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Opening Pandora's box: High-level resistance to antibiotics of last resort in Gram-negative bacteria from Nigeria

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ABSTRACT

Objectives: The aim of this study was to determine the percentage of antimicrobial-resistant isolates and the associated resistance mechanisms in Gram-negative bacteria from South Western Nigeria.
Methods: A total of 306 non-duplicate unbiased Gram-negative isolates were recovered from patients admitted to three teaching hospitals in South Western Nigeria in 2011 and 2013. Isolates were from clinical samples as well as from stool samples of inpatients without infection to assess antimicrobial resistance patterns in carriage isolates. Antimicrobial susceptibility testing was performed, and PCR and sequencing were used to identify genes encoding various known β-lactamases. Based on phenotypic and genotypic results, 10 isolates representing the diversity of phenotypes present were selected for whole-genome sequencing (WGS).
Results: Antimicrobial susceptibility testing revealed the following resistance rates: fluoroquinolones, 78.1%; third-generation cephalosporins, 92.2%; and carbapenems, 52.6%. More resistant isolates were isolated from stools of uninfected patients compared with clinical infection specimens. *Klebsiella* (10%) and *Escherichia coli* (7%) isolates produced a carbapenemase. WGS of selected isolates identified the presence of globally disseminated clones.
Conclusion: This study illustrates a crisis for the use of first-line antimicrobial therapy in Nigerian patients. It is likely that Nigeria is playing a significant role in the spread of antimicrobial resistance owing to its large population with considerable global mobility.

1. Introduction

Nigeria and other sub-Saharan African countries are facing an increasing number of healthcare-associated infections caused by multidrug-resistant Gram-negative bacteria (GNB) [1]. Pathogenic species have evolved resistance to multiple antimicrobial agents including the mainstays of treatment [2,3]. This is of concern as there are few new antibiotics in the development pipeline with activity against GNB [4].

Carbapenems have become a mainstay of therapy for the treatment of extended-spectrum β-lactamase (ESBL)-producing GNB, leading to an increase in carbapenem use for the treatment of serious infections [5]. As a result, there has been a selective pressure for carbapenem resistance, and carbapenem-resistant strains have spread globally [6]. Worryingly, carbapenem-resistant bacteria are often also resistant to other classes of antibiotics including aminoglycosides, fluoroquinolones and other β-lactams, often with colistin and tigecycline as the only effective drugs. Resistance to both of these antibiotics can also evolve easily, making them unreliable as 'last-resort' therapies [7].

GNB cause a significant number of infections in Nigerian hospitals and represent the majority of isolates both from wounds and urinary tract infections; these form the largest group of clinical specimens received in Nigerian clinical microbiology laboratories [1]. Carbapenem-resistant GNB have become prevalent in many parts of the world, including Nigeria and sub-Saharan Africa. However, to our knowledge there are few data and no organised antimicrobial resistance surveillance networks for Africa. Recently, we showed that carbapenem-resistant bacteria are present in Nigeria. However, details of the strains with this phenotype and the mechanisms of resistance have not been studied in detail [2,3].

The potential role of the Nigerian population in the global spread of antimicrobial resistance is great, but the local situation is

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not well understood. The aim of this retrospective study was to determine the percentage of antimicrobial-resistant clinical and commensal isolates and the associated resistance mechanisms in GNB from South Western Nigeria.

2. Materials and methods

2.1. Sample sites and bacterial isolates

Most of the Nigerian population is located in the southwest of the country, which is also where the major transportation hubs are located. GNB isolates for this study were recovered from patients admitted to three large teaching hospitals located in three states of South Western Nigeria from a range of clinical specimens from invasive infections and colonisation (Table 1 and Fig. 1). Olabisi Onabanjo University Teaching Hospital is a 185-bed tertiary healthcare centre and a major referral centre for Ogun State. University College Hospital, Ibadan, is an 850-bed federal teaching hospital in Oyo State. Obafemi Awolowo University Teaching Hospitals Complex is a 535-bed teaching hospital in Osun State.

In addition, isolates from stool samples sent for routine examination from patients without infection were also collected. Isolates were non-duplicate and unbiased (i.e. no selective criteria beyond being a GNB species were applied) and were randomly collected from the hospital laboratories in 2011 and 2013. A total of 306 isolates were recovered. No information regarding the antimicrobial susceptibility of any of the isolates was used as an inclusion criterion. The isolates comprised *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus* spp. and others (*Serratia*, *Citrobacter* spp. and *Providencia rettgeri*). Species assignment was confirmed for all isolates using standard biochemical tests and using API 20E strips (bioMérieux, Basingstoke, UK) for Enterobacteriaceae.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates to a panel of antibiotic classes commonly used in the study hospitals, such as fluoroquinolones, third-generation cephalosporins and carbapenems, was determined by the disc diffusion method on Mueller–Hinton agar using antibiotic disks from Oxoid Ltd. (Basingstoke, UK) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations and interpreted according to EUCAST breakpoints [8]. All antimicrobial susceptibility testing included the control organisms *E. coli* NCTC 10418 and *P. aeruginosa* NCTC 10662.

2.3. Identification of carbapenemase production

Enterobacteriaceae isolates were tested for carbapenemase production using the disc-based Carbapenemase Detection Set (Mast Group, Bootle, UK) and the results were interpreted using the Carbapenemase Detection Set Calculator (Mast Group) tool as per the manufacturer's guidelines.

2.4. Identification of known carbapenemase genes

Bacterial DNA was extracted by the crude boiling method. PCR and sequencing were used to identify genes encoding various known β-lactamases (including carbapenemases, NDM, VIM, KPC and GES) using the primers shown in Supplementary Table S1.

2.5. Random amplified polymorphic DNA (RAPD)-PCR

An RAPD-PCR typing approach was used for each species as a rapid and inexpensive way to assess the diversity of strains within each population. The primer sequences and PCR conditions used were according to Vogel et al. [9], modified to use 1 mL of 100 mM of primers at a final concentration of 0.02 mM. The experiment was repeated to ensure reproducibility.

2.6. Whole-genome sequencing (WGS) and bioinformatics analysis

To characterise the strain types, plasmid content and type of antimicrobial resistance genes present in the isolate collection, 10 isolates (a lack of resources limited the number sequenced) were selected for WGS based on their antimicrobial profile, carbapenemase production, genotype, clinical specimen, source and species. DNA was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Crawley, UK) according to the manufacturer's instructions. Paired-end Illumina sequencing was used to generate 250-bp high-quality reads. The genome was assembled using Velvet [10] and contigs were re-ordered against relevant reference genomes using MAUVE [11]. Assemblies were annotated using Rapid Annotation using Subsystem Technology (RAST) (http://rast.nmpdr.org/rast.cgi). Assemblies were used to search for plasmid content and to determine multilocus sequence typing (MLST) sequence types using the PlasmidFinder and MLST tools hosted at the Centre for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/PlasmidFinder and http://cge.cbs.dtu.dk/services/MLST, respectively). The Comprehensive Antibiotic Resistance Database (CARD) was searched to identify antimicrobial resistance genes [12]. Specific assembly of plasmids was attempted using plasmidSPAdes, and plasmid content was identified by plasmid network reconstruction

Table 1
Distribution and sources of bacterial isolates.

Specimen type	No. of isolates					
	Klebsiella spp.	*Escherichia coli*	*Pseudomonas* spp.	*Proteus* spp.	Other	Total
Wound	42	9	24	10	0	85
Urine	38	26	8	4	1	77
Blood culture	8	3	0	0	0	11
Sputum	7	2	1	1	3	14
HVS/ECS	2	3	0	0	0	5
Ear swab	6	0	15	8	0	29
Catheter	6	1	2	0	0	9
Aspirate	4	0	3	0	0	7
Other	0	0	1	0	0	1
Total	113	44	54	23	4	238
Stool[a]	9	52	6	1	0	68
Total (overall)	122	96	60	24	4	306

HVS/ECS, high vaginal swab/endocervical swab.
[a] Stool samples from healthy carriers.

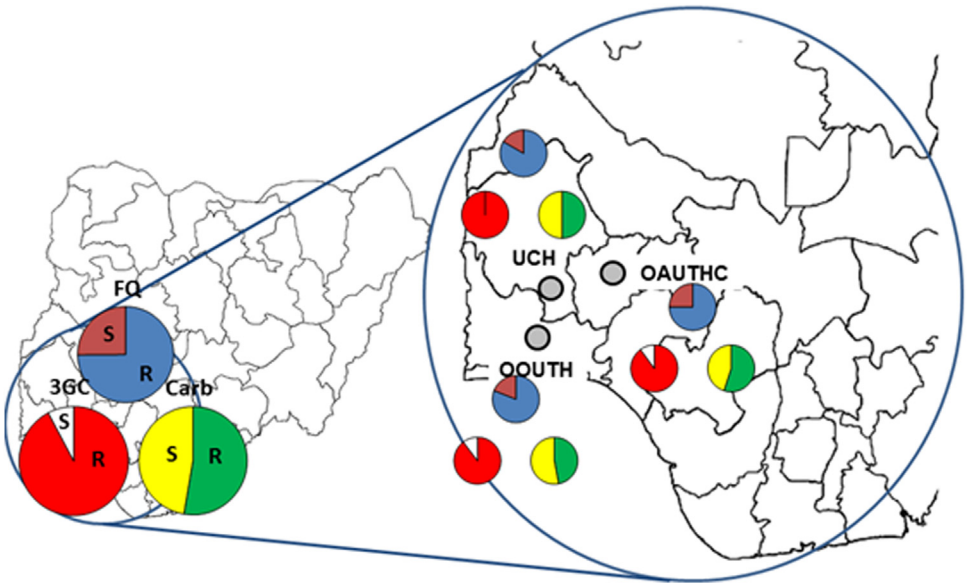

Fig. 1. Summary of antimicrobial resistance rates in South Western Nigeria. Pie charts show the proportion of isolates classified as resistant (R) or susceptible (S) to fluoroquinolones (FQ, blue and red), third-generation cephalosporins (3GC, red and white) and carbapenems (Carb, green and yellow). The left-hand figure shows data for the whole study (including all species and both years). The expanded insert (righthand side) shows data from each of the three hospitals (grey circles). UCH, University College Hospital (Ibadan); OOUTH, Olabisi Onabanjo University Teaching Hospital (Sagamu); OAUTHC, Obafemi Awolowo University Teaching Hospitals Complex (Ife).

using PLACNET (https://castillo.dicom.unican.es/upload) [13]. When necessary, reads were mapped against assemblies using Bowtie [14] and were visualised in Artemis [15].

3. Results

3.1. Antimicrobial resistance in Nigerian isolates

Among the entire collection of 306 isolates, 78.1% were resistant to fluoroquinolones, 92.2% to third-generation cephalosporins and 52.3% to carbapenems (Table 2). The high percentages of clinical isolates resistant to these drug classes were very similar in all three study sites (fluoroquinolone resistance, 75–83%; third-generation cephalosporin resistance, 90–100%; and carbapenem resistance, 50–55%).

Of concern was the observation that the percentages of isolates from stool samples of uninfected patients, i.e. those being carried as commensal bacteria, that were resistant to third-generation cephalosporins (100%) and carbapenems (69.1%) was actually higher than in isolates from patients being treated for an infection. The percentages of resistant isolates both from commensal and clinical samples are shown in Table 2.

When stratified by species, *E. coli* were most commonly resistant to third-generation cephalosporins (93.7%) and carbapenems (59.4%), followed by *Pseudomonas* spp. (91.7% resistant to third-generation cephalosporins and 51.7% to carbapenems) (Table 2). Of the 306 isolates, no species had ≥50% of isolates that were susceptible to all three classes of antibiotic. *Proteus* spp. isolates ($n = 24$) were least likely to be carbapenem-resistant; none the less, 45.8% of these isolates were resistant to this class of drug.

Isolates in this study were collected in two different years (2011 and 2013) and the percentages of fluoroquinolone-resistant isolates in the two years were very similar. However, the percentages of isolates resistant to cephalosporins in the two years decreased from 96.6% to 86.8% and the percentage of isolates resistant to carbapenems decreased from 59.3% to 43.0% (Table 2).

Table 2
Overview of antimicrobial susceptibility testing to clinically important antibiotics.

	No. of isolates	Resistant isolates (%)		
		FQ	3GC	CARB
Total isolates	306	78.1	92.2	52.6
Klebsiella spp.	122	75.4	92.6	50.8
Escherichia coli	96	81.4	93.7	59.4
Pseudomonas spp.	60	76.7	91.7	51.7
Proteus spp.	24	83.3	87.5	45.8
Others	4	75.0	75.0	0
Clinical samples	238	80.4	90	47.8
Klebsiella spp.	113	70.8	89.4	53.1
E. coli	44	77.2	84.1	40.9
Pseudomonas spp.	54	83.3	88.9	46.3
Proteus spp.	23	78.3	87	47.8
Others	4	75	75	0
Stool (healthy individuals)	68	69.1	100	69.1
Klebsiella spp.	9	100	100	0
E. coli	52	76.9	96.2	75
Pseudomonas spp.	6	16.7	100	83.3
Proteus spp.[a]	1	N/A	N/A	N/A
Others	0	–	–	–
By year of isolation				
2011	182	78.5	96.6	59.3
2013	124	76.9	86.8	43.0
By location				
Hospital I (UCH)	212	80.9	100	50
Hospital II (OOUTH)	6	83.3	100	50
Hospital III (OAUTHC)	20	75	90	55

FQ, fluoroquinolones; 3GC, third-generation cephalosporins; CARB, carbapenems; N/A, not applicable; UCH, University College Hospital (Ibadan); OOUTH, Olabisi Onabanjo University Teaching Hospital (Sagamu); OAUTHC, Obafemi Awolowo University Teaching Hospitals Complex (Ife).

[a] Percentage not calculated because of the small number.

3.2. Typing of isolates

RAPD-PCR was used to type 54 of the isolates, representing 18 each of *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa.* A wide variety of patterns were observed for each species, with only a small number of repeated patterns observed. For example, 13 RAPD

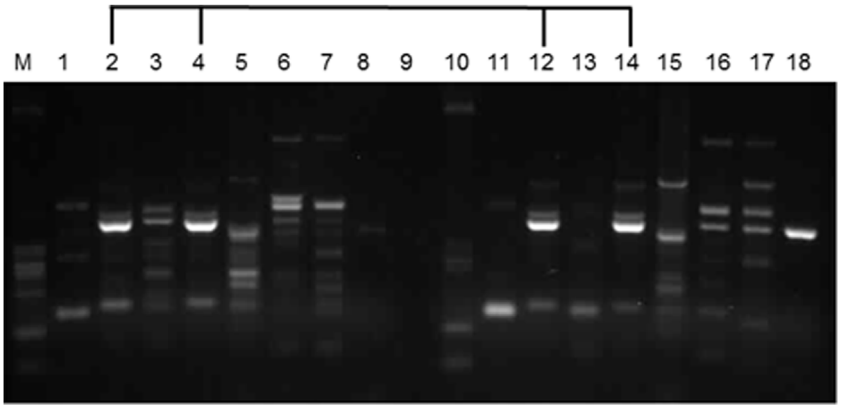

Fig. 2. Random amplified polymorphic DNA (RAPD) analysis of a selection of 18 *E. coli* isolates. Agarose gel electrophoresis of amplimers from RAPD-PCR. M, marker. The bold line above the gel shows four isolates with an identical banding pattern.

Table 3
Carbapenemase production and presence of carbapenemase genes.

Species	No. of isolates	No. of carbapenem-resistant isolates	No. of isolates with phenotypic carbapenemase activity		No. of isolates with carbapenemase genes by PCR		
			+ve	–ve	bla_{VIM}	bla_{GES}	bla_{NDM}
Klebsiella spp.[a]	122	62	34	28	2	3	2
Escherichia coli	96	57	32	25	3	1	0
Pseudomonas spp.[a]	60	31	22	9	4	6	0
Proteus spp.	24	11	5	6	0	0	0
Other	4	0	N/A	N/A	0	0	0
Total	306	161	93	68	9	10	2

N/A, not applicable.
[a] Two isolates carried two carbapenemase genes; *Klebsiella* spp., NDM + VIM; and *Pseudomonas* spp., GES + VIM.

patterns were identified from 18 isolates of *E. coli* demonstrating a lack of dominance by specific clones (Fig. 2).

3.3. Carbapenemase production and identification of carbapenemase genes

Although 40–60% isolates were phenotypically carbapenem-resistant, only 19 (6.2%) of the 306 isolates carried a known carbapenemase gene. Specific carbapenemase genes were amplified by PCR and the genes were verified by DNA sequencing. PCR revealed the presence of variants of VIM ($n = 9$), GES ($n = 10$) and NDM ($n = 2$) families (Table 3). These genes were detected in *K. pneumoniae* ($n = 6$), *E. coli* ($n = 4$) and *P. aeruginosa* ($n = 9$) isolates. Two isolates carried two carbapenemase genes ($bla_{NDM} + bla_{VIM}$ and $bla_{GES} + bla_{VIM}$). The bla_{KPC}, bla_{IMP} and bla_{OXA-48} genes were not detected in any isolate.

Since 161 (52.6%) of the 306 isolates were resistant to carbapenems but most did not appear to produce a known carbapenemase, the presence of other known resistance mechanisms was investigated. Primers specific for each of the CTX-M subgroups were used to detect various CTX-M genes. Of the total 218 *E. coli* and *K. pneumoniae* isolates, 173 (79.4%) contained a CTX-M allele. DNA sequencing of a random selection of 40 isolates revealed all to be CTX-M-15. None of these isolates demonstrated phenotypic derepression of efflux, but all showed either complete loss or reduced production of outer membrane porins (data not shown).

3.4. Characterisation of antimicrobial resistance mechanisms and strain types in representative isolates

WGS of 10 isolates, including two *K. pneumoniae*, two *E. coli*, three *P. aeruginosa*, two *Proteus mirabilis* and one *P. rettgeri* isolate (Table 4), identified some globally established strain types in circulation in Nigeria, notably *K. pneumoniae* ST11 and *P. aeruginosa* ST224 and ST233.

Both of the *K. pneumoniae* isolates belonged to ST11 and were from urine of different patients in 2011 from University College Hospital, Ibadan. Both genome assemblies were essentially identical, and both carried bla_{NDM-1} and $bla_{CTX-M-15}$ (Table 4). In addition, both isolates also carried bla_{OXA-1} and bla_{SHV-11}. Interestingly, there was direct evidence for mobility of the bla_{NDM-1} gene in this strain. Whilst the NDM-1-encoding gene was detected by PCR using a boiled colony preparation as a template in both isolates (U52 and U37), in both genome assemblies created after sequencing of isolated DNA preparations it was only initially seen in the genome assembly for U52. Analysis of the genetic location of this gene showed it to be present in a context similar to that previously observed by others in an operon with ble_{MBL} and associated with *trpF*, *dsbC* and *cutA* (Fig. 3). This region was absent in isolate U37, and no sequence reads mapped against the U52 reference (Supplementary Fig. S1), demonstrating likely mobility of this whole region as has been suggested previously [16]. IncFIB and FII plasmid replicons were present in both strains, supporting a plasmidic context for bla_{NDM-1} (Fig. 3). In addition to the β-lactamase genes, both isolates also carried resistance genes to trimethoprim (*dfrA12*), macrolides (*mphA*), aminoglycosides (*rmtF*), chloramphenicol (*cat*) and sulfonamides (*sul1*). Consistent with fluoroquinolone resistance, mutations in *gyrA* were observed.

One of the *P. aeruginosa* isolates belonged to ST244 and carried the mutant PDC-1 AmpC enzyme as well as genes contributing to resistance to chloramphenicol (*cmx*, *catB7*), aminoglycosides [*aph(3″)-I1*, *aph(6)-Id*] and fosfomycin (*fosA*). The other two isolates were both members of ST233 and both carried bla_{PDC-3}. These latter two isolates also carried bla_{VIM-2} and bla_{OXA-33}, were of the same MLST type and were both isolated from Olabisi Onabanjo University Teaching Hospital although 2 years apart. Reads from

Table 4
Strain types, β-lactamase genes and plasmid replicons present in representative isolates.

Strain	Species	MLST	MIC (μg/mL)			Carbapenemase gene(s)	β-Lactamase gene(s)	Plasmid replicon(s)
			IPM	MEM	ETP			
U37	*Klebsiella pneumoniae*	ST11	16	16	64	bla_{NDM-1}	bla_{OXA-1}, bla_{SHV-11}, $bla_{CTX-M-15}$	FIB (K), FII (K)
U52	*K. pneumoniae*	ST11	16	16	64	bla_{NDM-1}	bla_{OXA-1}, bla_{SHV-11}, $bla_{CTX-M-15}$	FIB (K), FII (K)
S46	*Escherichia coli*	ST226	16	0.12	0.12	–	bla_{AmpC}, bla_{TEM-1}	FII
F124	*E. coli*	ST156	0.5	0.06	0.06	–	bla_{TEM-1}, bla_{ACT-7}	Q1
F19	*Pseudomonas aeruginosa*	ST244	8	8	N/D	bla_{PDC-1}	–	–
F46	*P. aeruginosa*	ST233	32	64	N/D	bla_{VIM-2}, bla_{PDC-3}	bla_{OXA-33}	–
U36	*P. aeruginosa*	ST233	64	32	N/D	bla_{VIM-2}, bla_{PDC-3}	bla_{OXA-33}	–
F10	*Proteus mirabilis*	–	8	0.25	2	–	bla_{CMY-41}, bla_{CMY-31}, bla_{TEM-1}	Q1
F56	*P. mirabilis*	–	8	0.25	0.25	–	bla_{ACT-7}, bla_{TEM-1}	Q1, Col(BS512)
S39	*Providencia rettgeri*	–	16	2	4	–	bla_{SRT-2}	–

MLST, multilocus sequence typing; MIC, minimum inhibitory concentration; IPM, imipenem; MEM, meropenem; ETP, ertapenem.
N/D, not determined (as *Pseudomonas* spp. are intrinsically resistant to ETP).
–Indicates no gene or replicon detected, or for sequence type a species where no MLST scheme has been established.

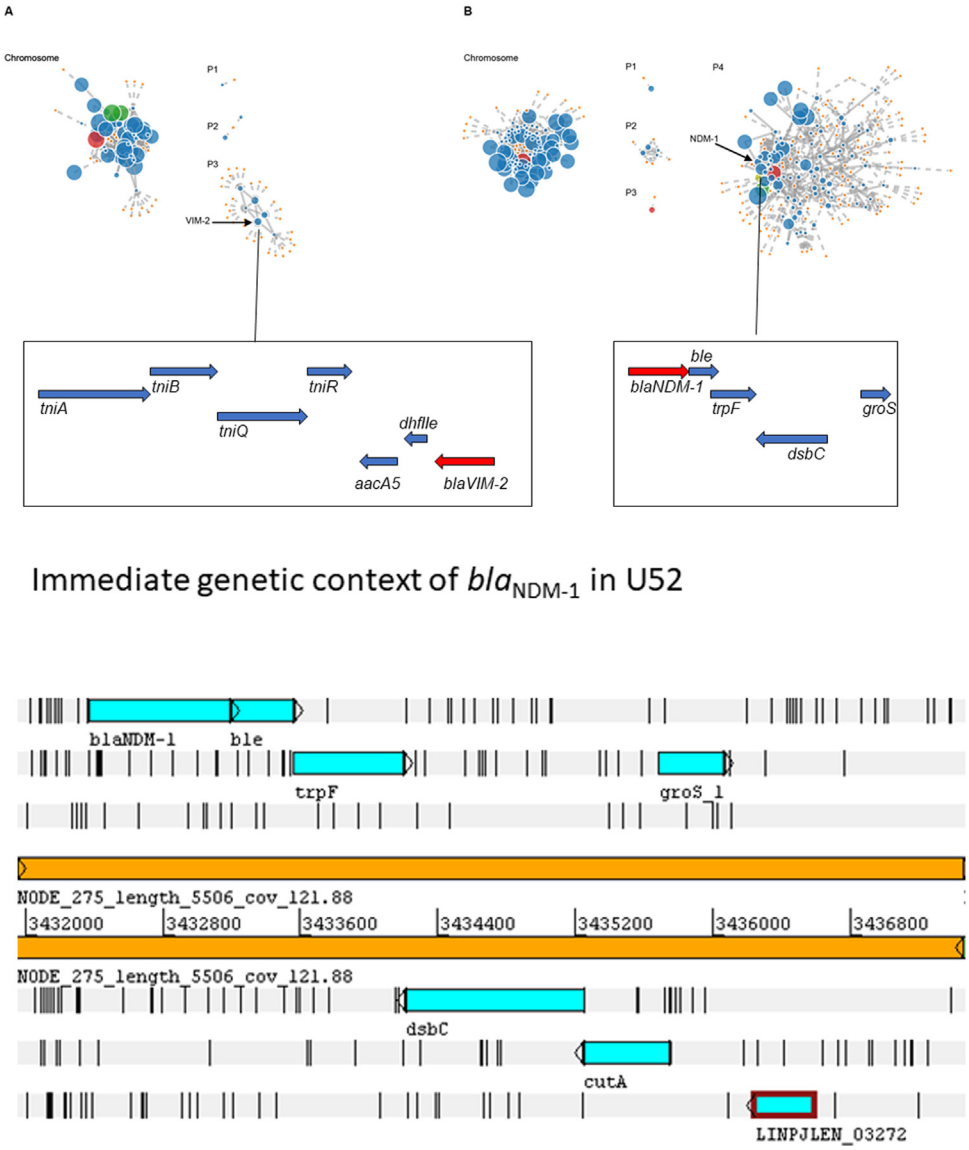

Fig. 3. (a) Plasmid content reconstructed using PLACNET. Panel (A) shows the network from *P. aeruginosa* F46 with a chromosomal network of 60 contigs totalling ~6.7 Mb and three discrete plasmid networks (P1–P3); P1 and P2 are both <5 kb, and P3 carrying VIM-2 consists of 8 contigs of ~31 kb. The bla_{VIM-2} gene in *Pseudomonas* was present on a small contig with homology to an integron. Trimethoprim and aminoglycoside resistance genes were also present in the assembled contig. Panel (B) shows the network from *K. pneumoniae* U52 with a chromosomal group of 87 contigs totalling 5.3 Mb and four plasmid networks (P1–P4); P1 and P3 are both ~5 kb, P2 is 9 kb, and P4 consists of a large network of 90 contigs totalling 1.9 Mb which is likely to represent more than one IncF type plasmid that have not been resolved. The bla_{NDM-1} gene in U52 was on a small contig in an operon with the *ble* gene. (b) Immediately downstream of bla_{NDM-1} in isolate U52 were the *trpF*, *dsbC* and *cutA* genes.

strain F46 carrying bla_{VIM-2} were assembled using both Velvet and SPAdes (using the plasmidSPAdes option); both resulted in assemblies with the bla_{VIM-2} gene present on a contig of ~7000 bp. When this sequence was compared with known sequences in GenBank using the BLAST algorithm, a perfect match for an integron carrying bla_{VIM-2} was found (accession no. KT768111.1). Fig. 3 shows a plasmid network reconstruction and the genetic context of the bla_{VIM-2} gene in these two isolates.

The two *E. coli* strains sequenced belonged to ST226 and ST156. Neither carried known carbapenemase genes, although both had multiple mutations within *ampD* suggesting de-repression of the chromosomal *ampC* gene. Both strains also carried bla_{TEM-1} and various other mobile resistance genes including genes conferring aminoglycoside resistance [*aph(6)-Id*, *aph(3″)-I1*]. An IncFII plasmid replicon was present in isolate S46 (the ST226 isolate).

Two *P. mirabilis* strains were sequenced. Isolate F10 carried two bla_{CMY} genes, namely bla_{CMY-41} reported once previously in a *Citrobacter freundii* isolated from food in Egypt [17] and bla_{CMY-31} previously reported in *Klebsiella* and *Salmonella* [18,19]. A Q1 plasmid replicon was present in isolate F10. This isolate also carried two separate aminoglycoside [*aadA5*, *aph(3″)-I1*], chloramphenicol (*catI*) and sulfonamide (*sul1*) resistance genes as well as a plasmid-mediated quinolone resistance gene (*qnrA1*). *Proteus mirabilis* isolate F56 was found to carry a novel CMY enzyme with a single substitution (glutamic acid for aspartic acid at codon 144) distinguishing this protein from CMY-48 isolated from *C. freundii*. Isolate F56 also carried a chloramphenicol acetyltransferase gene (*catI*) and three aminoglycoside resistance genes [*aadA5*, *aph(3″)-I1* and *aph(6)-Id*].

The sequenced *P. rettgeri* isolate (S39) carried an SRT-2 AmpC β-lactamase variant that has previously been described in *Serratia marcescens* [20]. No other β-lactamase genes or plasmid replicons were detected in this isolate.

4. Discussion

4.1. Population density of study areas

The study covered the southwest of Nigeria, where the population density of the country is highest with ~50 million people. The study area included major population centres close to other major cities with a diverse population in terms of culture, race, religion and social standing. The major international transportation hubs of Nigeria are also in the southwest of the country and more than 15,200 international flights leave annually to over 32 airports in 30 different countries, and more than 8.3 million passengers fly through Nigeria annually [21]. International destinations are varied, with Europe and the Middle East being most common, followed by destinations in Asia, and a smaller number of flights departing to North and South America [21].

4.2. Antimicrobial resistance of isolates

This study showed that there is a very high prevalence of resistance among Nigerian GNB isolates to three key classes of antibiotic. A high frequency of resistance to fluoroquinolones and cephalosporins has been seen in other areas of the world, increasing the reliance on carbapenems for the treatment of infections caused by GNB. In this study, >50% of the Nigerian isolates were carbapenem-resistant, and empirical use of these antibiotics for the treatment of serious infections is unlikely to be effective. Resistant isolates were prevalent both in commensal isolates from stools and clinical samples and were not restricted to patients receiving antibiotic treatment for infections. From these data, resistance to major antibiotics would appear to be the norm in GNB carried by the Nigerian population.

4.3. Mechanisms of carbapenem resistance

Characterisation of the mechanisms of carbapenem resistance in the collection of isolates showed that some well-known and globally disseminated carbapenemase genes are in circulation in Nigeria, including NDM, VIM and GES enzymes. However, <10% of the isolates in the study carried a known carbapenemase (according both to molecular and phenotypic testing) and none carried KPC or OXA family carbapenemases. Phenotypic detection of carbapenemase production agreed well with detection of genes by PCR and sequencing. All isolates with known carbapenemase genes showed phenotypic carbapenemase activity. A recent report from Edo State (in South Nigeria, further east from the locations in this study) reported the existence of OXA family carbapenemases of OXA-48 and OXA-181 as well as NDM-1 [22]. Carbapenem antibiotics are available in Nigeria but have historically not been widely used in hospital medicine as they have not been part of the common antibiotic formulary. Third-generation cephalosporins, aminoglycosides and fluoroquinolones are the most prescribed antibiotics in most Nigerian hospitals. There was essentially pandrug resistance to the cephalosporins and fluoroquinolones in the isolates in the current study. The high level of phenotypic resistance to carbapenems in this collection could be the result of carriage of carbapenemases that were not detectable by the methods currently used. However, we hypothesise that the very high frequency of carriage of ESBLs (~80% of isolates of Enterobacteriaceae contained CTX-M variants) and AmpC variants in combination with porin loss (in *Pseudomonas* isolates) selected by prolonged and heavy cephalosporin usage are the cause of carbapenem resistance in these isolates. A recent study described Enterobacteriaceae isolates from the USA that were carbapenem resistant without carriage of known carbapenemases [23].

4.4. Bacterial clones and antimicrobial resistance

Whilst local antibiotic use is likely to have made an impact on the incidence of antimicrobial resistance in the collection of isolates, globally disseminated strain types of high-risk clones, e.g. *K. pneumoniae* ST11 and *P. aeruginosa* ST244 and ST233, encoding important resistance genes were identified. This is highly relevant given the mobility of the Nigerian population and the implications of this mobility in global transfer of strains and genes. For example, a recent case report documented a Canadian visitor who suffered a lower leg fracture requiring surgical repair in Nigeria and was repatriated 2 months later with a wound infected with *Klebsiella*, *Pseudomonas* and *E. coli* isolates all carrying carbapenemases [24].

K. pneumoniae strains belonging to ST11 were detected. These have been associated with the carriage of CTX-M-15 and KPC enzymes, mainly in China. ST11 is also a single-locus variant of ST258, which has been associated with the international dissemination of KPC enzymes on the pKPQIL plasmid [25]. ST258 isolates have also been recently associated with NDM carriage in India, Sweden and the UK [26]. In this study, *E. coli* ST226 was recovered from an uninfected patient; this strain type has been found circulating in highly resistant diarrhoeagenic *E. coli* in China. The other *E. coli* ST identified (ST156) has previously been found in Bangladesh [27] and in NDM-1-carrying clinical *E. coli* isolates from the UK [28].

P. aeruginosa clone ST233 has been described as a dominant international 'high-risk clone' amongst metallo-β-lactamase-producing *P. aeruginosa*, and two bla_{VIM-2}-positive isolates were found in patients in the current study. Isolates of this ST have observed seen in the UK [29] and have also been reported previously as carrying bla_{VIM-2} or bla_{IMP-1} in Norway (in an isolate thought to be imported from Ghana) [30], Japan [31] and South Africa [32]. The other *P. aeruginosa* ST identified in this study (ST244) is a globally

disseminated *P. aeruginosa* clone identified in several countries and found to carry various carbapenemases including IMP and VIM enzymes as well as ESBLs such as PER-1 and VEB-1 [33].

5. Conclusion

This study demonstrates that antimicrobial resistance in GNB in Nigeria is commonplace and compromises the effectiveness of the mainstays of broad-spectrum empirical therapy. Perhaps most worryingly, this does not appear to be a problem restricted to hospital patients, with resistance rates in commensal isolates being equally high. Establishment of a reservoir of resistant strains and antimicrobial resistance genes has occurred in Nigeria and this reservoir is likely to be mobilised globally given the youthful population and mobility of Nigerians. Data from the current study underpin the urgent requirements for enhanced surveillance of drug resistance in sub-Saharan Africa and the need for interventions to minimise the selection and transmission of antimicrobial-resistant GNB.

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Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2019.10.016.

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