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Carbapenemase Genes in Gram-Negative Bacteria: Detection and Implications in Clinical Isolates from Patient Samples

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ABSTRACT

Background: Antimicrobial resistance is a great scourge on human health, exacerbated by the acquisition of resistance to carbapenems, the last resort treatment for infections caused by extended-spectrum beta-lactamase (ESBL) producing bacteria. **Objectives**: This cross-sectional study evaluated the prevalence of genes encoding ESBL and carbapenemase production in Gram-negative bacteria from clinical samples in Lagos state, Nigeria.**Method:** A total of 107 bacteria cultures were obtained from hospitals and clinical diagnostic laboratories. Isolate identification, antibiotics susceptibility testing, and phenotypic detection of ESBL production were done using standardized procedures. Multiplex polymerase chain reaction (PCR) was performed on ESBL-producing isolates to detect *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{OXA}$.**Result:** Among the 107 cultures, 83 isolates were obtained with 55 being Gram-negative. *Escherichia coli* (22; 40%) was the most prevalent species followed by *Klebsiella pneumoniae* (7; 13%). Multidrug resistance (MDR) was observed in 34 (62%) of the isolated bacteria with 14 (26%) not susceptible to meropenem. ESBL production was detected in 42 (76%) of the isolates of which 23 (55%) strains harboured one or more of the genes *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$. The carbapenemase genes *bla*$_{KPC}$ and/or *bla*$_{VIM}$ were observed in 11 (26%) isolates. No isolated bacteria were found to harbour *bla*$_{IMP}$ and/or *bla*$_{OXA}$.**Conclusion:** Genes encoding ESBL and carbapenemase production were detected in samples of human origin in Lagos state. Novel antibiotics and/or alternative therapy are necessary for infection therapy in the near future.

INTRODUCTION

Gram-negative bacteria (GNB) pathogens have been responsible for a wide range of infections including a few pandemics. Typhus, caused by *Rickettsia*, was a probable cause of the 430BC plague of Athens which killed 25% of Athens's population (Littman, 2009).

Yersinia pestis, has changed the path of our civilization through three pandemics; the Justinian plague of 541–750 A.D (Meier, 2016; Yang, 2018), the Bubonic plague or black death 1334 A.D (Eastman, 2009), and the third plague pandemic in 1855 (Eastman, 2009; Bramanti *et al*., 2019). Cholera by *Vibrio cholerae* was responsible for seven pandemics from 1817 to the 1960s (Chan *et al*., 2013). GNB has been majorly responsible for community and hospital-acquired infections ranging from urinary tract infections (UTIs), respiratory tract infections (RTIs), bloodstream infections, gastrointestinal infections, as well as wound and surgical site infections among others. Acquisition of antibiotics resistance by GNB poses a serious challenge to the effective prevention and treatment of a wide range of infections and has greatly increased particularly in the last two decades (O'Neill, 2016; Ponce-de-Leon *et al*., 2018). While there are yet no available studies outlining the full burden of antimicrobial resistance on the health and economy of Nigerians, however, Nigeria-specific reports demonstrate that antimicrobial resistance rates of many pathogens are untenably high in Nigeria (FMARDEH, 2017). Multidrug resistance has been defined by the European Centre for Disease Prevention and Control (ECDC) and Centers for Disease Control and Prevention (CDC) as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012). *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been identified as the major cause of multidrug-resistant (MDR) bacterial infections, increasingly posing challenges, particularly in intensive care units (Miller, 2016; Agyepong *et al*., 2018; Mamishi *et al*., 2019).

ESBL-producing GNB (EpGNB) is a critical concern as β-lactamases can inhibit the action of β-lactams, the most frequently used antibiotics worldwide, including those with extended-spectrum such as the oxyimino-cephalosporins and the monobactam aztreonam (Rahman *et al*., 2018). Genes encoding ESBL production are often on large plasmids cohabited by other genes encoding antibiotics resistance, thus giving rise to MDR bacteria also resistant to aminoglycosides, trimethoprim, sulphonamides, tetracyclines, chloramphenicol and fluoroquinolones (Rawat and Nair, 2010; Vasaikar *et al*., 2017). *Klebsiella* is an important reservoir for the dissemination of antibiotic-resistance genes into other Enterobacteriaceae and even more distantly related bacteria, making the expression of ESBL by the genus a critical concern (Karumidze *et al*., 2013; Ponce-de-Leon *et al*., 2018). MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been found to be resistant to nearly all classes of antibiotics including aminoglycosides, cephalosporins, fluoroquinolones and carbapenems (Ventola, 2015; Agyepong *et al*., 2018).

Carbapenems, the widest spectrum antibiotics among β-lactams, are resistant to hydrolysis by most β-lactamases including ESBLs, and in some cases can act as inhibitors of β-lactamases, and so, are drugs of choice for treatment of infections caused by multidrug-resistant GNB (Lima *et al*., 2020). Carbapenem resistance is a global health concern threatening the efficacy of available antimicrobial therapies and reducing patient treatment options. All the pathogens on the World Health Organization's list of critical bacteria for which new antibiotics are urgently required are carbapenem-resistant GNB but there are no antibiotics candidates sufficiently effective against these bacteria (WHO, 2021). The most frequent mechanism of carbapenem resistance is the production of carbapenemase enzymes, which has been reported in Gram-negative pathogens particularly *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* from countries worldwide, including Nigeria (Obasi *et al*., 2019; Lima *et al*., 2020). The magnitude of resistance is ever-increasing each year, presenting significant challenges in the effective control of infections as a specific therapeutic strategy

is dependent on the type of ESBL and carbapenemase (Sawa *et al*., 2020).

The objective of this study is to determine the prevalence of genes encoding ESBL and carbapenemase production in pathogenic Gram-negative bacteria within Lagos state.

MATERIALS AND METHODS

Ethical Approval:

This project was approved by Lagos University Teaching Hospital Health Research Ethics Committee (Approval number ADM/DCST/HREC/APP/5306). As no human sample or data was collected, no patient consent was sought. Patients or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Study Design:

This cross-sectional study was conducted over a two-month period in private hospitals and diagnostic laboratories in Lagos state, southwest Nigeria including Lifegate Specialist Hospital, Solid Rock Hospital, DFO Hospital, and Kowa Laboratories, as well as the National Institute of Medical Research over a two-month period. All non-fastidious aerobic bacteria samples obtained from patients of both genders and all ages were included in this study. Only non-duplicate samples were collected.

Sample Collection:

Bacterial 24-hour growth obtained from clinical samples was collected in Brain Heart Infusion broth (Oxoid, Basingstoke, Hampshire, United Kingdom), and incubated at 37^{o}C within 3 hours of collection. A total of 107 bacterial samples were collected from urine (55), throat swabs (3), wound swabs (3), high vaginal swabs (15), semen (6), stool (8) and sputum (17). Following incubation for 24 hours, the samples were inoculated on MacConkey agar (Oxoid, Basingstoke, Hampshire, United Kingdom) at 37^{o}C for 24 hours.

Bacterial Identification:

Isolates were Gram-stained and Gram-negative bacilli were further identified using the oxidase test and the API 20E Gram-negative bacteria identification kit (Biomérieux, Durham, USA).

Antibiotics Susceptibility:

The antibiotic susceptibility profile of each isolate was determined using Kirby Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (2020). Commercial antibiotics discs (Liofilchem, Roseto degli Abruzzi, Italy) including ceftazidime (30µg), gentamicin (10µg), amoxicillin-clavulanic acid (20/10µg), cefepime (30µg), ceftriaxone (30µg), meropenem (10µg), ciprofloxacin (5µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), cefotaxime (30µg), and doxycycline (30µg) were used.

Pure colonies were homogenized in 1ml of sterile water, standardized using 0.5 MacFarland turbidity standard and inoculated on Muller Hinton agar (MHA) plates after which antibiotics discs were placed and incubated for 24 hours at 37^{o}C. The diameter of growth inhibition around each disc was measured and interpreted as susceptible (S), intermediate (I), or resistant (R), according to CLSI standards. Isolates with intermediate sensitivity were regarded as resistant.

ESBL production was tested by placing cefepime and ceftriaxone discs 20mm apart edge to edge from amoxicillin-clavulanic acid disc on inoculated MHA plates and incubated for 24 hours at 37^{o}C. An enhanced zone of inhibition between any one of the cephalosporin discs and the amoxicillin-clavulanic acid disc was interpreted as presumptive evidence for the presence of an ESBL.

Identification of Resistance Genes:

GNB which tested positive for ESBL production or were not susceptible to meropenem were tested for the presence of resistance genes using polymerase chain reaction with specific oligonucleotide primers (Table 1). Bacterial DNA was extracted using the boiling method and PCR reaction was carried out using Solis Biodyne 5x HOT FIREPol Blend Master mix. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes using

100bp DNA ladder as DNA molecular weight standard. After electrophoresis, DNA bands were visualized by ethidium bromide staining.

Table 1. Primers used for the detection of ESBL and carbapenemase genes.

Target gene	Primer name	PRIMER SEQUENCE	Amplicon size (bp)	Annealing Temperature	REFERENCES
KPC	KPC-F	TGTTGCTGAAGGAGTTGGGC	340	56°C	Mlynarcik *et al*., 2016
	KPC-R	ACGACGGCATAGTCATTTGC			Mlynarcik *et al*., 2016
VIM	VIM-F	CGCGGAGATTGARAAGCAAA	247	58°C	Mlynarcik *et al*., 2016
	VIM-R	CGCAGCACCRGGATAGAARA			Mlynarcik *et al*., 2016
IMP	IMP-F	GAGTGGCTTAATTCTCRATC	183	56°C	Mlynarcik *et al*., 2016
	IMP-R	CCAAACYACTASGTTATCT			Mlynarcik *et al*., 2016
OXA	OXA-F	AACGGGCGAACCAAGCATTTT	585	58°C	Mlynarcik *et al*., 2016
	OXA-R	TGAGCACTTCTTTTGTGATGGCT			Mlynarcik *et al*., 2016
TEM	TEM-R	AGCGATCTGTCTAT	822	56°C	Al-Mayahieg, 2013
	TEM-F	AAACGCTGGTGAAAGTA			Al-Mayahieg, 2013
SHV	SHV-R	TGCTTTGTTATTCGGGCCAA	753	56°C	Al-Mayahieg, 2013
	SHV-F	ATGCGTTATATTCGCCTGT			Al-Mayahieg, 2013
CTX-M	CTX-M-R	CGATATCGTTGGTGGTGCCATA	590	56°C	Hackman *et al*., 2014
	CTX-M-F	TTTGCGATGTGCAGTACCAGTAA			Hackman *et al*., 2014

Statistical Analysis:

Data analysis was carried out using JMP^{R} version 16 by SAS. Chi-square and effect likelihood ratio tests were used for multivariance analysis of the data. P value of less than 0.05 ($p < 0.05$) was considered significant for the analysis.

RESULTS

Bacterial Distribution Among Samples:

A total of 55 GNB isolates were obtained belonging to 14 different species. Urine, with the highest number of isolates, was the most diverse, containing 10 different species while semen was the least with no GNB isolate obtained (Table 2). *Escherichia coli* (22; 40%) was the most prevalent bacteria followed by *Klebsiella pneumoniae* (7; 13%) and *Pantoea* spp. (6; 11%). No significant association existed between the type of sample collected and the species of bacteria isolated (p-value = 0.4, degree of freedom (df) = 52).

Antibiotic Resistance Pattern:

The highest antibiotic resistance observed was to the cephalosporins, fluoroquinolone, and trimethoprim-sulfamethoxazole (Fig. 1). Ceftriaxone had the highest number of resistant bacteria (52; 95%), followed by ciprofloxacin and cefotaxime (51; 93%). Lower resistance rates were recorded for doxycycline (26; 47%), amoxicillin-clavulanic acid (28; 51%) and gentamicin (31; 56%), with carbapenem resistance observed in 14 (25%) of the isolates. *K. oxytoca* was observed to be the most resistant species, with 100% resistance to third-generation cephalosporins and ciprofloxacin, and 75% resistance to cefepime and meropenem (Table 3). Isolates from stool were the most resistant while throat swabs and sputum had the highest prevalence of carbapenem-resistant GNB. ESBL production and was observed in 42 (76%) of the GNB isolates, and MDR in 49 (89%) of the isolates (Table 4).

Resistance Genes Detection:

Genes encoding ESBL and carbapenemase production were detected using PCR and agar gel electrophoresis (Fig. 2). At least one gene encoding ESBL production was detected in 24 (57%) of the 42 isolates tested. *Bla*$_{\text{CTX-M}}$ was the most

predominant gene encoding ESBL production followed by *bla*$_{TEM}$ (Table 5). All isolates carrying *bla*$_{TEM}$ cohabited *bla*$_{CTX-M}$. *Bla*$_{SHV}$ was the least prevalent gene, present in only two isolates. Carbapenem hydrolysis genes were detected in 11 (26%) of the tested isolates. *Bla*$_{VIM}$ (7; 17) and *bla*$_{KPC}$ (6; 14%) were the only carbapenemase genes observed in the isolates, with no *bla*$_{OXA}$ or *bla*$_{IMP}$ genes observed. Isolates carrying *bla*$_{KPC}$ also harboured at least two ESBL genes, most commonly *bla*$_{CTX-M}$ and *bla*$_{TEM,}$ while all *bla*$_{VIM}$ observed were in cohabitation with other genes, including those encoding ESBL and carbapenemase, except in a *Klebsiella oxytoca* isolate which had only *bla*$_{VIM..}$

Table 2. Frequency of Gram-negative Bacteria Isolated from Clinical Samples.

Organisms	Number of Isolates					
	Hvs	Stool	Urine	Sputum	Throat Swab	Total n (%)
Pantoea spp.	2	-	4	-	-	6 (11)
E. coli	3	4	13	-	2	22 (40)
P. fluorescens / putida	-	-	-	1	1	2 (3.6)
P. aeruginosa	-	-	1	2	-	3 (5.5)
P. luteola	-	-	1	1	-	2 (3.6)
K. pneumoniae	2	-	3	2	-	7 (12.7)
K. oxytoca	-	2	2	-	-	4 (7.3)
Aeromonas spp.	1	-	-	2	-	3 (5.5)
S. *plymuthica*	-	-	1	-	-	1 (1.8)
H. alvei	1	-	-	-	-	1 (1.8)
R. aquatilis	-	-	-	1	-	1 (1.8)
Acinetobacter spp.	-	-	1	-	-	1 (1.8)
Pasteurella spp.	-	-	1	-	-	1 (1.8)
P. penneri	-	-	1	-	-	1 (1.8)
Total n. (%)	9 (6.4)	6 (10.9)	28 (50.9)	9 (16.4)	3 (5.4)	55 (100)

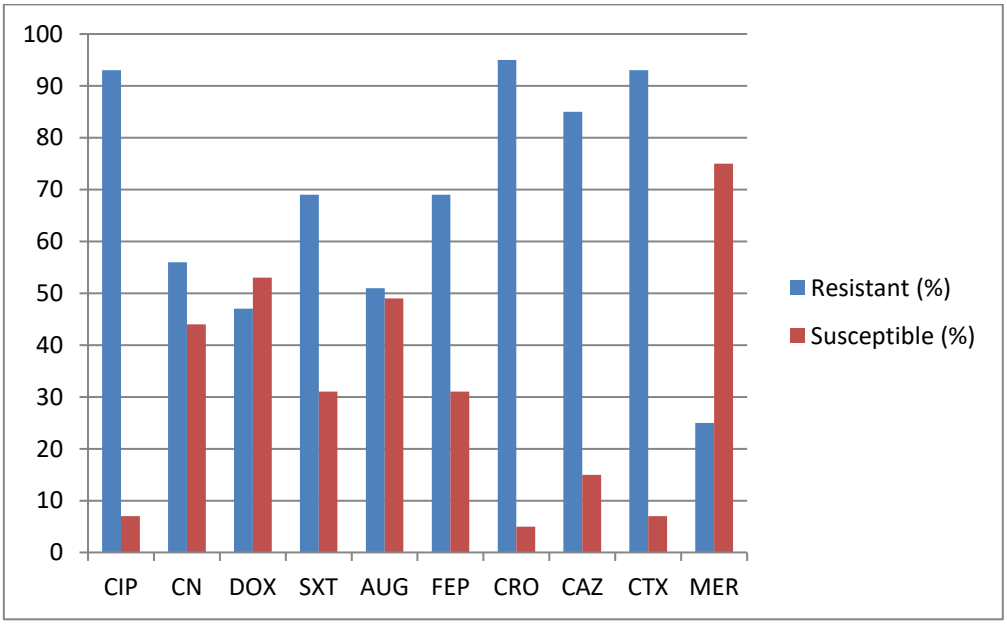

Fig. 1. Antibiotics Sensitivity of Isolated Bacteria. CIP – Ciprofloxacin, CN – Gentamicin, DOX – Doxycycline, SXT - Trimethoprim- sulfamethoxazole, AUG – Amoxicillin-clavulanic acid, FEP – Cefepime, CRO – Ceftriaxone, CAZ – Ceftazidime, CTX – Cefotaxime, MER – Meropenem.

Table 3: Overview of antimicrobial susceptibility testing to clinically important antibiotics.

	No. of Isolates	CIP	CN	DOX	SXT	AUG	FEP	CRO	CAZ	CTX	MER
Total Isolates	55	51 (92.7)	31 (56.4)	26 (47.3)	38 (69.1)	28 (50.9)	38 (69.1)	52 (94.5)	47 (85.4)	51 (92.7)	14 (25.5)
Pantoea spp	6	6 (100)	6 (100)	3 (50)	4 (66.7)	2 (33.3)	5 (83.3)	6 (100)	6 (100)	6 (100)	3 (50)
E. coli	22	22 (100)	11 (50)	10 (45.5)	16 (72.7)	15 (68.2)	16 (72.7)	22 (100)	17 (77.3)	20 (90.9)	2 (9.1)
P. aeruginosa	3	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	-	2 (66.7)	1 (33.3)	3 (100)	3 (100)	1 (33.3)
K. pneumoniae	7	7 (100)	4 (57.1)	5 (71.4)	5 (71.4)	5 (71.4)	5 (71.4)	7 (100)	6 (85.7)	7 (100)	2 (28.6)
K. oxytoca	4	4 (100)	3 (75)	3 (75)	4 (100)	3 (75)	3 (75)	4 (100)	4 (100)	4 (100)	3 (75)
Aeromonas spp.	3	3 (100)	1 (33.3)	-	3 (100)	-	2 (66.7)	3 (100)	2 (66.7)	2 (66.7)	-
Others *	10	7 (70)	4 (40)	3 (30)	5 (50)	3 (30)	5 (50)	9 (90)	9 (90)	9 (90)	3 (30)
By Samples											
HVS	9	9 (100)	5 (55.6)	3 (33.3)	6 (66.7)	5 (55.6)	7 (77.8)	9 (100)	8 (88.9)	8 (88.9)	2 (22.2)
SPUTUM	9	9 (100)	6 (66.7)	5 (55.6)	6 (66.7)	2 (22.2)	7 (77.8)	8 (88.9)	7 (77.8)	8 (88.9)	4 (44.4)
STOOL	6	6 (100)	4 (66.7)	3 (50)	6 (100)	5 (83.3)	5 (83.3)	6 (100)	4 (66.7)	5 (83.3)	2 (33.3)
THROAT SWAB	3	3 (100)	3 (100)	1 (33.3)	1 (33.3)	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)	2 (66.7)	2 (66.7)
URINE	28	25 (89.3)	13 (46.4)	14 (50)	19 (67.9)	14 (50)	17 (60.7)	26 (92.9)	26 (92.9)	28 (100)	4 (14.3)

CIP – Ciprofloxacin, CN – Gentamicin, DOX – Doxycycline, SXT - Trimethoprim-sulfamethoxazole, AUG – Amoxicillin-clavulanic acid, FEP – Cefepime, CRO – Ceftriaxone, CAZ – Ceftazidime, CTX – Cefotaxime, Mer – Meropenem.

* Grouped together due to small population, includes *P. fluorescens / putida*, *P. luteola*, *Aeromonas* spp., S. *plymuthica*, *H. alvei*, *R. aquatilis*, *Acinetobacter* spp., *Pasteurella* spp., *P. penneri.*

Table 4: Overall Prevalence of ESBL Production and Multidrug Resistance**.**

Organism	No. of Isolates	Overall Prevalence MDR n (%)	Overall Prevalence ESBL Production n (%)
Total Isolates	55	49 (89.1)	42 (76.4)
Pantoea spp.	6	6 (100)	5 (88.3)
E. coli	22	21 (95.5)	12 (21.8)
P. aeruginosa	3	2 (66.7)	3 (100)
K. pneumoniae	7	7 (100)	5 (71.4)
K. oxytoca	4	4 (100)	4 (100)
Aeromonas spp.	3	3 (100)	3 (100)
Others*	10	6 (60)	10 (100)

* Same as Table 3

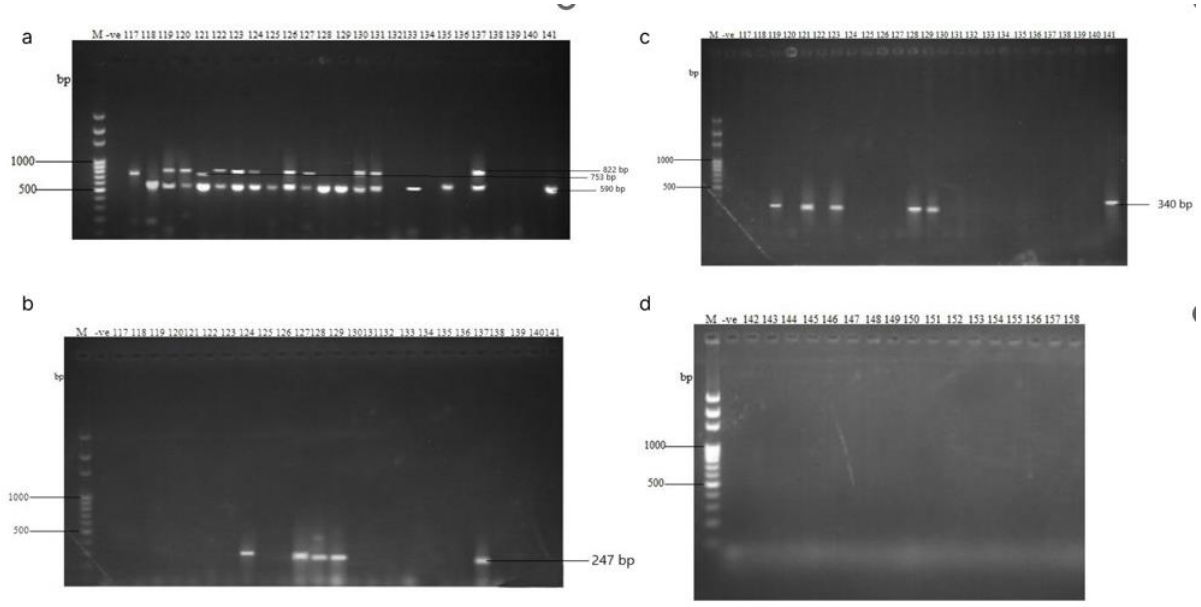

Fig. 2: Distribution of genes in ESBL producing Gram-negative bacteria. a – *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$, b – *bla*$_{OXA}$ and *bla*$_{VIM}$ (no *bla*$_{OXA}$ detected), c – *bla*$_{KPC}$ (340bp) and *bla*$_{IMP}$ (no *bla*$_{IMP}$ detected), d – no gene detected.

Table 5: Prevalence of ESBL and Carbapenemase Genes among ESBL producers.

Organism	No. of isolates	ESBL			Carbapenemase			
		bla$_{TEM}$	*bla*$_{SHV}$	*bla*$_{CTX-M}$	*bla*$_{KPC}$	*bla*$_{IMP}$	*bla*$_{OXA}$	*bla*$_{VIM}$
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total isolates	42	11 (26.2)	2 (4.8)	23 (54.8)	6 (14.3)	-	-	7 (16.7)
*Pantoea*spp	5	1 (20)	1 (20)	3 (60)	2 (40)	-	-	1 (20)
E. coli	12	3 (25)	-	5 (41.7)	1 (8.3)	-	-	1 (8.3)
P. aeruginosa	3	1 (33.3)	-	2 (66.7)	1 (33.3)	-	-	-
K. pneumoniae	5	-	-	2 (40)	-	-	-	1
K. oxytoca	4	-	1 (25)	2 (50)	-	-	-	1 (25)
Aeromonas spp.	3	2 (66.7)	-	2 (66.7)	-	-	-	1 (33.3)
Others*	10	4 (40)	-	7 (70)	2 (20)	-	-	2 (20)

DISCUSSION

Drugs of last resort such as carbapenems have become the mainstay for the treatment of infections with MDR bacterial aetiologies. However, reports of carbapenem-resistant bacteria have increased worldwide, including in Nigeria (Zubair and Iregbu, 2018; Ogbolu *et al*., 2020). Lagos state in southwest Nigeria is the largest city in Nigeria with a population of over 15 million people belonging to more than 250 ethnic groups due to large-scale migration from all parts of Nigeria and is therefore a good indication of the country's population. This study shows a 51.4% prevalence of GNB infections in the city of Lagos, predominantly urinary tract infections. UTI, with a prevalence of 50.9%, was caused by *E. coli* and *Klebsiella* species, as well as the Erwineaceae *Pantoea* spp. High rates of bacterial infection have been recognized as a problem in Nigeria (Raji *et al*., 2013; Abdu *et al*., 2019; Olowo-okere *et al*., 2020), albeit UTIs are the most common infections

diagnosed in outpatients worldwide (Flores-Mireles *et al*., 2015; Chu and Lowder, 2018; Ponce-de-Leon *et al*., 2018; Seifu and Gebissa, 2018).

Antimicrobial resistance, a worldwide threat, showed no respite in Lagos with average to very high resistance rates observed to all antibiotics. However, when comparing the resistance patterns in this study to existing antibiotics resistance rates within the country, our report varies considerably. The resistance patterns in this study differed from that of Bayelsa and Sokoto-based studies with high rates of ciprofloxacin and cephalosporins resistance, but lower amoxicillin-clavulanic acid resistance, unlike these studies which reported the opposite (Abdu *et al*., 2019; Olowo-okere *et al*., 2020). This might allude to a disparity in predominant infections and preferred treatment options within the country. MDR and ESBL-producing pathogens constituted 89%, and 76% of the isolates respectively, mostly *E. coli* and *Klebsiella* species. *Klebsiella*, particularly *K. oxytoca*, were the most resistant organisms in this study. All isolates of *K. oxytoca* were both multidrug-resistant and ESBL producers. All *Klebsiella* isolates were multidrug-resistant, with two *K. oxytoca* and one *K. pneumoniae* isolates resistant to all the antibiotics used. *E. coli*, though the most predominant GNB with all isolates but one being multidrug-resistant, was the least carbapenem-resistant while *K. oxytoca* was the most carbapenem-resistant with all but one isolate resistant to meropenem. All carbapenem-resistant isolates were resistant to the cephalosporins and ciprofloxacin, except an isolate of *K. pneumoniae* susceptible to ceftazidime, and *P. fluorescens/putida* susceptible to ceftazidime, cefotaxime and cefepime. Interestingly, three cephalosporin and carbapenem-resistant isolates were susceptible to amoxicillin-clavulanic acid. The burden of carbapenem-resistant pathogenic GNB from clinical samples in southern Nigeria is high with 25% prevalence in this study, 21.9% in Enugu, and 52.3% in a study comprising different southwest Nigerian states as opposed to northern Nigeria with 12% in Abuja and 6.5% in Sokoto state (Zubair and Iregbu, 2018; Ajuba *et al*., 2020; Ogbolu *et al*., 2020; Olowo-okere *et al*., 2020b). This disparity could be due to the higher prevalence of pharmaceutical companies in southern Nigeria, as only 47 of 165 pharmaceutical manufacturing facilities in Nigeria are in the north (NAFDAC, 2020). Epidemiological studies have demonstrated a direct relationship between the inappropriate consumption of antibiotics, including failure to complete treatment, re-use of leftover medicines, and overuse of antibiotics, use of counterfeit drugs often with low potency, as well as indiscriminate use of antibiotics in livestock farming, and the prevalence of antibiotic and multidrug resistance in pathogenic bacteria (Auta *et al*., 2013; Ventola, 2015; Efunshile *et al*., 2019; Olowo-okere *et al*., 2020;). Although all antibiotics are prescription-only medicines in Nigeria, there is next to zero enforcement which has resulted in a great abuse of antibiotics. The majority of Nigerians self-medicate on antibiotics purchased over the counter, often for fever, common cold and upper RTIs which are viral infections and do not respond to antibiotics, leading to the development of resistant strains of pathogenic bacteria (Auta *et al*., 2013; Dadari, 2020). Inadequate management of pharmaceutical wastes in Nigeria results in the presence of active pharmaceutical ingredients in water bodies and tap water which further aids the dissemination of resistance (Adesina and Felix, 2018; Ogunbanwo *et al*., 2020). Carbapenem-resistant GNB has been reported in pharmaceutical wastewater in southwestern Nigerian states (Obasi *et al*., 2019).

Bla$_{\text{CTX-M}}$ was the most predominant ESBL gene in this study, with the prevalence of genes encoding ESBL production within the range described for southwest Nigeria (Tanko *et al*., 2020). CTX-M are the most widespread β-lactamse enzymes, supplanting TEM and SHV families (Raji *et al*., 2015; Obasi *et al*., 2019; Olowo-okere *et al*., 2020b). *Bla*$_{\text{CTX-M}}$ was the only gene encoding ESBL production in 24% of the ESBL

producers of which 80% were resistant to the cephalosporins and amoxicillin-clavulanic acid. The majority of the isolates with *bla*$_{TEM}$ were resistant to cephalosporins, and amoxicillin-clavulanic acid. *Bla*$_{SHV}$ was the only gene observed in a strain of *K. oxytoca*, resistant to all cephalosporins except cefepime but susceptible to amoxicillin-clavulanic acid. MDR GNB constituted 87.5% of the isolates with genes encoding ESBL.

Genes encoding carbapenemase production aren't strangers to Nigeria with reports of *bla*$_{NDM,}$ *bla*$_{KPC}$, *bla*$_{VIM,}$ *bla*$_{GES}$ and *bla*$_{OXA}$ in studies from Enugu, Bornu[45], Bayelsa, Port Harcourt[46], Abuja, Sokoto, and other states (Mohammed *et al*., 2015; Zubair and Iregbu, 2018; Abdu *et al*., 2019; Ajuba *et al*., 2020; Igunna *et al*., 2020; Ogbolu *et al*., 2020; Olowo-okere *et al*., 2020b). However, the 23.6% prevalence in GNB from clinical samples recorded in this study is much higher than any previously reported in Nigeria to the best of our knowledge. *Bla*$_{KPC}$, detected in this study, appears to be rare in clinical samples in Nigeria as the only records of it to our knowledge are reports from two studies, one in Bornu state and the other in Port Harcourt (Mohammed *et al*., 2015; Igunna *et al*., 2020). Like other Nigerian clinical studies, no *bla*$_{IMP}$ was discovered. *Bla*$_{IMP}$ has however been reported in strains of *E. coli* from rectal swab samples of cows in the abattoir in Abakaliki (Ejikeugwu *et al*., 2020). The high prevalence of carbapenemase encoding genes recorded is attributable to not limiting genotypic analysis to only bacteria exhibiting carbapenem resistance in this study as carbapenemase genes were observed in some isolates susceptible to meropenem. Similarly, studies from Uganda[48] and Saudi Arabia[49] which used both phenotypic and genotypic methods to determine the carbapenemase prevalence reported higher rates with the genotypic method (Okoche *et al*., 2015; AlTamini *et al*., 2017).

The prevalence of multidrug resistance, coupled with ESBL and carbapenemase production is distressing as they severely limit the treatment options, particularly in Nigeria where funding for antibiotic alternatives is inadequate (FMARDEH, 2017) The clinical implication is that many patients stand the risk of treatment failure, a significant increase in mortality and lower quality of life as the available options for infections with carbapenem-resistant aetiologies, colistin and polymyxin B which are known to have severe adverse effects including nephrotoxic and neurotoxic reactions(Raji *et al*., 2015) There is a momentous need for new antibiotics to be produced to combat the prevalence of antibiotic resistance. Alternative therapies could also be considered for the treatment of microbial infections.

Limitations: This study was limited to hospitals and diagnostic laboratories on the Lagos mainland. Age, gender and previous antibiotic exposure were not taken into consideration.

Conclusion

The prevalence of MDR, ESBL and carbapenemase production in Lagos state, Nigeria is untenably high, particularly in *Klebsiella* species. Gram-negative bacteria with carbapenemase genes are not always carbapenem-resistant and so both phenotypic and genotypic analysis should be employed in determining carbapenemase production. New therapies are required for the control of bacteria that are resistant to all known antibiotics. Surveillance is required to curtail the further spread and emergence of new resistance mechanisms and conserve drug efficacy. Strict enforcement of regulations on the distribution of antibiotics and disposal of pharmaceutical waste, as well as educating the general public on the effects of inappropriate antibiotic consumption is also necessary.

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