abdullahi Memorial Hospital, Sabon Gari, Zaria	11	2	2	3	9
St. Luke's Anglican Hospital, Wusasa, Zaria.	14	10	2	7	11

Table 3: Biochemical characterization of isolates

Organisms	Isolate source				
	Urine (n=58)	Blood (n=28)	HVS (n=13)	Sputum (n=22)	Wound (n=43)
Klebsiella pneumonia	11	1	2	5	2
Klebsiella oxytoca	14	2	1	2	4
Klesiella ozaenae	1	0	0	0	1
Others	32	25	10	15	36

Table 4: Distribution of *Klebsiella species* in Different Hospitals Evaluated

Hospitals Evaluated	K. pneumonia	K. oxytoca	K. ozaenae
Ahmadu Bello University Teaching Hospital, Shika	6	10	0
Hajia Gambo Sawaba General Hospital, Kofar Gayan, Zaria	7	9	0
Major Ibrahim Abdullahi Memorial Hospital, Sabon Gari, Zaria	5	3	0
St Lukes Anglican Hospital, Wusasa, Zaria	3	1	2

The antibiotic susceptibility profile of the isolates showed that largest 89.1% were resistant to Ceftriaxone, while least 8.7% of the isolates were resistant to Imipenem.

Table 5: Antibiotic Resistant Profile of *Klebsiella* spp. Isolated

Antibiotics	Class of Antibiotics	Percentage of *Klebsiella spp.* Resistant to various Antibiotic Tested			
		K. pneumonia (n=21)	*K. oxytoca* (n=23)	*K. ozaenae* (n=2)	Total resistant (n=46)
Meropenem	Carbapenem	12 (57.14)	5 (21.74)	0 (0.00)	7 (15.22)
Imipenem	Carbapenem	4 (19.05)	0 (0.00)	0 (0.00)	4 (8.70)
Amox/clav.	Penicillin	9 (42.86)	13 (56.52)	1 (0.50)	23 (50.00)
Ceftriaxone	Cephalosporin	18 (85.67)	22 (95.65)	1 (0.50)	41 (89.13)
Cefoxitin	Cephalosporin	11 (52.38)	5 (21.74)	0 (0.00)	16 (34.78)
Cefixime	Cephalosporin	17 (80.95)	19 (82.61)	2 (1.00)	38 (82.61)
Ofloxacin	Fluoroquinolone	9 (42.86)	1 (4.35)	0 (0.00)	10 (21.74)
Gentamicin	Aminoglycoside	7 (33.33)	5 (21.74)	0 (0.00)	12 (26.09)

Out of the 46 isolates confirmed as Klebsiella species, 17(37.0%) *Klebsiella species* were resistant to Meropenem, 11(23.91%) produce Carbapenemase enzymes, 6 (13.04%) are non- Carbapenemase producers, while 29 (63.04%) were neither resistant to Meropenem nor produce Carbapenemase (Fig. 1)

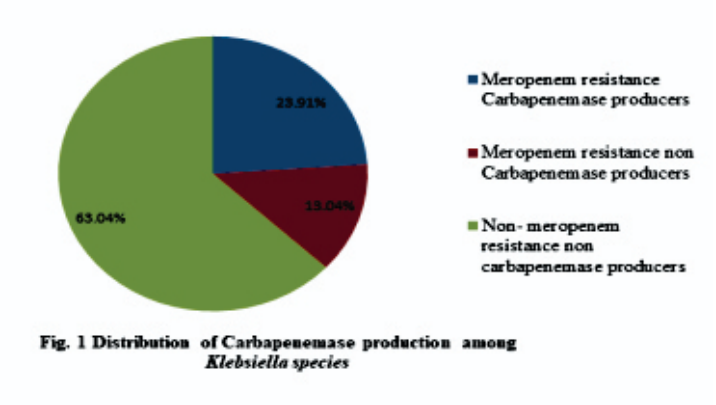

Fig. 1 Distribution of Carbapenemase production among *Klebsiella species*

Table 6: Antibiotics resistant Pattern of *Klebsiella* isolates

Isolate	Antibiotics resistant to	NART
ABW4	AMC, CRO, FOX, CFM, OFX, CN	6
ABU13	AMC, CRO,FOX, CFM, OFX,CN	6
STU4	MEM, AMC, CRO, FOX, CFM, OFX, CN	7
ABB4	AMC, CRO, FOX, CFM,OFX, CN	6
MISP8	AMC, CRO, FOX, CFM, CN	5
STU10	MEM, AMC, CRO, FOX, CFM	5
STB2	CFM	1
ABU9	MEM, AMC,CRO, FOX, CFM, CN	6
GSW2	AMC, CRO	2
GSU10	CRO, CFM, OFX	3
GSVS2	AMC, CRO, CFM, CN	4
STU9	MEM, AMC, CRO,CFM	4
ABW3	MEM, AMC, CRO,CFM, OFX, CN	6
STW1	MEM, CRO, FOX, CFM,	4
MIVS1	CRO	1
MIU1	MEM, AMC, CRO, FOX, CFM	5
ABU5	CRO, CFM	2
GSU5	CRO	1
GSU3		0
ABVS3	MEM	1
MIU10	FOX	1
ABSP2	MEM, IMP, AMC, CRO, CFM	5
ABSP5	MEM, CRO, FOX, CFM	4
STU3	CRO	1
STSP1	IMP, CRO, FOX, CFM, OFX	5
GSW5	AMC, CRO, CFM, OFX	4
MIU7	IMP, AMC,CRO, FOX, CFM, OFX, CN	7
MIU8	IMP, CRO, FOX, CFM, CN	5
GSU7	MEM, AMC, CRO, FOX, CFM	5
GSSP2	CRO, FOX, CFM	3
STW5	MEM, CRO, FOX, CFM	4
STVS1	MEM, CRO, FOX, CFM, OFX, CN	6
ABSP1	AMC,CRO, CFM	3
ABU3	AMC, CRO, FOX, CFM, OFX	5
GSU11	CRO, FOX,	2
GSU8	AMC, CRO, FOX, CFM	4
GSU13	MEM, CRO, FOX, CFM	4
ABU7	CRO, FOX, CFM, CN	4
MIW3	MEM, AMC, CRO,	5
MIW2	FOX, CFM MEM, AMC, FOX, CFM	4
STU8	AMC, CRO, FOX, CFM	4
STU6	AMC, CRO, FOX, CFM	4
ABU10	CRO, FOX, CFM	3
ABU8	FOX	1
GSW1	CRO, FOX, CFM	3
MISP5	MEM, AMC, CRO, FOX, CFM	5

Keys: NART = Number of antibiotics resistant by each isolates, AMC= Amoxicillin Clavulanic acid, CFM= Cefixime, CRO= Ceftriaxone, CN= Gentamicin, FOX= Cefoxitin, IMP= Imipenem, MEM= Meropenem OFX= Ofloxacin

Table 7: Percentage Multiple Antibiotics Resistant Index of Carbapenameses and non Carbapenemases Producing *Klebsiella* Isolates

MARI	NCCP	Percentage (%)	NCNCP	Percentage (%)
0	0	0	1	2.2
0.1	0	0	7	15.2
0.2	0	0	0	0
0.3	0	0	3	6.5
0.4	0	0	5	10.9
0.5	3	6.5	9	19.6
0.6	7	15.2	3	6.5
0.7	0	0	0	0
0.8	0	0	6	13.0
0.9	1	2.2	1	2.2

Key: NCCP = Number of occurrence of Carbapenemese producer, NCNCP = Number of occurrence of non-Carbapenamese producer.

Discussion

In this study, the order of isolation of *Klebsiella species* were from urine - 58> Sputum - 43>Blood -28 >Wound- 22> High vaginal swab - 13. This report is in agreement with the report of Thosar and Kamble (2014) that isolated *Klebsiella* from urine (37.8%), wound swabs (29.7%) and sputum (16.5%). Iroha et al., (2011) reported similar trend in a study conducted in Ebonyi State, South eastern Nigeria.

Identification of *Klebsiella* isolates in this study showed that *Klebsiella oxytoca* was most frequently found followed by *Klebsiella peumoniae* and least prevalence was *Klebsiella Ozaenae*. This result was similar to that obtained by Rosool (2003) who reported the high prevalence of *K. oxytoca* (52%) followed by *K. pneumonia* (42%) and *K. ozanae* (6%) among the clinical isolates of *Klebsiella* collected from various hospital and laboratories of Karachi city in Pakistan. The result is in contrast with what was reported by Thousar and Kamble (2014) who found *Klebsiella pneumoniae* (35.6%) the most prevalent followed by *Klebsiella oxytoca* (4.7%) and K. ozaenae (0.09%) from samples in India. Higher prevalence of *Klebsiella oxytoca* obtained in this study may be due to the fact that *Klebsiella oxytoca* being an opportunistic pathogen that cause primarily hospital-acquired infections, most often involving immune compromised patients or those requiring intensive care. It is also a causative agent of colitis and sepsis (Hogenauer et al., 2006).

The result of antibiotics susceptibility in this study showed Imipenem to be very effective. This was however lower than the 100% activity reported by Olowe et al., (2012) for *Klebsiella* isolates from clinical samples at Ile-ife, Nigeria, but comparable with those of Manikandan and Asmath (2013) and Ejikeugwu et al., (2015) who reported 86.1% and 87.2% susceptibility of *Klebsiella species* isolated from clinical samples in India and Nigeria respectively. The susceptibility of *Klebsiella species* to Carbapenems, aminoglycosides and quinolones and cefoxitin as obtained in this study may be due to the fact that, Carbapenems are expensive and not commonly sold over the counter, while quinolones such as ofloxacin are rearly prescribed due series of contraindications attached to it administration. The parenteral routes of gentamicin reduce the abuse of the antibiotics. Also concentrations dependent bactericidal activity of gentamicin, it has extended post antibiotics effect and the possibility of reduced nephrotoxicity and ototoxicity also affect the recommendations of gentamicin (Behm-Dillon, 2000).

This study recorded resistance to penicillins and Cephalosporins The result varied from the resistance reported by Orhue and Aiu (2015) who found that Amoxi/clavulcanic acid showed 100%activity, Cefuroxime 60% activity and 50% activity against Cefotaxime by *Klebsiella species* isolated from different clinical specimens in healthcare centre's of Etsako west, Edo state, Nigeria. Also Olusola et al., (2013) who reported 69% resistance to Amoxi/clavulanic acid, 27% resistance of Ceftazidime, 32% resistance to Ceftriaxone, 41% resistance of Cefuroxime by *Klebsiella species* in tertiary hospital in Abeokuta, Nigeria. The slight decrease in antibiotics resistance obtained in this study compared to other studies may be due to differences in multiple antibiotics resistance (MAR) of *Klebsiella species*. The differences in MAR may be due to the differences in the place and the time of the study.

Incidence of Carbapenemases recorded among *Klebsiella* isolates in this study concurs with 25.0% reported by Yusuf et al, (2012)

among *Klebsiella pneumoniae* in Kano, Nigeria. But less than 35.7% reported by Dahiya et al., (2015) among Klebsiella species isolates in India. The higher percentage of Carbapenemases among Meropenem resistant *Klebsiella species* shows that Carbapenemases mediated resistance is more frequent than the non-carbapenemase mediated mechanism of resistance among the isolates. Also the incidence of Meropenem resistant but Non-Carbapenemases production among *Klebsiella* isolates, may be mutational resistant due to loss of porin proteins and this may cause resistance of the organisms to other classes of antibiotics such as; Cephalosporins (Cao et al., 2000).

Conclusion

The study showed that *Klebsiella oxytoca* has higher incidence among species of *Klebsiella* isolates from the sampled hospitals in Zaria. The isolates are resistant to penicilins and cephalosporins but most relatively sensitive to Imipenem, Ofloxacin, Cefoxitin, Meropenem and Gentamicin. There is incidence of Carbapenemase production among the isolates and higher Meropenem resistant among isolates may have resulted from Carbapenemase mediated resistant more frequent than non-Carbapenemase mediated mechanism of resistance.

Recommendation

There is need for more studies to ascertain the incidence of Carbapenemase production among *Klebsiella* isolates in Zaria, Nigeria. In order to determine the prevalence and provide adequate control measures to prevent the dissemination of the plasmid that carries the gene that code for the enzyme in the environment.

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