

ORIGINAL ARTICLE**Phenotypic Detection of Extended Spectrum Beta lactamase and Carbapenemase Co-producing Clinical Isolates from Two Tertiary Hospitals in Kano, North West Nigeria****Yusuf Ibrahim^{1*}, Yahaya Sani¹, Qabli Saleh², Algarni Saleh², Gbadamosi Hakeem³****OPEN ACCESS**

Citation: Yusuf Ibrahim, Yahaya Sani, Qabli Saleh, Algarni Saleh, Gbadamosi Hakeem. Phenotypic Detection of Extended Spectrum Beta lactamase and Carbapenemase Co-producing Clinical Isolates from Two Tertiary Hospitals in Kano, North West Nigeria. *Ethiop J Health Sci* 2017;27(1):3-10. doi: <http://dx.doi.org/10.4314/ejhs.v27i1.2>

Received: September 15, 2015

Accepted: December 1, 2015

Published: January 1, 2017

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Funding: Nil

Competing Interests: The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

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ABSTRACT

BACKGROUND: *Continue rise in unprofessional use of antibiotics in our hospitals and communities is worrisome. A research study was therefore conducted to detect extended spectrum beta-lactamase (ESBL), carbapenemase, metallobeta lactamase and their co-production phenotypically from isolates obtained from patients admitted to or attending two tertiary hospitals in Kano, Nigeria.*

METHOD: *A total of 248 isolates of Escherichia coli and Klebsiella pneumoniae were screened phenotypically for ESBL production and carbapenemase production according to CLSI 2012 breakpoints using double disk synergy test and modified Hodge test (MHT) respectively. Antibiotic susceptibility of the organisms was tested against colistin, tigecycline and 3 flouroquinolones.*

RESULT: *The result shows that 58.0% of the isolates were ESBL producers with higher percentage in K. pneumoniae (62.9%). Further, about 40.3% and 36.6% of the isolates were resistant to meropenem and imipenem respectively. However, E. coli showed higher resistance to meropenem (47.1%) while K. pneumoniae showed higher resistance to imipenem (44.4%). Co-productions of carbapenemase and ESBL were observed in both E. coli and K. pneumoniae. Carbapenemase producing isolates were more obtained from uro-pathogens and wound isolates, with almost all the cases of co-production of the β lactamases occurring in urine and cathertips isolates. Overall susceptibilities of the isolates to colistin and tigecycline were 64.6 and 70.0% respectively, but isolates were less susceptible to flouroquinolones.*

CONCLUSION: *The finding of the study therefore indicates that carbapenem resistance is mediated by carbapenemase production and or overproduction of ESBL coupled with reduced porins. Co-production of carbapenemase, MBLs and ESBLs by some of the isolates is worrisome. Susceptibility to colistin and tigecycline was still promising, but increasing resistance to flouroquinolones has been observed.*

KEYWORDS: *Carbapenemase, ESBL, colistin, tigecycline, flouroquinolones, phenotypic, Kano*

INTRODUCTION

Hospital wards, especially those in low income countries where infection prevention and control (IPC) protocols are lacking have become the main foci for developing multidrug resistance such as multidrug-resistant Gram negative bacilli (1). Community acquisitions of these hard to treat bacteria are highly possible due to inadequate environmental/personal sanitations and lack of surveillance (1).

Increasing rates of Gram negative bacteria resistance to beta lactam antibiotics such as cephalosporins and carbapenems are of great concern not only to health care workers (HCWs) but to the public. This is because they are the drug of choice for the treatment of very stubborn infection by many Gram negative bacteria (2). However, the increasing use of these antibiotics in some countries has led to the development of carbapenem resistant pathogens, which have already found their ways to low income countries where treatments with such antibiotics (carbapenems) are not frequent. However, carbapenem-resistant strains are emerging worldwide, and resistance rates have reached 50-60% in some institutions (3). However, data regarding carbapenemase and other beta lactamase mediated resistance are scarce in overburden low income healthcare facilities. This paucity of information in our facilities poses a great danger to general health care delivery system (4-6).

During the last decade, carbapenem resistance has been increasingly reported among Enterobacteriaceae and is largely attributed to the production of class A β -lactamase, the *Klebsiella pneumoniae* carbapenemase (KPC) and Ambler class B metallo- β -lactamases (MBLs) requiring metal ion for action (7). In addition, some clinical isolates like *E. coli* and *K. pneumoniae* are capable of co-producing both MBL and KPC carbapenemases in addition to ESBL, which may further narrow down treatment options with β -lactam and non- β -lactam antibiotics.

The mechanism of carbapenem resistance is either through the acquisition of carbapenem-hydrolysing β -lactamases like carbapenemase or through production of ESBL in association with impaired permeability via loss of porins

(8). Infections caused by organisms producing such enzymes have resulted in poor outcomes, reduced rate of clinical and microbiological responses, longer hospital stays and greater hospital expenses (9). Physical contact is the most likely mode of transmission and the gastrointestinal tract of colonized or infected patients is the most frequent reservoir while transient carriage of bacteria on the hands of healthcare workers may lead to transmission to patients (10).

The lack of screening for these multi-drug resistant pathogens in our hospitals coupled with poor antibiotic stewardship and surveillance systems has increased their burden in our hospitals and community in recent years. The traditional susceptibility testing protocol being used currently in many clinical laboratories in Nigeria cannot detect bacteria harboring these enzymes. This will eventually lead to inappropriate diagnosis, unsuccessful therapy of patient and unnecessary use of drugs. Since molecular detection and genotyping of resistant isolates are expensive and unavailable in most hospitals, this study aimed to detect carbapenemase and ESBL production among the clinical isolates phenotypically in the two tertiary hospitals in Nigeria and to relate the gross antibiotic resistance being observed in the hospitals to carbapenemase and ESBL or co-production of two or more by the pathogens, and to test the susceptibility of the carbapenem and ESBL producing pathogens to colistin and tigecycline.

MATERIALS AND METHOD

Collection of Clinical Isolates: A total of two hundred and forty-eight (248) consecutive non-repetitive clinical bacterial isolates of *Escherichia coli* (140) and *Klebsiella pneumoniae* (108) were derived from samples of urine, catheter tips and wounds, and abscesses were collected from pathology department of the hospital A and B over a period of 12 months. Hospital A (Aminu Kano Teaching Hospital) is a teaching hospital with about 600 bed capacity while hospital B (Murtala Muhammad Specialist Hospital, Kano) is a general specialist hospital with about 700 bed capacity. The two hospitals receive patients from all over the country and neighboring countries. The demographic information and the history of

each patient was obtained from his/her records. The isolation and identification of the bacterial pathogens were made in the laboratory by the routine standard method used to identify pathogens in the two hospitals. All urine and catheter samples were cultured on cysteine lactose electrolyte deficient (CLED) and nutrient agar (NA) while wound and abscess samples were cultured on blood, nutrient and McConkey agar for bacterial isolation. Gram negative bacterial pathogens isolated were identified by biochemical tests according to CLSI protocols. The following antibiotics were tested against the isolates in the laboratory as part of their routine practice using Kirby-Bauer's disc diffusion method: gentamicin (10 µg), co-trimoxazole (25µg), ciprofloxacin (5 µg), cefpodoxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), nalidixic acid (30 µg), amoxicillin (20 µg), nitrofurantoin (300 µg), tetracycline (30 µg), chloramphenicol (30 µg) and augmentin (30 µg). Isolated pathogens included *E. coli*, *K. pneumoniae*, *Serratia* sp, *Proteus* sp, *Citrobacter* sp. and *Pseudomonasaeruginosa*. Only *K. pneumoniae* and *E. coli* were selected because they were the highest prevalent uropathogen and of wound and abscess in the two hospitals, as shown in previous studies, and they showed high resistance to many of the routine antibiotics in addition to *P. aeruginosa*. Because few *P. aeruginosa* was obtained, it was not included in the study. However, during the selection, attention was given to isolates that were at least resistant to 3 classes of antibiotics including beta-lactams.

Test for Extended Spectrum Beta lactamase (ESBL): All the clinical pathogens were screened for ESBLs using phenotypic methods according to CLSI guidelines. In the presumptive test to detect potential ESBL producers, all the isolates were screened for susceptibility to cefpodoxime (10 µg), ceftazidime (30 µg) and the ceftriaxone (30 µg) antibiotic discs (Oxoid, UK). Results were interpreted based on the CLSI guidelines as follows: zones of inhibition of ≤ 17 mm for cefpodoxime; ≤ 22 mm for ceftazidime and ≤ 25 mm ceftriaxone or combination of two or more. The less susceptible or resistant isolates were subjected to confirmatory

test using the double discs synergy test as described in the CLSI guidelines.

Carbapenem Susceptibility Testing: Susceptibility of all the isolates to carbapenems was tested in accordance with the current CLSI guidelines (11). Carbapenem antibiotics MEM and IPM were used. The discs were placed on the surface of inoculated Mueller Hinton Agar (MHA) (Hi media, India) plates using sterile forceps. The discs were placed about 25mm apart, and the plates were incubated for 24hours at 37 °C after which zones of inhibition in diameter were recorded using a ruler according to CDC guidelines (12). *E. coli* ATCC 25922 strain was used as a control culture. Isolates that showed a zone of inhibition ≤ 21 mm in diameter for meropenem or ≤ 23 mm in diameter for imipenem were considered as carbapenem resistant.

Screening for carbapenemase production

The selected carbapenem-resistant pathogens were screened for carbapenemase production phenotypically using Modified Hodge Test (MHT) as recommended by the CLSI guidelines (12). This is necessary in order to determine if the reduced susceptibility to carbapenems observed is mediated by a β lactamase-carbapenemase.

Detection of class B metallo β -Lactamases (MBLs):

The carbapenemase producing organisms detected were further screened for Class B metallo-carbapenemases (MBLs), which are characterised by inhibition by metal chelators, EDTA. EDTA-disc synergy based method described by Walsh *et al* (13) and CLSI (11) was employed. The method involves streaking a carbapenemase producing isolate on MHA, followed by placing two MEM discs; one containing EDTA and the other without EDTA at a distance of about 30 mm apart. The EDTA disc was prepared in-house by adding 250 µl of neutral EDTA to MEM. The plates were incubated for 24 hours at 37 °C and read for zones of inhibition after incubation. Control disc containing EDTA alone was used to determine the activity of the EDTA to ensure that it does not cause false-positive results by inhibiting the test isolate. Organisms that showed difference in diameter of zone of inhibition by ≥ 4 mm on the MEM containing EDTA and MEM alone were confirmed for the production of MBLs.

Screening of Carbapenemase, MBL and ESBL Producers for Susceptibility to Colistin, Tigecycline and Flouroquinolones: The susceptibility of the carbapenemase (MBL inclusive) and ESBL producers to colistin (10µg), tigecycline (15µg), ciprofloxacin (5 µg), levofloxacin (30 µg) and ofloxacin (30 µg) (Oxoid UK), was performed by disc diffusion method. The zone diameters of each of the antibiotics were interpreted as per CLSI 2012 recommendations. Isolates producing a diameter of ≥ 14 mm and ≥ 21 mm for colistin and tigecycline respectively were considered susceptible. *E. coli* ATCC 25922 was used as the control strain for the antimicrobial susceptibility testing.

RESULTS

Distribution and carbapenem-susceptibility of the isolates: The distribution of the 248 isolates obtained from patients from the two hospitals were 140 *E. coli* (56.5%) and 108 *K. pneumoniae* (43.5%), which were obtained from patients with a mean age of 37.3 years. Sixty-six (66) of the *E.*

coli and 59 of the *K. pneumoniae* were obtained from surgical wards, 28 and 33 from out patients (OP) while the rest of 46 *E. coli* and 16 *K. pneumoniae* were obtained from other wards (inpatients). Most of the isolates were from urine and catheter tip samples (83 *E. coli* and 61 *K. pneumoniae*), and others included wound swabs (16 *E. coli* and 25 *K. pneumoniae*) and abscess (41 *E. coli* and 24 *K. pneumoniae*).

Of the total 248 isolates screened for ESBL production, 58.0% were ESBL producers with higher percentage in *K. pneumoniae* (62.9%). Meanwhile, a total percentage of 40.3% and 36.6% of the isolates were resistant to MEM and IMP respectively with *E. coli* showing higher resistance to MEM (47.1%) and *K. pneumoniae* showing higher resistance to IMP (44.4%) (Table 1). In out of the 66 *E. coli* that showed positive cut-off zone breakpoints for MEM, 32 (48.5%) of the isolates were also resistant to IMP, but in the case of *K. pneumoniae*, 23 (67.6%) were also resistant to IMP.

Table 1: resistance of the pathogens to carbapenems

Bacterial isolates	No. of isolates screened	No. resistant to meropenem (Zones <21mm)	No. resistant to imipenem (Zone <23mm)	No resistant to both
<i>E. coli</i>	140	66(47.1)	43 (30.7)	38 (27.1)
<i>K. pneumoniae</i>	108	34(31.4)	48 (44.4)	19 (17.6)
Total	248	100 (40.3)	91 (36.6)	57 (22.9)

Of the total 191 carbapenem-resistant isolates screened for carbapenemase production, 41 (37.6%) and 52 (63.4%) of *E. coli* and *K. pneumoniae* respectively, produces carbapenemase. Furthermore, out of the total 93 isolates that were carbapenemase screened positive, 38.7% were MBL producers. Some of the isolates had the intrinsic capacity to produce two or three of the enzymes. Combined production of ESBL and carbapenemase was detected in 19 *E.*

coli and 16 *K. Pneumoniae* and a combination of ESBL, carbapenemase and MBL were detected in 13 *E. coli* and 11 *K. pneumoniae* (Table 2).

The majority (109, 75.7%) of the 144 ESBL producers and 39 (53.4%) of the 73 carbapenemase producers were from surgical wards, followed by from inpatients from other wards (20.1% *E. coli* and 46.5% *K. pneumoniae*) then outpatients with only 2.8% ESBL but no carbapenemase. About 63.5% (61/96) of the isolates from urine samples

Table 2: Prevalence of ESBL and carbapenemase-MBL mediated carbapenem resistance

Bacterial Species	Screening for ESBL		Screening for carbapenemase production		Screening of carbapenemase producers (CP) for MBL		Combination of CP, MBL and ESBL	
	No. screened	No positive for ESBL	No screened	No positive for carbapenemase (%)	No of CP screened	No positive for MBL (%)	CP+ESBL	CP+MBL+ESBL
<i>E. coli</i>	140	76(54.2)	109	41 (37.6)	41	19(46.3)	19(13.6)	13 (9.2)
<i>K. pneumoniae</i>	108	68(62.9)	82	52 (63.4)	32	17 (53.1)	16 (14.8)	11 (10.1)
Total	248	144(58.0)	191	93 (48.7)	93	36 (38.7)	35 (14.1)	24 (9.6)

Note: figures in parenthesis are percentages

were ESBL positive, 57.3% (47/82) of isolates from catheter tips samples were also ESBL positive while 43.1% (22/51) wound swabs and 75.6% (14/19) of the abscesses samples were ESBL positive (Table 3). Carbapenemase producers from urine, catheter tips and wound

swabs followed a similar pattern with ESBL with being the highest in urine (33.3%), followed by catheter tips (15.8%) and wound swabs (9.8%).

Table 3: Distribution carbapenemase, MBL and ESBLs producers among clinical samples

Clinical samples	No. screened	CP	MBL	ESBL	CP+ESBL	CP+MBL+ESBL
Urine	96(38.7)	32 (33.3)	18 (18.7)	61(63.5)	15 (15.6)	8 (8.3)
Catheter tips	82 (33.0)	26 (31.7)	13(15.8)	47 (57.3)	14(17.0)	13 (15.8)
Wound discharge	51 (20.5)	12 (23.5)	5 (9.8)	22 (43.1)	6 (11.7)	3 (5.8)
Abscesses fluid	19 (7.6)	3 (15.7)	0 (0.0)	14 (73.6)	0 (0.0)	0 (0.0)
Total	248 (100)	73 (29.4)	36 (14.5)	144 (41.1)	35 (14.1)	24 (9.6)

Note: figures in parenthesis are percentages. CP=carbapenemase

Antibacterial susceptibility of all the isolates resistant to IPM and MEM, including those that produced ESBL and or carbapenemase to selected members of the class: polymyxin, glycolcycline, and flouroquinolones were determined. Susceptibility of *E. coli*, and *K. pneumoniae* to colistin and tigecycline, irrespective of type of β -

lactamase they produced, was higher compared with their susceptibility to flouroquinolones. However, *K. pneumoniae* was less susceptible to colistin (62.5%) and tigecycline (67.5%) compared with *E. coli* (66.7% and 72.6%), but with a little higher susceptibility to flouroquinolones than *E. coli* (Table 4).

Table 4: Susceptibility of carbapenemase (MBL inclusive) and ESBL producing isolates to selected polymyxin, glycolcycline, and flouroquinolones

Bacterial Species	Antimicrobial Susceptibility				
	Colistin	Tigecycline	Ciprofloxacin	Levofloxacin	Ofloxacin
	No.susceptible (%)	No. susceptible (%)	No. susceptible (%)	No. susceptible (%)	No. susceptible (%)
<i>E. coli</i> (117)	78 (66.7)	85 (72.6)	57(48.7)	63 (53.8)	43 (36.7)
<i>K.pneumoniae</i> (120)	75 (62.5)	81 (67.5)	77(64.1)	53 (44.1)	60 (50.0)
Total (237)	153 (64.6)	166 (70.0)	134 (56.5)	116 (48.9)	103 (43.4)

DISCUSSION

The occurrence and continual spread of ESBL and AmpC mediated beta lactam resistance in many hospitals in Nigeria is known only to few. The rise in treatment failures in low income countries with over stretched healthcare facilities, typical of those in the study area, has continued to make life difficult for patients, their relatives and the practicing HCWs. In addition to inappropriate and personal prescriptions of antibiotics, lack of proper and sustainable infection prevention and control (IPC) measures in place has made the situation worse. In the study, it was observed that nearly all of the isolates were either totally resistant or slightly susceptible to commonly prescribed antibiotics in the two hospitals. The first and second line treatment drugs such as amoxicillin, augmentin, ceftazidime, cefpodoxime and ceftriaxone are now fast approaching clinically irrelevant drugs (data not shown).

Carbapenems are considered to be one of the antibiotics of last resort for treatment of infections caused by multi drug resistant bacteria such as *E. coli* and *K. pneumoniae* (14). Alarms have been raised over and over on the dangers of spreading of carbapenem resistant bacteria in hospitals and communities, where they can be acquired if IPC programs are not in place. A survey of the two hospitals showed that usage of carbapenems is limited, probably due to their non-availability or their high cost. In hospital A, carbapenems are reserved only for very stubborn infections. Similarly, specific screening for highly resistant pathogens such as ESBL, AmpC producers are not conducted currently in the laboratories of the two hospitals, despite the several local reports on their existence and continual rise in the hospitals (15-17). While the traditional antibiotic susceptibility testing technique in use has failed to detect them, the spread continues undetected and false diagnosis is being issued. In an ideal situation, isolates screened positive for ESBL or carbapenemase phenotypically need to be confirmed by a polymerase chain reaction-based molecular test, but this technique is not really available in routine laboratory investigations in the study area. Therefore, the study restricted the screening to phenotypic confirmation tests.

The prevalence of ESBL and carbapenemase among the isolates was 38.4 and 39.2% respectively. This is very high when compared with previous figures of 14.4% and 11.9% respectively reported in 2011 in same hospitals combined. Patients colonised with β lactam-resistant ESBL, AmpC or carbapenemase KPC producers could be an important source for further propagation of these pathogens among patients.

The carbapenem susceptibility of various pathogens obtained from various clinical samples of patients, irrespective of their ailment, decreased compared with previous reports of carbapenem resistance in none ICU isolates in 2011 in which IMP susceptibility in Kano was 92.6% (18). The decrease of carbapenem susceptibility in few years in our study is worrisome given the very disturbing fatality ratio of 33.3:9 for patients infected with imipenem-resistant KPC producing *Enterobacter* spp and those infected with imipenem-susceptible strains as reported by Marchaim *et al.* (19). In the case of the two hospitals and many others in the country and the entire region, mortality data regarding use of any antibiotic is lacking or at least inadequate.

E. coli was more resistant to MEM than to IMP while, in *K. pneumoniae*, the reverse was the case. Poor hygienic practices by HCWs are some of the driving forces of carbapenem resistance in our hospitals despite their low or no usage. The resistant genes in these pathogens can be easily transmitted to other patients in the same or other wards if proper care is not taken. The concurrent production of the duo makes the whole scenario more disturbing. However, the remaining 98 isolates of the 191 carbapenem resistant isolates that are not carbapenemase producers were treated as carbapenemase non-producers but were resistant to carbapenems and third generation cephalosporins probably due to production of ESBLs in association with impaired permeability via loss of porins. About 48% of the carbapenemase producers, produced MBLs, the most prevalent type reported in different countries as a source of several nosocomial outbreaks (8).

B-lactamase mediated enterobacterial infections in this study were found in almost all the samples from the patients, which suggest that the β -lactamase producers seem not to be specific

to sites, tissue or organs. This coincides with the view of Nordmann *et al.* 2009 (8). However, high prevalence was observed in patients with urinary tract related problems which may involve use of invasive devices such as catheter. In addition, since previous treatment with carbapenems was ruled out, other risk factors that might be associated with the acquisition of these bacteria in the hospitals are prolonged hospitalisations, ICU stay, and immune-suppression as gathered from their history. On the contrary, other studies attributed carbapenem-resistant *K pneumoniae* infections in patients to previous therapy with carbapenem (20) or increased use of carbapenems in a hospital (21). All attempts to link the hospitalised patients from hospital A to B or vice versa were unsuccessful, making the common source of infection still unclear to us. The possibility of community acquisition is also not ruled out due to the lifestyle of most of the patients. Factors like consumption of foodstuffs or animals treated with antibiotics, or frequent patient contact with healthcare facilities are all possibilities. Only five of the patients had history of previous travel to India, one of the endemic areas in the world and place of birth of NDM-1. Three (3) of the patients only studied in India and had never have access to any hospitals except the initial medical examination which was carried out in school clinics. Only one (1) was treated earlier in India for an unconfirmed illness. In all the patients, the overall history of recent hospitalization was just 5.6%(14/248). Rural dwellers constituted 20.9%(52/248) of the total patients, where they might have been exposed to any of the resistant strains due to multiple antibiotic treatments in local nursing homes due to the unrestricted over-the-counter availability of drugs or self-medication.

Antibiotic treatment options for carbapenem-resistant bacteria are limited and are highly costly where available. However, combination therapy with active drugs such as colistin, tigecycline, and flouroquinolones has been recommended (22). Susceptibility to colistin and tigecycline was still promising irrespective of the type of beta lactamase they produced. While the expanded spectrum of tigecycline has been reported to produced 100% susceptibility *invitro* against many

Enterobacteriaceae producing ESBLs and carbapenemase, treatment failures *invitro* have also been recorded. Similarly, colistin susceptibility and resistance in carbapenemase, ESBL and MBLs producing Enterobacteriaceae has been observed and reported previously. Despite their high activity in treating most of the uro-pathogens *invitro* in this study, tigecycline usage is limited in treating urinary tract infections *in vivo* due to its low urine concentration while colistin-polymyxins member have high neurotoxicity and nephrotoxicity (8). Their successful use *in vivo* was not documented in this study.

There is a need for urgent intervention in our hospitals as the data generated on increasing antibiotic resistance from 2007 to-date suggest. It is envisaged for the next decade that the emergence of NDM, MBL, XDR and KPC in our health care facilities will significantly escalate healthcare costs associated with laboratory detection and salvage combination therapy for infections due to these pathogens.

It is concluded that carbapenemase-ESBL mediated carbapenem resistant pathogens have occurred among the pathogens commonly isolated in the two tertiary hospitals in Nigeria. Co-production of two or more β lactamase occurred among some of the isolates, raising fears of resistance to a multitude of antibiotics in the treatment of clinical infections. There is a sharp decrease in carbapenem susceptibility among the pathogens despite low carbapenem usage in the hospitals, which can make treatment of infections caused by these pathogens difficult. Colistin and tigecycline are still promising while resistance to flouroquinolones, which are mostly used in the hospitals, is increasing.

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