Suan Sunandha Science and Technology Journal

Extended-spectrum beta lactamase and carbapenemase-producing *Klebsialla* spp. in urine and fecal samples obtained from hospitals and communities in Lagos, Nigeria

Tenny Obiageli Egwuatu[1]*, Godwin Ochuko Orkeh [2], Chibuzor Ifeanyichukwu Okeke[3], Amudat Aladesokun [4], Tochukwu Frank Egwuatu[5], Chisom Freda Egwuatu[6]

[1]Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Nigeria.
[2]International Health and Development Consult (IHDC) Los Angeles, USA
[3]Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Nigeria.
[4]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Idi-Araba, Nigeria
[5]Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Akoka, Nigeria
[6]Minerva Schools at KGI, San Francisco, USA.
Corresponding author e-mail: *tenoglad@yahoo.com

Received: *2 July, 2018* / **Revised***: 20 November, 2018* / **Accepted***: 3 December, 2018*

Abstract

The use of beta-lactams has tremendously increased since the discovery of antibiotics. This has led to the emergence of certain resistant genes such as Extended Spectrum Beta-Lactamase (ESBL) which confer resistance to third generation cephalosporins. The objective of this study was to determine the prevalence of Extended Spectrum Beta Lactamase (ESBL) genes, Carbapenem resistance genes (blaKPC, blaOXA and IMP) and outer membrane porins genes (OMP-35, OMP-36, and OMP-36N) from different hospitals and laboratories in Ikeja-Lagos, Nigeria. A total of 177 bacterial isolates were collected between May 2017 to July 2017 from patients with urinary tract infections (UTI) and gastroenteritis. They were identified biochemically and investigated for ESBL and Carbapenemase production using phenotypic Double Disk Synergy Test (DDST) and Modified Hodges' Test respectively. Antibiotics susceptibility profile was also investigated. Multiplex PCR was used to detect the genes responsible for the resistant genes. Out of 177 bacterial isolates, 47 (26.6%) were identified as *Klebsiella* spp and 17 (36.1%) were ESBL positive and then 5 (29.4%) were positive for carbapenem resistance. Multiplex PCR revealed that 3 (27.3%) possessed both $bla_{\text{CTX-M}}$ and bla_{SHV} genes, 6 (54.5%) possessed only $bla_{\text{CTX-M}}$ gene while only 2 (18.2%) possessed only bla_{SHV} gene. Also, 13 (76.5%) possessed only bla_{KPC} gene. However, bla_{TEM} as well as IMP, OXA-48, OMP 35, OMP 36 and OMP 36N genes were not detected. This study revealed that antibiotic resistance is on the rise and preventive measures should be put in place by both government and health care providers to curtail this trend.

Keywords: Extended Spectrum Beta-Lactamase, Urinary Tract Infections, Carbapenemase, *Klebsiella* spp,

1. Introduction

ESBL has generally been defined as transmissible β-lactamases that can be inhibited by clavulanic acid, tazobactam or sulbactam, and which are encoded by genes that can be exchanged between bacteria (1). The most common genetic variant of ESBL is CTX-M (1, 2). Beta-lactamases are commonly classified according to two general schemes: the Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification (3, 4). The Ambler scheme classifies β -lactamases into four classes according to the protein homology of enzymes. Beta-lactamases of class A, C, and D are serine β -lactamase and class B enzymes are metallo-β -lactamases. The Bush–Jacoby–Medeiros functional scheme is based on functional properties of enzymes. The SHV family of β -lactamases are to be derived from Klebsiella spp. The progenitor of the SHV class of enzymes, SHV-1, is universally found in K. pneumonia (5). The SHV-1 β –lactamase

Vol.06, No.1 DOI: 10.14456/ssstj.2019.2

is responsible for up to 20% of the plasmid-mediated ampicillin resistance in K. pneumoniae species (6).

TEM-1, first reported from an E. coli isolate in 1965, has substrate and inhibition profiles similar to those of SHV-1 (7). TEM-1 is capable of hydrolyzing penicillins and first generation cephalosporins but is unable to attack the oxyimino cephalosporin. The first TEM variant with increased activity against extended spectrum cephalosporins was TEM-3 (8,9). TEM-2 the first derivative of TEM-1, had a single amino acid substitution from the original β -lactamase (10). This caused a shift in the isoelectric point from a pH of 5.4–5.6, but it did not change the substrate profile. TEM-3, originally reported in 1989, was the first TEM-type β -lactamase that displayed the ESBL phenotype (8). Klebsiella oxytoca, harboring a plasmid carrying a gene encoding ceftazidime resistance, was first isolated in Liverpool, England, in 1982. The responsible β -lactamase was what is now called TEM-12. Interestingly, the strain came from a neonatal unit which had been stricken by an outbreak of K. oxytoca producing TEM-1. This is a good example of the emergence of ESBLs as a response to the selective pressure induced by extended-spectrum cephalosporins (9).

A new family of b-lactamases that preferentially hydrolyzes cefotaxime has arisen. It has been found in isolates of Salmonella enterica serovar, Typhimurium, E. coli mainly and some other species of Enterobacteriaceae (10,11). These are not very closely related to TEM or SHV b-lactamases (12). In addition to the rapid hydrolysis of cefotaxime, another unique feature of these enzymes is that they are better inhibited by the β -lactamase inhibitor tazobactam than by sulbactam and clavulanate (13). CTX-M β -lactamases are found exclusively in the functional group 2 (14) and thought to originate from chromosomal ESBL genes found in Kluyvera spp., an opportunistic pathogen of the Enterobacteriaceae found in the environment. The first CTX-M proteins were discovered in the late 1980s and today more than 100 variants have been sequenced (15). Based on their amino acid sequences, they can be divided into five groups (CTX-M group 1, 2, 8, 9, and 25) (15). The origin of the CTX-M enzymes is different from that of TEM and SHV ESBLs. While SHV-ESBLs and TEM-ESBLs were generated by amino acid substitutions of their parent enzymes, CTX-M ESBLs were acquired by the horizontal gene transfer from other bacteria using genetic apparatuses such as conjugative plasmid or transposon. The gene sequences encoding CTX-M enzymes show a high similarity to those of b-lactamases of Kluyvera species (16). In addition, the gene sequences adjacent to the CTX-M genes of Enterobacteriaceae are also similar to those surrounding the β -lactamase genes on the chromosomes of Kluyvera species (17). Also, blaKPC is a gene that codes for carbapenemase production and also OMP 35, OMP 36 and OMP 36N. The OXA-type b-lactamases are so named because of their Oxacillin-hydrolyzing abilities. These b-lactamases are characterized by hydrolysis rates for Cloxacillin and Oxacillin greater than 50% as that for benzyl penicillin (4). They predominantly occur in P. aeruginosa but have been detected in many other Gram-negative bacteria. Extended Spectrum Beta- Lactamase have arisen as a result of different factors of which human related-factors play vital roles. They include overuse of antibiotics, inappropriate prescription, lack of infection control measures in healthcare facilities and the use of sub therapeutic doses of antibiotics for the promotion of animal growth in the agricultural sector. The rise of ESBL producing Enterobacteriaceae has mounted a selective pressure in the usage of Carbapenem as a drug of last resort for the treatment of infections caused by ESBL. Also the sporadic reports of carbapenem resistant Gram negative organisms is seriously posing a therapeutic issues in the health centres as previously almost abandoned antibiotics which were toxic to humans are now being recruited for treatment of infectious diseases.

The aim of this study was to determine the prevalence and types of Extended Spectrum Beta Lactamase (ESBL) encoding genes and carbapenem resistant encoding genes existing in the hospitals and laboratories in communities in Ikeja, Lagos environs, Nigeria.

Suan Sunandha Science and Technology Journal

2. Materials and Methods

2.1 Clinical Isolates:

A total of 177 bacterial isolates were collected between May 2017 to July 2017 from different private hospitals and laboratories in Ikeja, Lagos, Nigeria after obtaining permission. All 177 bacterial isolates were collected on nutrient agar and incubated at 37°C for 24 hours. The isolates were from samples of patients suffering from different infections such as urinary tract infections (UTI) and gastroenteritis. All bacterial isolates were identified using standard microbiological and biochemical tests (Cheesbrough, 2006).

2.2 Detection of ESBL Producers

The production of ESBL was detected by the double disk synergy test according to CLSI guidelines using a disk of amoxicillin/clavulanic acid along with ceftazidime, ceftriaxone, cefotaxime and aztreonam/cefuroxime (18). A Mueller Hinton agar plate was inoculated with each isolate as described above and labeled properly. Next, an amoxicillin/clavulanic acid disc was placed in the centre of the plate, and ceftazidime, ceftriaxone, cefotaxime, and aztreonam/cefuroxime discs were placed 25mm (center to center) from the amoxicillin/clavulanic acid disc. After overnight incubation at 37°C, any distortion or increase in the zone of inhibition (i.e., augmentation of inhibition) towards the amoxicillin/clavulanic acid disc was considered a positive result for the ESBL production.

2.3 Detection of Carbapenemase Producers

Carbapenemase production was tested using the Modified Hodge Test according to CLSI guidelines (2012). A 0.5 McFarland suspension of each isolate in 5 mL of sterile saline was prepared. A 1:10 dilution was prepared by adding 0.5mL of the 0.5 McFarland suspension to 4.5mL sterile saline, this was then used as inoculum for an MH agar plate. The plate was dried for 5mins and a disk of meropenem (10µg), was placed in the center of the agar plate. The colonies of the test organism were selected and streaked in a straight line, from the edge of the disk, up to the edge of the plate. After overnight incubation at 37°C, Carbapenemase production was identified by observing a clover leaf-like indentation of *Escherichia coli* 25922 (susceptible strain) growing along the test organism growth streak within the disk diffusion zone.

2.4 Antibiotic Susceptibility Testing

Susceptibility was determined by Kirby Bauer disc diffusion method as described by Clinical and Laboratory Standard Institute (CLSI) (2012). All Isolates were grown on nutrient broth for 18 hours at 37ºC. The suspension was visually adjusted with normal saline to equal that of 0.5 McFarland turbidity standard. The inoculum was swabbed across the entire surface of Muller Hinton agar plate using sterile swab stick and the plate was rotated approximately 60ºC between streaking to ensure an even distribution.

The inoculated plates were left to stand for 5 minutes before the discs were applied. Commercial antibiotics discs (Oxoid) used contained ceftazidime (30µg), cefotaxime gentamicin (10µg), ciprofloxacin (5µg), amoxicillin clavulanic acid (30µg), ceftriazone (30µg) and meropenem (10µg). The plates were incubated within 15 minutes before the application of the discs at 37ºC and subsequently incubate at the same temperature for 18 hours after discs' application. The inhibition zone diameter around the disc was measured and interpreted according to the CLSI guidelines. Isolates were considered as multidrug resistance when it showed resistance to ≥3 antimicrobial agents.

2.5 DNA Extraction (By Boiling)

Cells were harvested into 1000µl of sterile water. They were vortexed until it was completely dissolved. It was centrifuged for 5 minutes at 10,000rpm. The supernatant was then discarded and 1000µl of sterile water was added. It was vortexed and centrifuged, the supernatant was decanted and then 200µl of sterile water was then added and vortexed till it was thoroughly mixed. It was heated for 10 minutes at 100°C and cool immediately on ice and vortexed. It was centrifuged for 5 minutes at

Vol.06, No.1 DOI: 10.14456/ssstj.2019.2

Suan Sunandha Science and Technology Journal

10,000 rpm and the supernatant was transferred into another Eppendorf tube and the pellet was discarded.

2.6 Multiplex PCR amplification of ESBL and Carbapenemase genes

A multiplex PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 20 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer (Solis Biodyne), 2.0mM MgCl2, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 20pmol of each primer (Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), 5µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture.

Thermal cycling was conducted in a Pielter Thermal Cycler PTC 100 (MJ Research Series) for an initial denaturation of 95°C for 5 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 59°C and 62°C and then 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by Ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard. Table 1 shows the list of primers used.

Table 1. list of primers for Multiplex PCR amplification of ESBL and Carbapenemase genes

Primers	Product size (bp)	Reference
SHV-F ATGCGTTATATTCGCCTGT		
SHV-R TGCTTTGTTATTCGGGCCAA	753	Sharma *et al*., 2010
CTX-M-F TTTGCGATGTGCAGTACCAGTAA		
CTX-M-R CGATATCGTTGGTGGTGCCATA	543	Edelstein *et al*., 2003
TEM-F AAACGCTGGTGAAAGTA		
TEM-R AGCGATCTGTCTAT	822	Nasehi *et al*., 2010
OXA-48 F TGTTTTTGGTGGCATCGAT		
OXA-48 R GTAAMRATGCTTGGTTCGC	177	Monteiro *et al*., 2012
IMP-F GAGTGGCTTAATTCTCRATC		
IMP-R ACCTAYCCAARATCCAATATRTAAC	120	Mendes *et al*., 2007
KPC-F GATACCACATTCCGTCTGG		
KPC-R GCAGGTTCCGGTTTTGTCTC	246	Anne and Karen, 2007

Data analysis: Data was analyzed using simple percentage.

3. Results

Out of 177 bacterial isolates, 47 bacterial isolates were biochemically identified as spp and 17 out of the 47 bacterial isolates were ESBL producers. The biochemical patterns of the 17 ESBL producers are shown in table 2.

Vol.06, No.1 DOI: 10.14456/ssstj.2019.2

Suan Sunandha Science and Technology Journal

Table 2. Biochemical patterns of the ESBL producers

s/n	Butt	Slope	H_2S	Gas	Indole Test	Citrate Test	Lactose	Glucose	Organism
1	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
2	Y	Y	-	+	-	+	+	+	*Klebsiella pneumoniae*
3	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
4	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
5	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
6	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
7	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
8	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
9	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
10	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
11	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
12	Y	Y	-	-	-	+	+	+	*Klebsiella ozaenae*
13	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
14	Y	Y	-	-	-	+	+	+	*Klebsiella ozaenae*
15	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
16	Y	Y	-	-	+	+	+	+	*Klebsiella oxytoca*
17	Y	Y	-	+	-	+	+	+	*Klebsiella pneumoniae*

KEY: Y=Yellow

Each of the organisms showed varying zones of inhibition to the various antibiotics used as shown in Table 3.

Table 3. Representation of organisms and their zones of inhibition (mm) (ESBL)

S/N	Specimen	Age	Gender	CAZ	CRO	CTX	AMC	ATM/CXM	MEN	CIP	CN	Organism
1	Urine	40	F	15	17	15	25	17	23	21	15	*K. oxytoca*
2	Stool	5	F	18	29	10	13	20	29	23	18	*K. pneumoniae*
3	Urine	71	F	23	34	24	18	17	33	25	20	*K. oxytoca*
4	Urine	33	M	17	22	20	15	30	14	16	16	*K. oxytoca*
5	Stool	40	M	13	13	15	10	24	30	15	22	*K. oxytoca*
6	Stool	49	F	20	26	30	10	11	10	20	4	*K. oxytoca*
7	Stool	36	F	15	8	8	12	0	15	23	23	*K. oxytoca*
8	Urine	31	F	10	17	29	28	13	25	27	12	*K. oxytoca*
9	Urine	70	F	10	9	9	10	0	24	10	13	*K. oxytoca*
10	Urine	65	F	11	20	25	10	24	0	25	14	*K. oxytoca*
11	Stool	75	M	12	28	27	17	10	28	17	17	*K. oxytoca*
12	Urine	45	F	21	26	10	19	23	32	24	19	*K. ozaenae*
13	Stool	30	F	15	20	23	22	20	35	27	21	*K. oxytoca*
14	Urine	60	M	20	25	28	10	10	37	15	10	*K. ozaenae*
15	Urine	77	M	26	14	24	23	10	19	10	13	*K. oxytoca*
16	Urine	38	M	16	24	14	21	29	26	30	0	*K. oxytoca*
17	Urine	70	M	27	12	26	27	3	22	0	0	*K. pneumoniae*

Key: CAZ (Ceftazidime;R,I,S=≤17,18-20,≥21), CRO (Ceftriazone;R,I,S=≤22,23-25,≥26), CTX (Cefotaxime;R,I,S=≤22,23-25,≥26), AMC (Amoxicillin-Clavulanic acid;R,I,S=≤13,14-17,≥18), ATM/ CXM (Aztreonam/ Cefuroxime;R,I,S=≤17,18-20,≥21), MEM (Meropenem;R,I,S=≤19,20-22,≥23), CIP (Ciprofloxacin) ;R,I,S=≤15,16-20,≥21, CN (Gentamicin;R,I,S=≤10,13-14,≥15). R=Resistant;I=Intermediate;S=Sensitive.

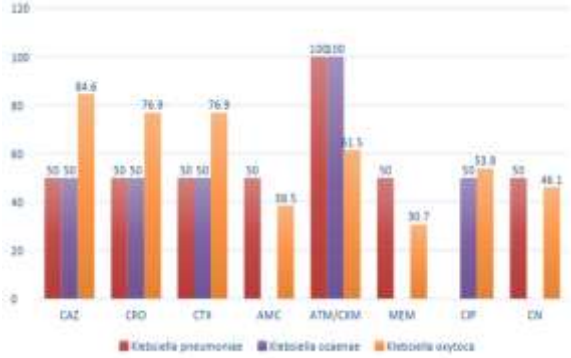
In the case of carbapenemase production, 5 (29.4%) out of 17 (36.1%) isolates were positive. They were 1 (5.9%) *Klebsiella pneumoniae* and 4 (23.5%) *Klebsiella oxytoca*. The antibiotic susceptibility profile of the organisms showed varying degrees of Multidrug Resistance among the isolates as shown in Figure 1. *Klebsiella pneumoniae* showed complete resistance to aztreonam/ cefuroxime (100%) and it was absolutely susceptible to ciprofloxacin (0%). *Klebsiella pneumoniae* was resistant to ceftazidime (50%), ceftriaxone (50%), cefotaxime (50%), meropenem (50%), amoxicillin-clavulanic acid (50%) and gentamicin (50%). *Klebsiella ozaenae* was absolutely resistant to aztreonam/cefuroxime (100%) and absolutely susceptible to amoxicillin-clavulanic acid (0%), meropenem (0%) and gentamicin (0%). *Klebsiella ozaenae* was also resistant to ceftriaxone (50%), ceftazidime (50%), cefotaxime (50%) and ciprofloxacin (50%). *Klebsiella oxytoca* showed high level of resistance to ceftazidime (84.6%) and it was susceptible to meropenem (30.7%). It was also resistant to ceftriaxone (76.9%), cefotaxime (76.9%), aztreonam/cefuroxime (61.5%), amoxicillin-clavulanate (38.5%), ciprofloxacin (53.8%) and gentamicin (46.1%).

Vol.06, No.1 DOI: 10.14456/ssstj.2019.2

This is a PDF file of an unedited manuscript that has been accepted for publication.

Suan Sunandha Science and Technology Journal

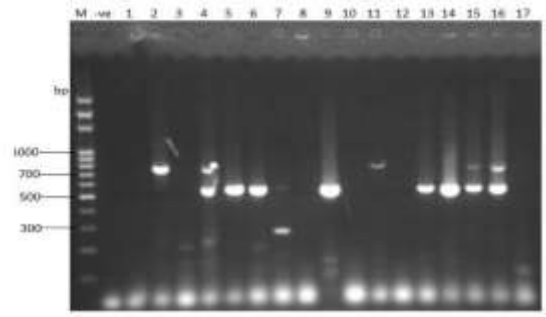
Figure 1. Antibiotic susceptibility profile of the ESBL producing *Klebsiella* spp.



Key: CAZ = Ceftazidime, CRO = Ceftriaxone, CTX = Cefotaxime, ATM/CXM = Aztreonam/ Cefuroxime, MEM = Meropenem, CIP = Ciprofloxacin, CN = Gentamicin.

Genotypic characterization of the isolates was performed by Multiplex PCR and specific primers such as: SHV, TEM and CTX-M were used to detect the genes responsible for ESBL resistance. Figure 1 shows CTX-M gene distribution among ESBL producing isolates. Multiple genes were detected in 3 (27.3%) of the isolates which possessed both $bla_{\text{CTX-M}}$ and bla_{SHV}. However, 6 (54.5%) possessed only $bla_{\text{CTX-M}}$. Two (18.2%) possessed only bla_{SHV} consisting of 1 (50%) *K. pneumonia* and 1 (50%) *K. oxytoca* as shown in Table 2. However, no TEM gene was displayed by any of the isolates in this study.

Figure 2. Gene Distribution among ESBL producing isolates.



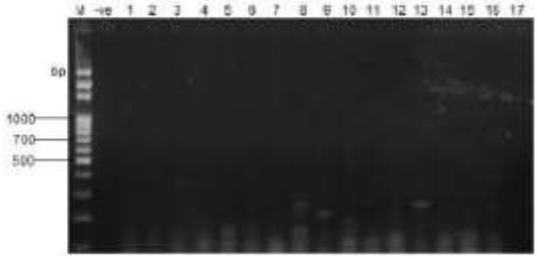
Lane M: DNA Marker (100bp), Lane –VE: Negative Control, Lane 1-17: Test organisms, Lane 5, 6, 7, 9, 13, 14: CTX-M positive Lane 2 and 11: SHV positive, Lane 4, 15 and 16: CTX-M and SHV positive.

Table 2. Genotypic characterization of ESBL and Carbapenemase genes

S/N	Organism	ESBL genes	KPC & OMP
1	*Klebsiella oxytoca*	-VE	-VE
2	*Klebsiella pneumoniae*	-VE	+VE (KPC)
3	*Klebsiella oxytoca*	-VE	+VE (KPC)
4	*Klebsiella oxytoca*	-VE	+VE (KPC)
5	*Klebsiella oxytoca*	-VE	+VE (KPC)
6	*Klebsiella oxytoca*	-VE	+VE (KPC)
7	*Klebsiella oxytoca*	-VE	-VE
8	*Klebsiella oxytoca*	-VE	+VE (KPC)
9	*Klebsiella oxytoca*	-VE	+VE (KPC)
10	*Klebsiella oxytoca*	-VE	-VE
11	*Klebsiella oxytoca*	-VE	+VE (KPC)
12	*Klebsiella ozaenae*	-VE	+VE (KPC)
13	*Klebsiella oxytoca*	-VE	+VE (KPC)
14	*Klebsiella ozaenae*	-VE	+VE (KPC)
15	*Klebsiella oxytoca*	-VE	+VE (KPC)
16	*Klebsiella oxytoca*	-VE	+VE (KPC)
17	*Klebsiella pneumoniae*	-VE	-VE

Also using Multiplex PCR the genes responsible for Carbapenem resistance was also determined using primers such as OXA- 48 and IMP. However, no gene was detected for these primers (Figure 3.0. Plate 2).

Figure 3. Plate 2.0: Gene distribution among Carbapenemase producing isolates

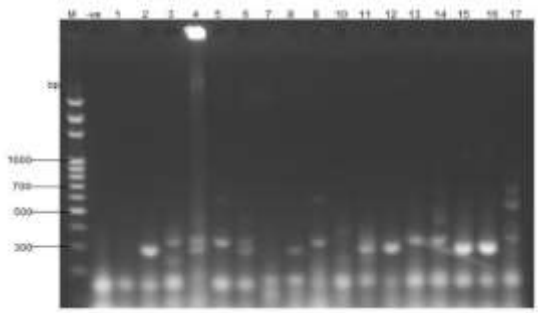


Lane M: Marker (100bp), Lane –VE: Negative control, Lane 1-17: Test organisms (All negative)

Additionally, KPC primers were used to determine the other causes of Carbapenem resistance. Plate 3 revealed that out of 17 (36.1%) isolates, only 13 (76.5%) exhibited the KPC gene which were 1 (7.69%) *Klebsiella pneumoniae*, 2 (15.4%) *Klebsiella ozaenae* and 10 (76.9%) *Klebsiella oxytoca*.

Suan Sunandha Science and Technology Journal

Figure 4. Gene distribution among KPC (*Klebsiella pneumoniae* carbapenemase) producing isolates



Lane M: Marker (100bp), Lane –VE: Negative control, Lane 1-17: Test organism, Lane 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16: KPC +ve

4. Discussion

The types of Extended Spectrum Beta-Lactamase (ESBL) in Ikeja, Lagos community is now recognized. ESBL are plasmid mediated bacterial enzymes typically found in enteric Gram negative important pathogens involved in nosocomial infections. They are able to hydrolyse a wide range of beta-lactam antibiotics including third and fourth generation cephalosporins (Rahal, 2000). The widespread use of broad spectrum beta- lactam antibiotics has led to a marked increase in the incidence of ESBL producing Gram negative microbe especially *Klebsiella* spp. (Shakil e*t al*., 2010). This study was carried out to determine the presence and prevalence of these enzymes among isolates obtained from Ikeja, Lagos community. From the result obtained in this study, greater part of the organisms were least resistant to meropenem. *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Klebsiella ozaenae* showed 5.9%, 23.5%, and 0% resistance to meropenem respectively. This result obtained from *K. pneumoniae* is consistent with the findings of Abdullah and colleagues (Abdullah *et al*., 2015) which had a resistance of 4% while the result obtained from *K. oxytoca* is similar to the report by Shakti and associates (Shakti *et al*., 2014) which had a prevalence of 23% against meropenem. The reason of low rate of resistance to meropenem could be that the drug has not been used extensively in this community. This study also revealed that most of the isolates were resistant to ceftazidime and this agrees with the study of Rabindra and colleagues (Rabindra and Sibanarayan, 2014) which reported ceftazidime resistance rates of 59% in *Klebsiella pneumoniae*, 48% in *Klebsiella ozaenae* and 76% in *Klebsiella oxytoca* from a community in India. In previous years, no ceftazidime resistance was detected in isolates of *K. pneumoniae, K. ozaenae* and *K. oxytoca* isolated from a Chicago hospital, however, in recent times, 27% of these isolates were resistant to ceftazidime (Shakti *et al*., 2014). The result from this study showed high carriage of CTX-M gene (54.5%) in *K. pneumoniae, K. oxytoca* and *K. ozaenae*. This is contrary to the findings of Maninder and Aruna (Maninder and Aruna, 2013) which had a prevalence of 25% of CTX-M among ESBL positive *Klebsiella*.spp. From this study, CTX-M and SHV genes were observed in 27.3% of all the isolates obtained. This is contrary to that reported by Maninder and Aruna (Maninder and Aruna, 2013) that showed a prevalence of 12.5% among their isolates. This shows the continued increase and high dissemination of genes responsible for resistance in the community. Also, this study revealed a prevalence of 76.5% of *bla*KPC gene in *K. pneumoniae, K. ozaenae, Klebsiella oxytoca* and this contradicts the findings of Masoume and colleagues (Masoume, 2015) that recorded no presence of *bla*KPC in any of their isolates. Interestingly, this study recorded no presence of the TEM gene in any of the isolates.

5. Conclusion

The results obtained from this study shows that ESBL resistance is on the rise not only among *Klebsiella* species. Their genes which are plasmid borne are easily transferred through horizontal gene transfer and could pose serious threats to global health. Therefore, the identification and treatment of patients infected with these organisms is of prime necessity.

6. Acknowledgement

The authors wish to thank the hospitals and laboratories from where the isolates were collected.

7. References

Abdullah, E., Syeda, S., Waseema, A. & Fatima, S. (2015). Identification of Klebsiella pneumoniae and Klebsiella oxytoca in urine specimens from a laboratory in Karachi. *Microbiology Research International*, *3*(3), 37 - 40.

Ambler, R. P. (1980). The structure of b-lactamases. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 289, 321–331.

Anne, M. Q and Karen, B. (*2007).* Carbapenemases: the versatile beta-lactamases. *Clinical Microbiology Review*, *20*(3), 440-458.

Bharat, P. M., Janak, K., Rajan, D. K., Shyam, M. K., Prem, K. K., & Tuladhar, N. R. (2006). Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing Salmonella enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. *International Journal of Infectious Diseases*, *10*, 434-438.

Bonnet, R. (2004). Growing group of Extended-Spectrum β-lactamases: the CTX-M enzymes. Antimicrobial Agents and Chemotherapy, *48*, 1-14.

Bora, A., Hazarika, N. K., Shukla, S. K., Prasad, K. N., Sharma, J. B., Ahmed, G. (2014). Prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in clinical isolates of Escherichia coli and *Klebsiella pneumoniae* from Northeast India. *Journal of Pathology and Microbiology, 57*(2), 249-254.

Bradford, P. (2001). Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical Microbiology Revolution, *14*(4): 933-51.

Bush, K. and Jacoby, G. (2010). An updated functional classification of b-lactamases. Antimicrobial Agents and Chemotherapy, *54* (3), 969–976.

Bush, K., Jacoby, G. A., Medeiros, A. A. (1995). A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*, 39 (6): 1211–33.

Clinical and Laboratory Standards Institute. (2012). Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. CLSI document M100-S22 Clinical and Laboratory Standards Institute, Wayne, PA.

Datta, N., & Kontomichalou, P. (1965). Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. *Nature International Journal*, *208*, 239–241.

Garrec, H., Drieux-Rouzet, L., Golmard, J., Jarlier, V. & Robert, J. (2011). Comparison of nine phenotypic methods for detection of extended-spectrum beta-lactamase production by Enterobacteriaceae. *Journal of Clinical Microbiology*, *49*, 1048 - 1057.

Gazouli, M., Tzelepi, E., Markogiannakis, A., Legakis, N. J., & Tzouvelekis, L. S. (1998). Two novel plasmid-mediated cefotaxime hydrolyzing b-lactamases (CTX-M-5 and CTX-M-6) from Salmonella typhimurium. Oxford University Press, University of Oxford. *FEMS Microbiology Letters*, *165*, 289–293.

Livermore, D. M. (1995). Beta-Lactamases in laboratory and clinical resistance. *Clinical Microbiology Revolution, 8*(4), p. 557-84.

Maninder, K., & Aruna, A. (2013). Occurrence of the CTX-M, SHV and the TEM genes among the Extended Spectrum Beta- Lactamase producing isolates of Enterobacteriaceae in a Tertiary Care in Hospital of North India. *Journal of Clinical and Diagnostic Research*, *7*(4), 642-646.

Masoume, B., Abazar, P., Shiva, M., Malihe, T. & Gholamreza, I. (2015). Detection of the Klebsiella pneumoniae Carbapenemase (KPC) in K. pneumoniae Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. *Iranian Journal of Pathology*, *10* (3): 199-205.

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology Reviews, 18*(4), 657-86.

Paterson, D. L. & Bonomo, R. A. (2005). Extended-spectrum beta lactamases: a clinical update. *Clinical Microbiology Revolution*, 18, 657-686.

Pitout, J., Hossain, A. & Hanson, N. (2004). Phenotypic and molecular detection of CTX-M-beta-lactamases produced by Escherichia coli and Klebsiella spp. *Journal of Clinical Microbiology*, *42*, 5715 - 5721.

Rabindra, N. & Sibanarayan, R. (2014). Monitoring invitro Antibacterial Efficacy of twenty six Indian Spices against Multidrug Resistant Urinary Tract Infecting Bacteria. *Integrative Medicine Research*, *3*(3), 133-141.

Rahal, J. J. (2000). Extended Spectrum Beta-Lactamase: How big is the problem? Clinical Microbiology and Infection, *6*(2), 2-6.

Shakil, S., Ali, S., Akram, S. & Khan, A. (2010). Risk Factors for Extended-Spectrum Beta-Lactamase Producing Escherichia coli and Klebsiella pneumoniae Acquisition in a Neonatal Intensive Care Unit. *Journal of Tropical Pediatrics*, *56* pg 2.

Shakti, R., Debasmita, D., Mahesh, C., Sahu, R. & Padhy, N. (2014). Surveillance of ESBL producing Multidrug Resistant Escherichia coli in a teaching hospital in India. *Asian Pacific Journal of Tropical Diseases*, *4* (2), 140 - 149.

Soughakoff, W., Goussard, S., & Courvalin, P. (1998). TEM-3 beta lactamases which hydrolyzes broad-spectrum cephalosporins is derived from the TEM-2 penicillinases by two amino acid substitutions. *FEMS Microbiology Letters*, *56*, 343–348.

Tzouvelekis, L.S., & Bonomo, R.A. (1999). SHV-type b-lactamases. *Current Pharmacological Descriptions*, *5*, 847–864.

Tzouvelekis, L.S., Tzelepi, E., Tassios, P.T., & Legakis, N.J., (2000) CTX-M type sbeta-lactamases: an emerging group of Extended Spectrum enzymes. *International Journal of Antimicrobial Agents*, *14*, 137–143.

Walsh, C. (2003). Antibiotics: actions, origins, resistance. The American Society for Microbiology (ASM Press). Washington, DC.