*JAC Antimicrob Resist*
https://doi.org/10.1093/jacamr/dlad038

JAC-Antimicrobial Resistance

# High incidence of carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from Lagos, Nigeria

**Adesola Olalekan [1,2]*, Baris Kai Bader[2], Bamidele Iwalokun[3], Sophia Wolf[2], Albert Lalremruata[4], Adobi Dike[5], Morounke Mannie-Udoh[6], Libera Lo Presti[7], Jan Liese[2], Julia Guther [2], Paul D'alvise[2]† and Silke Peter[2,7,8]†**

*[1]Department of Medical Laboratory Science, College of Medicine, University of Lagos, Lagos, Nigeria; [2]Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany; [3]Molecular Biology & Biotechnology Department, Nigerian Institute of Medical Research, Lagos, Nigeria; [4]Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany; [5]Department of Microbiology, Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria; [6]Medical Microbiology Unit, National Orthopaedic Hospital, Lagos, Nigeria; [7]Cluster of Excellence EXC2124: Controlling Microbes to Fight Infections, Tübingen University, Tübingen, Germany; [8]German Center for Infection Research (DZIF), Partner site Tübingen, Tübingen, Germany*

*Corresponding author. E-mail: adesolaolalekan@unilag.edu.ng
@adesolaolalekan
†These authors contributed equally.

*Received 3 October 2022; accepted 14 March 2023*

**Background:** Carbapenem-resistant *Pseudomonas aeruginosa* strains are on the rise worldwide. This study characterized clinical isolates of *P. aeruginosa* from three Nigerian hospitals for carbapenem resistance.

**Methods:** Strains isolated from wounds ($n=88$), urine/catheter tips ($n=25$), sputum/tracheotomy aspirates ($n=5$), ear swabs ($n=4$) and vaginal swabs ($n=1$) were identified by MALDI-TOF and antibiotic susceptibility testing was performed using the VITEK 2 system. The genomic DNA of each isolate was subject to sequencing using Illumina and Oxford nanopore technology. Bioinformatics analyses were performed to detect antimicrobial resistance genes, clonal affiliations and phylogenetic relations of 123 non-duplicate *P. aeruginosa* isolates, whereas assembly of the nanopore reads using the plasmIDent pipeline enabled the identification of plasmids.

**Results:** Forty-three percent of the isolates were resistant to all antibiotic categories tested. More than 40% of the isolates were resistant to the carbapenems imipenem and/or meropenem (39% and 44%, respectively). Among the meropenem-resistant isolates, 48 (89%) carried at least one carbapenemase gene. The predominant one was $bla_{NDM-1}$ ($n=34$), which conferred resistance to all five antibiotic categories and highly increased the MICs of both meropenem and imipenem. The other recurrent carbapenemase genes were $bla_{VIM-2}$ ($n=4$), and $bla_{VIM-5-like}$ ($n=11$), which co-existed with $bla_{NDM-1}$ in two isolates.

**Conclusions:** The study revealed a high rate of carbapenem resistance and conjugative, broad host range plasmids carrying carbapenemase-encoding genes, especially the NDM-1 type, among isolates of *P. aeruginosa*. This may forebode the emergency of ubiquitous carbapenem resistance urging the implementation of infection control and antimicrobial stewardship strategies in Nigerian hospitals.

## Introduction

*Pseudomonas aeruginosa* is a commonly multi-drug-resistant (MDR) bacterial pathogen that causes serious infections, including ventilator-associated pneumonia, in hospitalized patients and immunodeficient individuals, but it is also prevalent in the environment.[1–3] Infections with MDR *P. aeruginosa* are difficult to treat due to limited treatment options and are associated with prolonged hospitalization and increased mortality.[4] The widespread occurrence and rising prevalence of MDR *P. aeruginosa* have become a major concern of public health practitioners, clinicians and infection control experts worldwide.[5] Carbapenems are widely used in the treatment of infections with MDR *P. aeruginosa*, and colistin is used as an antibiotic of last resort. However, the emergence and spread of carbapenem-resistant *P. aeruginosa* necessitates periodic surveillance to understand the mechanisms of carbapenem resistance and extent of dissemination.[6,7]

Carbapenemases are resilient β-lactamases that hydrolyse most β-lactam antibiotics, i.e. penicillins, cephalosporins and carbapenems. The first carbapenemases in *P. aeruginosa* and members of the Enterobacteriaceae were reported during the 1990s, and for the past two or three decades these enzymes have evolved, diversified and spread on a global scale. Apart from simple spread of introduced carbapenemase-carrying bacteria, which typically leads to outbreaks with monoclonal population structure, horizontal transfer of carbapenemase genes is an important route of dissemination.[6] Carbapenemase genes, as well as other resistance determinants, are frequently found on conjugative plasmids with broad host range and can be readily exchanged between the plasmids and host chromosomes by transposons or integrons. Thereby, locally adapted populations of pathogenic bacteria can, under the selective pressure of antibiotics, acquire multiple antibiotic resistances. In recent studies, plasmid-borne carbapenemases such as $bla_{\text{GPC-1}}$, $bla_{\text{VIM-1}}$, $bla_{\text{VIM-4}}$, $bla_{\text{VIM-11}}$, $bla_{\text{GIM}}$, $bla_{\text{SIM}}$, $bla_{\text{IMP-13}}$ and $bla_{\text{NDM-type}}$ have been reported as causes of carbapenem resistance in *P. aeruginosa* in Germany,[8] Egypt,[9] Algeria,[10] Argentina,[11] UK,[12] India[13] and South Korea.[14,15]

Based on multi-locus sequence typing (MLST), some of these studies also reported the involvement of high-risk epidemic clones of MDR *P. aeruginosa* in the dissemination of carbapenem resistance. These clones include sequence types (STs) 111, 175, 233, 235, 244, 277, 357, 381, 654, 773 and 1076. Members of these high-risk clones have an increased tendency to acquire resistance genes from other bacteria within the same hospital facilities.[16]

New Delhi metallo-β-lactamase (NDM) is a type of carbapenemase that was first isolated in 2008 in Sweden from a patient who had recently been discharged from a hospital in India.[17] Since its encoding gene is located on a multitude of transferable plasmids, it has now spread globally among various species of bacteria.[18] In central Europe, $bla_{\text{NDM-1}}$ has been detected only sporadically, e.g. in France, in a *P. aeruginosa* isolate from a patient who returned from Serbia.[19]

In Nigeria, $bla_{\text{NDM-1}}$ has been documented in *Escherichia coli* and *Klebsiella pneumoniae* before 2020.[20,21] Among the very few publications describing the molecular epidemiology of clinical MDR *P. aeruginosa* isolates in Nigeria, a study reported the presence of $bla_{\text{NDM-1}}$ in one isolate of *P. aeruginosa*.[22] Others reported the presence of $\text{bla}_{\text{VIM-1}}$,[23] $bla_{\text{VIM-2}}$ and the extended-spectrum β-lactamase (ESBL) $bla_{\text{GES-1}}$,[24,25] Since 2021, there has been an increasing number of publications describing highly resistant *P. aeruginosa* hospital isolates from Nigeria and highlighting the increased occurrence of the carbapenemase-encoding genes $bla_{NDM}$, $bla_{KPC}$ and $bla_{VIM\text{-}5}$.[26–29] Whole-genome sequencing was, to our knowledge, performed only once on a collection of 66 carbapenem-non-susceptible, Gram-negative clinical isolates from Northeast Nigeria[30]; one *P. aeruginosa* isolate, which belonged to ST-773, carried $bla_{NDM\text{-}1}$. In view of the rising prevalence of MDR *P. aeruginosa* in Nigeria,[23,26] knowledge of the mode of transmission, sources of infection, mechanisms of resistance and genetic relatedness of the isolates in local and global context is crucial for the development of appropriate mitigation and control measures in the country and provides a perspective on the current international dissemination of resistance genes.

This study aimed to determine (i) the antimicrobial susceptibility pattern and (ii) the content of antimicrobial resistance genes, with particular focus on those encoding carbapenemases, in clinical isolates of *P. aeruginosa* from tertiary hospitals in Nigeria, and (iii) to elucidate their clonal affiliation and phylogenetic relatedness. We show that a major proportion of the isolates were resistant to multiple categories of antibiotics and harboured a multitude of resistance genes. Resistance to carbapenems was common and conferred by different types of ESBL and carbapenemase-encoding genes spreading both clonally and horizontally.

## Methods

### Bacterial isolates

One-hundred and twenty-three non-duplicate isolates of *P. aeruginosa* were collected from three hospitals in Lagos State, Nigeria, between September 2018 and June 2019. The sampling was non-biased, the isolates have not been pre-selected in any way, all *P. aeruginosa* isolates obtained during the collection period from adult patients (>18 years) were included. The hospitals, namely the National Orthopaedic Hospital (NOH) ($n=58$), a specialist hospital for orthopaedic surgery, the Lagos University Teaching Hospital (LUTH) ($n=59$), a tertiary generalist hospital affiliated to the college of medicine of the University of Lagos, and the Federal Medical Centre (FMC), a secondary generalist health institution, all located in Lagos State metropolis. The isolates were obtained from both patients on admission (88) and outpatients (35). Sample materials were urine/catheter tips (25), wound swabs (88; including deep surgical swabs, diabetic foot swabs, burn wounds, crush injuries and ulcers), ear swabs (four), sputum/tracheotomy aspirates (five) and one vaginal swab. The protocol was approved by the Health Research Ethics Committee of the College of Medicine University of Lagos (approval no., CMUL/HREC/05/17/136). The species of the isolates was confirmed with MALDI-TOF mass spectrometry (Microflex LT, Bruker Daltonics, Germany), following a standard protocol.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on a VITEK 2 system, using card N-232 (both bioMerieux SA, France). The minimal inhibitory concentrations were interpreted according to the clinical breakpoints and interpretation guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, version 13.0). *P. aeruginosa* ATCC 27853 was used for quality control. The isolates tested as intermediate according to EUCAST were regarded here as susceptible. Resistance towards different classes of antibiotics was inferred from resistance to piperacillin (for penicillins), ceftazidime and/or cefepime (for cephalosporins), imipenem and/or meropenem (for carbapenems) and ciprofloxacin (for quinolones) according to the MDR Gram-negative (MRGN) classification system for Gram-negative bacteria.[31] In addition, we included aminoglycosides (derived from tobramycin resistance, EUCAST) as a fifth antibiotic category.

### mCIM test

To test for phenotypic carbapenemase activity, 10 carbapenemase or ESBL-encoding isolates of different STs were selected for mCIM-tests (modified Carbapenem Inactivation).[32,33] For each isolate, one inoculation loop of colony mass (~1 µL) was homogenized in 2 mL of soy broth by vortexing, one 10 µg-meropenem disc was added and incubated for 4 h at 35°C. A suspension of the indicator strain *E. coli* ATCC25922 in sterile 0.9% NaCl solution (McFarland 0.5) was spread on a Müller–Hinton agar plate and the pre-exposed meropenem disc was placed on the agar. After incubation of the test plate for 18–24 h at 35°C, the diameter of the inhibition zone around the disc was measured. Diameters of 15 mm

or less indicate meropenem inactivation, diameters of 19 mm or more indicate no carbapenemase activity.

### DNA extraction, library preparation and whole-genome sequencing (WGS)

For all 123 study isolates, genomic DNA was extracted from discrete colonies of overnight subcultures using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany). The genomic DNA was sheared (Covaris M220, Woburn, USA) to obtain 550-bp fragments, and libraries were prepared using the TruSeqNano DNA LT Kit (Illumina, San Diego, USA) according to the manufacturer's standard protocol. The prepared barcoded libraries were quantified on the Invitrogen Qubit 4 fluorometer (Thermo Fisher Scientific, Germany) and analysed on the QIAxcel Advanced capillary electrophoresis instrument (Qiagen, Hilden, Germany). All libraries were sequenced bidirectionally, either on an Illumina NextSeq instrument with 2×150 bp reads using the NextSeq 500/550 High Output Kit v2.5 (300 cycles) (Illumina, San Diego, USA), or on an Illumina MiSeq instrument with 2×250 bp reads (500 cycles). For determination of the plasmid content, the same 10 isolates as selected for the mCIMtests were subjected to long-read sequencing using Oxford Nanopore Technologies MinION (chemistry SQK-LSK109, flowcell R9.4.1/FLO-MIN106D) or PromethION (chemistry SQK-LSK109, flowcell R9.4.1/FLO-PRO002) following standard protocols.

### Sequence assembly and bioinformatic analysis

The sequencing reads were assembled using the A5 pipeline (v.20140604)[34] and SPAdes v.3.7.0,[35] as previously described.[36] The core genome of all isolates was calculated using Spine (v.0.1.2).[37] applying the default settings except for the segment length, which was adjusted to 500 bp and an identity of 85%. Prophage regions were detected using PHASTER[38] and removed from the core genomes using a customized script. This resulted in a core genome of 5.182 MB. Single nucleotide polymorphisms were called applying a customized script comprising GATK (v.3.2–2) and SAM tools (v.0.1 19).[39,40] A maximum likelihood estimation was performed using IQ tree v.1.6.3[41–43] (UF boot mode with parameters model Finder and 1000 bootstraps) followed by visualization applying Figtree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). For additional confirmation of the species identification, the average nucleotide identity based on the ANI algorithm was calculated using JSpecies v.1.2.[44] For the identification of resistance genes and ST, the assembled WGS dataset was uploaded to ResFinder v.2.1 on the Centre of Genomic Epidemiology website (https://cge.food.dtu.dk/services/ResFinder/).[45] The current distinction of β-lactamases into carbapenemases and ESBLs was adopted from the European beta-lactamase database (March 2023).[46] STs were assigned using the PubMLST website (https://pubmlst.org/).[47] The nanopore reads were assembled using the plamIDent pipeline for identification of plasmids.[48] Putative plasmid sequences were used to BLAST-search the NCBI nucleotide database for homologous sequences. Homology with known plasmids and rearrangements of syntenic sequences were visualized using progressive MAUVE.[49] Prophages were identified in isolates 17 and 84 (both 5386 bp) and in isolate 24 (7849 bp). They did not harbour any resistance genes and were not further analysed.

## Results

### General characteristics and antimicrobial susceptibility

A total of 123 unique *P. aeruginosa* isolates were recovered from clinical samples of 65 male and 58 female patients from 20 to 85 years of age (Table 1). Most of the isolates originated from wounds (71.5%) and from urine or urinary catheters (20.3%). For all *P. aeruginosa* isolates, the minimal inhibitory concentration (MIC) of 10 antibiotics was determined (Table 2). Remarkably, >70% of the isolates were resistant to the fluoroquinolones, ciprofloxacin and levofloxacin, 59%/55% were resistant to the aminoglycosides gentamicin and tobramycin and 39%/44% of the isolates were resistant to the carbapenems imipenem and meropenem. Thirty-one isolates (25%) were susceptible to all antibiotics tested, while 11 isolates (9%) were resistant to one antibiotic category, mostly to fluoroquinolones. Eighty-one (66%) were resistant to fluoroquinolones and at least one more antibiotic category, and 53 of these (44%) showed resistance towards all five of the tested antibiotic categories, i.e. penicillins, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones, and are thus classified as 4MRGN (Table 3).

### Antimicrobial resistance gene content of the isolates

Fifty-four (44%) of the 123 *P. aeruginosa* isolates from this study were resistant to meropenem, in 48 (89%) of the meropenem-resistant isolates at least one carbapenemase gene was identified, and 3 (6%) of the meropenem-resistant isolates encoded an ESBL. The most prevalent carbapenemase gene in the genomes of all isolates was the New Delhi metallo-β-lactamase gene $bla_{\text{NDM-1}}$. This was present in 34 isolates, which all belonged to ST-773, were resistant to all five antibiotic categories, and had high MICs for both meropenem and imipenem. Thirty-two of these isolates harboured $bla_{\text{NDM-1}}$ as the only carbapenemase gene. The second most prevalent carbapenemase gene was $bla_{\text{VIM-5}}$, which was found in the genomes of 11 isolates. $bla_{\text{VIM-2}}$ was found in four isolates. Two isolates carried two carbapenemase genes ($bla_{\text{NDM-1}}$ and $bla_{\text{VIM-5-like}}$). The ESBL $bla_{\text{GES-9}}$, conferred the lowest levels of carbapenem resistance (meropenem and imipenem intermediate). Detailed records of the antibiotic resistance genes detected in the carbapenem-resistant isolates are summarized in Table S1 (available as Supplementary data at *JAC* Online). The other antibiotic resistance genes found in the genomes of the $bla_{\text{NDM-1}}$—carrying isolates were $bla_{\text{CARB-2}}$, $bla_{\text{CARB-4}}$, $bla_{\text{GES-5}}$, $bla_{\text{GES-9}}$, $bla_{\text{NPS-like}}$, $bla_{\text{OXA-4}}$, $bla_{\text{OXA-10}}$, $bla_{\text{TEM-1B}}$ (beta-lactam resistance), *aph* (3′)-IIb-like, *aph (3′)-III-like*, aph(3′)-XV, *aadB*-like, *aadA1*-like, *aac*(3)-IIa, *rmtB*-like (aminoglycoside resistance), *qnrVC1* (fluoroquinolones resistance), *fosA-like* (fosfomycin resistance), *sul1* (sulfonamide resistance), *tetG* (tetracycline resistance), *catB7* (chloramphenicol resistance) and *arr2* (arsenate resistance).

### Carbapenemase production (mCIM test)

A phenotypic test for carbapenemase production, the modified Carbapenem Inactivation Method (mCIM) was performed on 10 carbapenem-resistant isolates from different STs (Table 4). The isolates with $bla_{NDM-1}$ and $bla_{VIM-5}$ tested positive for carbapenemase production. Isolate number 7 with the carbapenemase $bla_{\text{VIM-2}}$ tested borderline negative. The isolates harbouring extended-spectrum β-lactamases (ESBLs) tested negative, although some were highly carbapenem resistant.

### Multi-locus sequence typing (MLST)

MLST analysis allowed the classification of the 123 isolates in 47 STs, 13 of which were newly assigned. The most prevalent was

**Table 1.** Origin of the *P. aeruginosa* isolates

| | Hospital A | Hospital B | Hospital C | Total |
|---|---|---|---|---|
| Catheter tip/urine | 9 (7.3%) | 14 (11.4%) | 2 (1.6%) | 25 (20.3%) |
| Wound swab | 49 (39.8%) | 37 ((30%) | 2 (1.6%) | 88 (71.5%) |
| Ear swab | — | 2 (1.6%) | 2 (1.6%) | 4 (3.2%) |
| High vaginal swab | — | 1 (0.8%) | — | 1 (0.8%) |
| Sputum/tracheotomy tube | — | 5 (4.1%) | — | 5 (4.1%) |
| Sex: | 31/27 | 25/34 | 2/4 | 58/65 |
| Female/male | | | | 47.1%/52.8% |
| Mean age (years) | 45 | 45 | 44 | 45 |
| Meropenem-resistant | 23 (39.6%) | 27 (45.8%) | 4 (66.7%) | 54 (43.9%) |
| Period of sample collection | 8/9/2018 -19/6/2019 | 5/11/2018 -18/5/2019 | 14/2/2019 - 6/3/2019 | |
| Total number | 58 (47.6%) | 59 (47.6%) | 6 (4.8%) | 123 (100%) |

**Table 2.** Proportions of resistant *P. aeruginosa* isolates in this study

| Category | Antibiotic | MIC range (mg/L) | Breakpoint (mg/L) (resistant >) | *n* of resistant (total *n* = 123) | Percentage resistant (%) |
|---|---|---|---|---|---|
| Penicillins | Piperacillin | ≤4–>128 | 16 | 76 | 62 |
| (+β-lactamase inhibitor) | Piperacillin/Tazobactam | ≤4–>128 | 16 | 73 | 59 |
| Cephalosporins | Ceftazidime | ≤1–>64 | 8 | 64 | 52 |
| | Cefepime | ≤1–>64 | 8 | 55 | 45 |
| Carbapenems | Imipenem | ≤0.25–>16 | 4 | 48 | 39 |
| | Meropenem | ≤0.25–>16 | 8 | 54 | 44 |
| Aminoglycosides | Gentamicin | ≤1–>16 | 8 | 72 | 59 |
| | Tobramycin | ≤1–>16 | 8 | 68 | 55 |
| Fluoroquinolones | Ciprofloxacin | ≤0.5–>4 | 0.5 | 87 | 71 |
| | Levofloxacin | 1–8 | 2 | 91 | 74 |

ST-773 (35 isolates), followed by ST-2613 (15), ST-381 (6), ST-244 (6), ST-233 (6), ST-357 (4), ST-1203 (3), ST-277 (3) and ST-654 (3), ST-238 (2), ST-316 (2), ST-1076 (2). Others were ST-217, ST-234, ST-242, ST-258, ST-260, ST-261, ST-270, ST-303, ST-308, ST-463, ST-487, ST-569, ST-571, ST-639, ST-903, ST-1117, ST-1400, ST-2021, ST-2329, ST-2340, ST-3004, ST-3069, ST-3205 and the 13 newly assigned ST-3391 to ST-3402. There was no noticeable bias in the distribution of STs, both between hospitals and sample types. Interestingly, 34 of 35 isolates belonging to ST-773 carried the $bla_{NDM-1}$ carbapenemase gene, all isolates of ST-654 carried $bla_{VIM-5-like}$, all isolates of ST-1203 carried the ESBL gene $bla_{GES-9}$, and 4 of 6 isolates belonging to ST-233 carried $bla_{VIM-2}$. This pattern of different carbapenemase and ESBL-types being restricted to specific STs indicates clonal spread of recently introduced strains as a major source of carbapenem resistance within the study population.

### *Phylogeny analysis*

The genetic relatedness of the isolates was assessed by using the core genome polymorphism data to construct a phylogenetic tree (Figure 1) further details can be found at https://microreact.org/project/fJtGKnBHjPvEZJaTowFkZZ. The $bla_{NDM-1}$-carrying isolates, which all belonged to ST-773, clustered closely together. This indicates very close relatedness of the $bla_{NDM-1}$ carrying isolates, which probably share a recent common ancestor and thus form a clonal complex. However, also singletons were observed among the carbapenem-resistant isolates, such as ST-639, which originates from ST-773. Phylogenetic diversity was highest among the carbapenem-susceptible isolates, which scattered all over the dendrogram, unlike the carbapenem-resistant isolates, which clustered together and likely constitute clonal complexes.

### *Plasmids*

Long-read sequencing was performed on 10 carbapenemase and ESBL-harbouring isolates (same isolates in mCIM test) from different STs that represent the different clades of the ML phylogeny (Table 5, Figure 2). This approach allowed analysis of plasmid content and locating the carbapenemase genes to plasmid or chromosome. Overall, circular DNA structures were found in six of 10 isolates, plasmids were identified in five of 10 isolates, and circular prophages were detected in three isolates. Three of five identified plasmids contained resistance genes.

**Table 3.** Patterns of co-occurring resistances in selected antibiotic categories among the *P. aeruginosa* isolates ($n=123$), according to the MRGN-classification system for MDR Gram-negative bacteria[31]

| Number of resistances (antibiotic categories) | Number of isolates (%) | Penicillins[a] | Cephalosporins[a] | Carbapenems[a] | Amino-glycosides[a] | Fluoro-quinolones[a] |
|---|---|---|---|---|---|---|
| 0 | 31 (25%) | 0 | 0 | 0 | 0 | 0 |
| 1 | 11 (9%) | 0 | 0 | 0 | 1 | 10 |
| 2 | 8 (6%) | 3 | 0 | 0 | 5 | 8 |
| 3 | 9 (7%) | 9 | 6 | 0 | 3 | 9 |
| 4 | 11 (9%) | 11 | 11 | 1 | 10 | 11 |
| 5 | 53 (43%) | 53 | 53 | 53 | 53 | 53 |
| Total | 123 | 76/62% | 70/57% | 54/44% | 72/59% | 91/74% |

[a]Number of isolates resistant to the respective class of antibiotics.

**Table 4.** Carbapenemase production of selected isolated; mCIM test results. A 10 µg meropenem paper disc is incubated in a suspension of the isolate and is thereafter laid on a lawn of the meropenem-susceptible *E. coli* indicator strain. A lack of inhibition (<15 mm; positive) indicates meropenem inactivation by carbapenemase activity

| Isolate number | Inhibition zone (mm) *E. coli* ATCC25922 | Interpretation | Meropenem MIC (mg/L) | Imipenem MIC (mg/L) | Beta-lactamase or carbapenemase | Sequence type |
|---|---|---|---|---|---|---|
| 3 | 0 | **positive** | ≥16 | ≥16 | ***blaNDM-1*** | ST-773 |
| 7 | 18 | borderline | ≥16 | ≥16 | *blaVIM-2* | ST-233 |
| 19 | 25 | negative | 4 | 2 | *blaOXA-10* | ST-233 |
| 20 | 0 | **positive** | 4 | ≥16 | ***blaVIM-5-like*** | ST-654 |
| 24 | 25 | negative | 4 | 2 | *blaGES-9* | ST-1203 |
| 32 | 0 | **positive** | ≥16 | ≥16 | ***blaNDM-1, blaVIM-5-like*** | ST-773 |
| 40 | 0 | **positive** | ≥16 | ≥16 | ***blaVIM-5-like*** | ST-639 |
| 84 | 0 | **positive** | ≥16 | ≥16 | ***blaNDM-1*** | ST-773 |
| 96 | 0 | **positive** | 8 | ≥16 | ***blaVIM-5-like*** | ST-2613 |
| 119 | 22 | negative | ≥16 | ≥16 | *blaGES-5* | ST-2613 |

Bold font (isolates that are positive for mCIM test with their corresponding carbapenemase gene).

Isolate 32 contained a small plasmid of 20 197 bp carrying the carbapenemase gene $bla_{NDM-1}$, in addition to other antibiotic resistance genes [*aac*(3), *tet*(G), *floR*2] and rich in transposase sequences (5800/20 197 bp). The full plasmid sequence is identical (99.9%) to a transposase-flanked scaffold, probably a plasmid, in the genome of *P. aeruginosa* strain PSE6684 from South Korea (CP053917). Isolate 84 harboured a large plasmid of 200 693 bp, encoding a beta-lactamase (NPS-like) and resistance against aminoglycosides and trimethoprim. Of its sequence, 75% is homologous to a plasmid of the extensively drug resistant *P. aeruginosa* hospital isolate PA83 from Germany (CP017294).[55]

The plasmid in isolate 40 is a conjugative, broad host range megaplasmid of 453 289 bp. It is widely identical to the MDR *P. aeruginosa* megaplasmids pBT2436 (Figure 2; CP039989; 83% coverage, 99% identity) and pBT2101 (CP039991; 81% cov, 99% id), which were isolated in Thailand,[51] the carbapenemase-containing *P. aeruginosa* plasmid pPUV-7 (83% cov, 98.5% id), which was isolated in Poland,[52] and with the *P. aeruginosa* MDR plasmid pPAG5 (83% cov, 98.5% id), which was isolated in China (Figure 2).[53] However, the MDR region has different resistance determinants in any of these plasmids ($bla_{VIM-5}$, *qnrVC*1, *aadA*1, *qacE*, *sul*1), and is flanked by transposase genes and insertion sequences that facilitate exchange of resistance cassettes. One locus in the plasmid of isolate 40 (242 000…289 000), which contains genes involved in plasmid transfer and partition (e.g. *trb*-family members) and components of efflux transporter systems, does not align with other known *Pseudomonas* plasmids. It is identical (99.9%) to the broad host range plasmid pE33, which was isolated from *Citrobacter freundii* in Australia (CP042518),[54] indicating possible horizontal gene transfer with a wide range of Gram-negative bacteria.

## Discussion

Studies in Nigeria have shown that *P. aeruginosa* is a leading cause of morbidity and mortality among hospitalized patients in health institutions.[56–60] However, data on antibiotic susceptibility and resistance mechanisms, especially for carbapenem antibiotics, are limited in many of the local hospitals, which impedes adequate case management and efficient containment strategies for extensively antibiotic resistant strains. This is

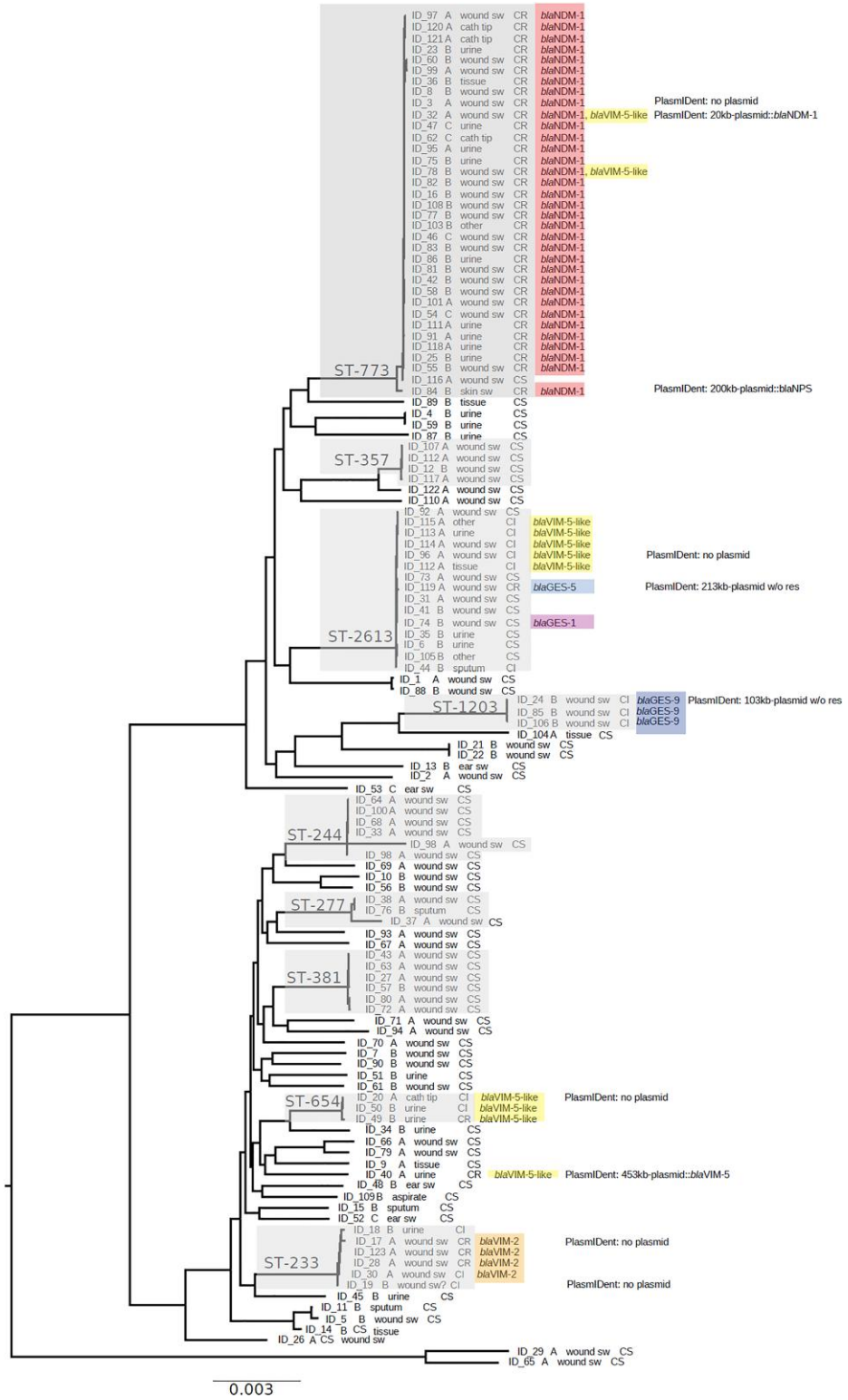


**Figure 1.** Maximum likelihood phylogeny generated from the core genomes of the 123 *P. aeruginosa* isolates of this study. Clusters of equal sequence types are marked by grey boxes with ST-numbers. Leaf labels denote isolate ID, origin (Hospital A, B, C), sample material, carbapenem susceptibility (according to EUCAST; CS: carbapenem susceptible, CI: carbapenem intermediate, CR: carbapenem resistant) and, if detected, the type of carbapenemase (red: $bla_{\text{NDM-1}}$, yellow: $bla_{\text{VIM-5-like}}$, light blue: $bla_{\text{GES-5}}$, ochre: $bla_{\text{VIM-2}}$) or ESBL (purple: $bla_{\text{GES-1}}$, blue: $bla_{\text{GES-9}}$). Ten isolates were selected for Nanopore sequencing to generate long reads for plasmid identification using the PlasmIDent tool;[48] plasmid size and plasmid-encoded carbapenemase are stated after the respective leaf labels. Further details can be found https://microreact.org/project/fJtGKnBHjPvEZJaTowFkZZ.

the first comprehensive, whole-genome-sequence-based report of numerous carbapenemase-producing, clinical isolates of *P. aeruginosa* from Nigeria, which elucidates their resistance mechanisms and their evolutionary trajectory.

In the present study, the prevalence of carbapenem resistance in clinical *P. aeruginosa* isolates was 39% for imipenem and 44% for meropenem. In 2018, a report from central Nigeria described 200 isolates with 12% imipenem and 28% meropenem resistance, which could be ascribed to the sole presence of the carbapenemase $bla_{VIM-1}$.[23] A recent study from Southwest Nigeria found that 17% of clinical *P. aeruginosa* isolates were extensively drug resistant and harboured metallo-β-lactamase genes.[28] Thirteen different carbapenemase and ESBL genes were detected in a collection of 430 isolates, while in the present study four different carbapenemase and two different ESBL genes were identified in 123 isolates. A recent study from Northeast Nigeria reported $bla_{NDM-1}$ in one clinical *P. aeruginosa* isolate of ST-773, in addition to other carbapenemase-encoding genes ($bla_{NDM}$, $bla_{KPC}$) found in other Gram-negative clinical isolates.[27]

The samples for our study were taken in secondary and tertiary hospitals. These hospitals may have a larger fraction of patients with serious infections and therefore an increased use of carbapenems, selecting for resistant bacteria that may cause additional nosocomial infections. This could explain the considerable difference in the fractions of highly resistant, carbapenemase-producing isolates between the studies. Moreover, the samples were taken in Lagos, the economic hub of the country, where international exchange and a greater population density may accelerate import and spread of resistant bacteria and antibiotic resistance genes, and periodical flooding events may facilitate genetic exchange among pathogenic bacteria. Nonetheless, local small-scale differences aside, our study contributes to the growing scientific evidence that antibiotic resistance of hospital isolates in Western Africa has dramatically increased.[61] Most of the carbapenemase-encoding isolates in our study were resistant to all the antibiotics tested, especially the isolates carrying the carbapenemase $bla_{NDM-1}$, which indicates spread of carbapenemase-encoding genes as central driver of multiple antibiotic resistance.

**Table 5.** Plasmids of selected isolates, as identified by PlasmIDent[48] analysis of Nanopore reads, and plasmid-bound resistance, as identified by ResFinder.[45,50] Prophages are not shown

| Isolate ID | Plasmid size (bp) | Resistance genes |
|---|---|---|
| 3 | no plasmid | |
| 17 | no plasmid | |
| 19 | no plasmid | |
| 20 | no plasmid | |
| 24 | 103 560 | none |
| 32 | 20 197 | ***bla*$_{NDM-1}$**, *tetG*, *floR2*, *aac3* |
| 40 | 453 289 | ***bla*$_{VIM-5}$**, *qnrVC1*, *aadA1*, *qacE*, *sul1*, *terD* |
| 84 | 200 693 | $bla_{NPS}$, *dfrB5*, *ant(2'')-Ia* |
| 96 | no plasmid | |
| 119 | 213 132 | none |

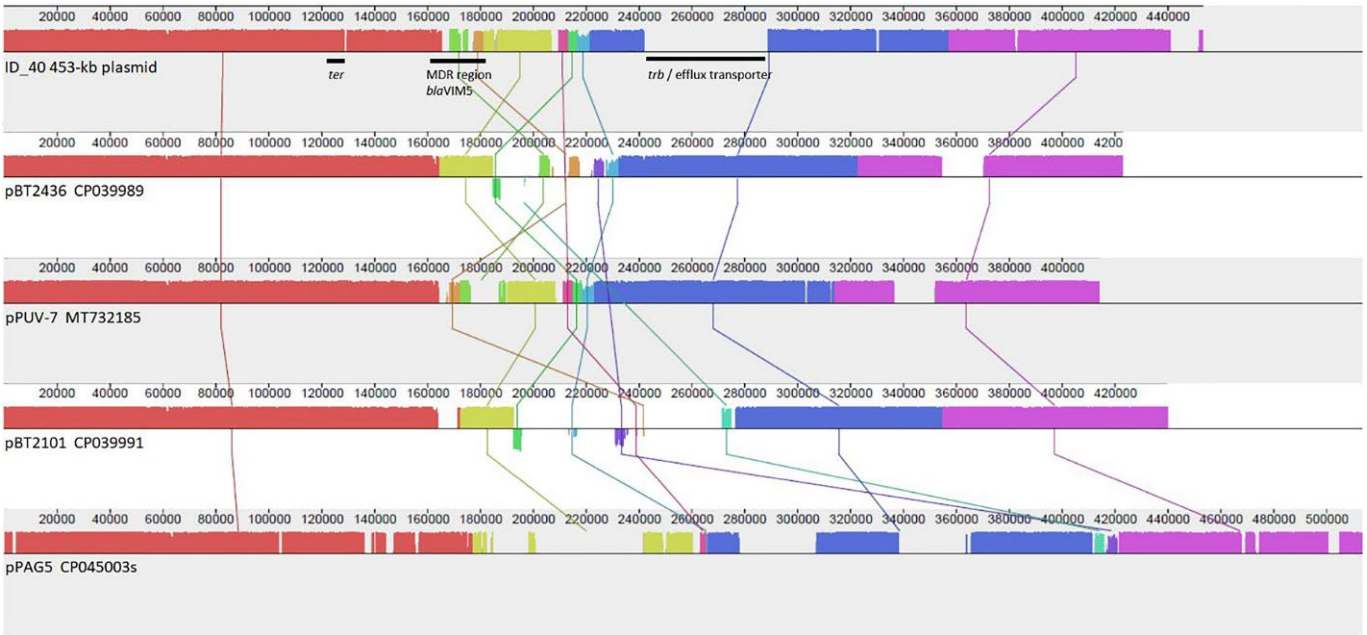


**Figure 2.** Alignment of syntenic sequences (locally colinear blocks) of the $bla_{VIM-5}$-containing megaplasmid of isolate 40 (ID_40) with the closely related *P. aeruginosa* plasmids pBT2436, pBT2101 from Thailand (CP039989, CP039991),[51] pPUV-7 from Poland (MT732185)[52] and pPAG5 from China (CP045003),[53] The $bla_{VIM-5}$ gene is situated, together with other resistance determinants, in a plastic MDR region that shows several traces of horizontal gene transfer. There is a 47-kb region (242'000..289'000) that encodes numerous factors mediating plasmid partition and conjugative transfer (e.g. trb-family genes) as well as efflux transporter components. This region does not align with other *Pseudomonas* plasmids, but it is 99.9% identical to a region in a 243-kb conjugative megaplasmid that was isolated from *Citrobacter freundii* in Australia (CP042518).[54].

In a setting where almost half the *P. aeruginosa* isolates are carbapenem resistant, treatment options are very limited, and antibiotic susceptibility testing is crucial. Ceftazidime/avibactam may be an option for *P. aeruginosa* strains producing Ambler class-B ESBLs/carbapenemases (e.g. GES, KPC type). For the highly resistant $bla_{NDM-1}$ and $bla_{VIM-5}$-producing strains, cefiderocol or colistin could be tried as last-resort antibiotics for life-threatening infections. Unfortunately, in MDR *P. aeruginosa* resistance against both cefiderocol and colistin can arise rapidly.[55]

In contrast to these findings from Nigeria, carbapenem resistance in *P. aeruginosa* isolates from European countries and the US is mostly conferred by loss or decreased expression of the outer membrane porin OprD, often in combination with upregulation of the MexAB-OprM efflux system.[62–64] Also, the inducible, chromosomal-encoded beta-lactamase AmpC of *P. aeruginosa* plays a role in resistance to carbapenems. In our isolate collection, however, the carbapenemase $bla_{NDM-1}$ is the dominant mechanism of carbapenem resistance.

The STs of the isolates, as revealed by MLST analysis, agree with the phylogeny reconstructed from their core genomes and also with the distribution of carbapenemase and ESBL gene types (Figure 1). Every carbapenemase is specific to one ST, except $bla_{VIM-5}$, which was found in isolates of four different STs. $bla_{NDM-1}$ was only present in ST-773, and $bla_{VIM-2}$ was only found in ST-233. Both ST-233 and ST-733 were described as 'international high-risk clones', i.e. internationally dispersed clones that readily acquire and frequently carry carbapenemase genes and other resistance determinants.[12,16,65] ST-773 seems to have originated from Asia,[66] but it has since been detected worldwide.[12,16,66] ST-773 was initially described to carry the carbapenemase $bla_{VIM-2}$. The combination of ST-773 and $bla_{NDM-1}$ was first reported in Hungary in 2019,[16] and in 2022 from Nigeria.[30] An introduction from Asia seems plausible considering common business travels from and to Asian countries and the previous records of the respective sequence and carbapenemase types. However, due to the limited data in the database and the high mobility of both plasmids and strains of *P. aeruginosa*, the source of the original $bla_{NDM-1}$-carrying ST-773 strain that was introduced to Nigeria cannot be determined. Other international high-risk clones in our isolate collection are ST-654 (three isolates with $bla_{VIM-5}$), ST-277 and ST-357 (both of the latter without carbapenemase). Taken together, 51 of our 123 isolates (41%) belong to previously described international high-risk clones. The uniformity in resistance determinants and close phylogenetic relation of the isolates within each of these clones indicates that they may have been introduced to Nigeria recently, and have spread clonally due to a selective advantage, probably their increased antibiotic resistance. In contrast, the isolates of the 13 newly assigned STs (ST3391-3402) within our collection, which are mostly susceptible to all the antibiotics tested (four are resistant to quinolones), may represent the primary local population of *P. aeruginosa*. The backbones of plasmids from our isolate collection that encode carbapenemases are highly identical to other plasmids of isolates from different continents (Asia, Australia, Europe). Therefore, both the resistance genes and their vectors indicate that antibiotic resistances are subject to dispersal and exchange on a global scale. The observed spread of $bla_{NDM-1}$ and other carbapenemases on plasmids with broad host range such as the $bla_{NDM-1}$-carrying pE33-like plasmid of our isolate 32 is worrisome, considering the possible transfer to other pathogenic bacteria in the environment, or in a hospital setting. This is exemplified by the finding that multiple *Vibrio parahaemolyticus* and *V. vulnificus* strains carrying $bla_{NDM-1}$ and other carbapenemases were isolated from seawater on different beaches in Lagos State, Nigeria.[67]

In conclusion, the exceptional prevalence of carbapenemases, particularly $bla_{NDM-1}$, in Nigerian hospitals highlights the global rise in carbapenem resistance mediated by carbapenemases and emphasizes the necessity of limiting the continuing spread of antimicrobial resistance. This can be achieved by controlling and reducing antibiotic use in agriculture, by adequate wastewater treatment, and by implementing routine susceptibility testing, antibiotic stewardship programmes and improved hygiene measures in hospitals to limit the application of last-resort antibiotics and the spread of highly resistant bacteria.

## Data availability

The data for this study have been deposited in the European Nucleotide Archive at EMBL/EBI under accession number PRJEB59020.

## Acknowledgement section

We thank Jennifer Müller and Ulrich Schoppmeier (NGS Competence Center Tübingen, NCCT) for their help with the sequence data submission. Some results of this study were presented as a poster (Abstract Number 6275) at the 30th ECCMID 2020 conference.

## Funding

A.O. was supported by German Academic Exchange Service (DAAD) for a research visit to Germany (grant 91732270). S.P. was supported by infrastructural funding from the German Research Foundation (DFG), Cluster of Excellence EXC 'Controlling Microbes to Fight Infections' (grant number 2124 – 390838134).

## Transparency declarations

S.P. consulted for IDbyDNA and received speaker's honoraria from bioMerieux. This did not have any influence on the study design, data interpretation and manuscript preparation. All other authors declare no competing interests.

## Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online.

## References

**1** Pang Z, Raudonis R, Glick BR *et al*. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnol Adv* 2019; **37**: 177–92. https://doi.org/10.1016/j.biotechadv.2018.11.013

**2** Pérez A, Gato E, Pérez-Llarena J *et al*. High incidence of MDR and XDR *Pseudomonas aeruginosa* isolates obtained from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the

MagicBullet clinical trial. *J Antimicrob Chemother* 2019; **74**: 1244–52. https://doi.org/10.1093/jac/dkz030

**3** Adesoji AT, Ogunjobi AA, Olatoye IO. Molecular characterization of selected multidrug resistant *Pseudomonas* from water distribution systems in southwestern Nigeria. *Ann Clin Microbiol Antimicrob* 2015; **14**: 1–11.

**4** Sanders CD, Guimbellot JS, Muhlebach MS *et al*. Tracheostomy in children: epidemiology and clinical outcomes. *Pediatr Pulmonol* 2018; **53**: 1269–75. https://doi.org/10.1002/ppul.24071

**5** Cerceo E, Deitelzweig SB, Sherman BM *et al*. Multidrug-resistant gram-negative bacterial infections in the hospital setting: overview, implications for clinical practice, and emerging treatment options. *Microb Drug Resist* 2016; **22**: 412–31. https://doi.org/10.1089/mdr.2015.0220

**6** Yoon EJ, Jeong SH. Mobile carbapenemase genes in *pseudomonas aeruginosa*. *Front Microbiol* 2021; **12**: 1–21. https://doi.org/10.3389/fmicb.2021.614058

**7** Labarca JA, Salles MJ, Seas C *et al*. Carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in the nosocomial setting in Latin America. *Critic Rev Microbiol* 2016; **42**: 276–92.

**8** Schauer J, Gatermann SG, Hoffmann D *et al*. GPC-1, a novel class A carbapenemase detected in a clinical *Pseudomonas aeruginosa* isolate. *J Antimicrob Chemother* 2020; **75**: 911–16. https://doi.org/10.1093/jac/dkz536

**9** Hassuna NA, Darwish MK, Sayed M *et al*. Molecular epidemiology and mechanisms of high-level resistance to meropenem and imipenem in *Pseudomonas aeruginosa*. *Infect Drug Resist* 2020; **13**: 285–93. https://doi.org/10.2147/IDR.S233808

**10** Merradi M, Kassah-Laouar A, Ayachi A *et al*. Occurrence of VIM-4 metallo-β-lactamase-producing *Pseudomonas aeruginosa* in an Algerian hospital. *JIDC* 2019; **13**: 284–90. https://doi.org/10.3855/jidc.10679

**11** Elena A, Quinteros M, Di Conza J *et al*. Full characterization of an IncR plasmid harboring qnrS1 recovered from a VIM-11-producing *Pseudomonas aeruginosa*. *Rev Argent microbiol* 2020; **52**: 298–304.

**12** Wright LL, Turton JF, Livermore DM *et al*. Dominance of international 'high-risk clones' among metallo-β-lactamase-producing *Pseudomonas aeruginosa* in the UK. *J Antimicrob Chemother* 2015; **70**: 103–10. https://doi.org/10.1093/jac/dku339

**13** Verma N, Prahraj AK, Mishra B *et al*. Detection of carbapenemase-producing *Pseudomonas aeruginosa* by phenotypic and genotypic methods in a tertiary care hospital of East India. *J Lab Physicians* 2019; **11**: 287–91. https://doi.org/10.4103/JLP.JLP_136_19

**14** Choi YJ, Kim YA, Junglim K *et al*. Emergence of NDM-1-producing *Pseudomonas aeruginosa* sequence type 773 clone: shift of carbapenemase molecular epidemiology and spread of 16S rRNA methylase genes in Korea. *Ann Lab Med* 2023; **43**: 196–99. https://doi.org/10.3343/alm.2023.43.2.196

**15** Hong JS, Song W, Park MJ *et al*. Molecular characterization of the first emerged NDM-1-producing *Pseudomonas aeruginosa* isolates in South Korea. *Microb Drug Resist* 2021; **27**: 1063–70. https://doi.org/10.1089/mdr.2020.0374

**16** Kocsis B, Toth A, Gulyas D *et al*. Acquired qnrVC1 and blaNDM-1 resistance markers in an international high-risk *Pseudomonas aeruginosa* ST773 clone. *J Med Microbiol* 2019; **68**: 336–38. https://doi.org/10.1099/jmm.0.000927

**17** Yong D, Toleman MA, Giske CG *et al*. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 2009; **53**: 5046–54. https://doi.org/10.1128/AAC.00774-09

**18** Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. *J Med Microbiol* 2013; **62**: 499–513. https://doi.org/10.1099/jmm.0.052555-0

**19** Flateau C, Janvier F, Delacour H *et al*. Recurrent pyelonephritis due to NDM-1 metallo-beta-lactamase producing *Pseudomonas aeruginosa* in a patient returning from Serbia, France, 2012. *Euro Surveill* 2012; **17**: 20311. https://doi.org/10.2807/ese.17.45.20311-en

**20** Ogbolu DO, Webber MA. High-level and novel mechanisms of carbapenem resistance in Gram-negative bacteria from tertiary hospitals in Nigeria. *Int J Antimicrob Agents* 2014; **43**: 412–7. https://doi.org/10.1016/j.ijantimicag.2014.01.014

**21** Olalekan A, Onwugamba F, Iwalokun B *et al*. High proportion of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* among extended-spectrum β-lactamase-producers in Nigerian hospitals. *JGAR* 2020; **21**: 8–12.

**22** Shettima SA, Tickler IA, Dela Cruz CM *et al*. Characterisation of carbapenem-resistant Gram-negative organisms from clinical specimens in Yola, Nigeria. *J Glob Antimicrob Resist* 2020; **21**: 42–5.

**23** Zubair KO, Iregbu KC. Resistance pattern and detection of metallo-beta-lactamase genes in clinical isolates of *Pseudomonas aeruginosa* in a central Nigeria tertiary hospital. *Nigerian J Clin Pract* 2018; **21**: 176–82.

**24** Cholley P, Ka R, Guyeux C *et al*. Population structure of clinical *Pseudomonas aeruginosa* from west and central African countries. *PLoS ONE* 2014; **9**: 1–9. https://doi.org/10.1371/journal.pone.0107008

**25** Ogbolu DO, Piddock LJV, Webber MA. Opening Pandora's box: high-level resistance to antibiotics of last resort in Gram-negative bacteria from Nigeria. *JGAR* 2020; **21**: 211–17.

**26** Akinloye AO, Adefioye JO, Adekunle CO *et al*. Multidrug-resistance genes in *Pseudomonas aeruginosa* from wound infections in a tertiary health institution in Osogbo, Nigeria. *Infect Disord Drug Targets* 2021; **21**: 90–8. https://doi.org/10.2174/1871526520666200117112241

**27** Onipede A, Odetoyin B, Ojo O *et al*. Detection of NDM-1 in metallo-beta-lactamase-producing strains of *Pseudomonas aeruginosa* isolates from a tertiary hospital in Southwest Nigeria. *West Afric J Med* 2021; **38**: 1072–79. https://doi.org/10.55891/wajm.v38i11.16

**28** Olaniran OB, Adeleke OE, Donia A *et al*. Incidence and molecular characterization of carbapenemase genes in association with multidrug-resistant clinical isolates of *Pseudomonas aeruginosa* from tertiary healthcare facilities in Southwest Nigeria. *Curr Microbiol* 2021; **79**: 27.

**29** Karlowsky JA, Bouchillon SK, El Mahdy Kotb R *et al*. Carbapenem-resistant enterobacterales and *Pseudomonas aeruginosa* causing infection in Africa and the Middle East: a surveillance study from the ATLAS programme (2018–20). *JAC Antimicrob Resist* 2022; **4**: 1–8. https://doi.org/10.1093/jacamr/dlac060

**30** Tickler IA, Shettima SA, Dela Cruz CM *et al*. Characterization of carbapenem-resistant gram-negative bacterial isolates from Nigeria by whole genome sequencing. *Diagn Microb Infect Dis* 2021; **101**: 1–6. https://doi.org/10.1016/j.diagmicrobio.2021.115422

**31** Kaase M. MRGN: new classification for multidrug-resistant Gram-negative bacteria. *Lab Medizin* 2016; **37**: 1–5.

**32** van der Zwaluw K, de Haan A, Pluister GN *et al*. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS ONE* 2015; **10**: e0123690. https://doi.org/10.1371/journal.pone.0123690

**33** Pierce VM, Simner PJ, Lonsway DR *et al*. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol* 2017; **55**: 2321–33. https://doi.org/10.1128/JCM.00193-17

**34** Coil D, Jospin G, Darling AE. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics* 2015; **31**: 587–9. https://doi.org/10.1093/bioinformatics/btu661

**35** Nurk S, Bankevich A, Antipov D *et al*. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol* 2013; **20**: 714–37. https://doi.org/10.1089/cmb.2013.0084

**36** Liese J, Schüle L, Oberhettinger P *et al*. Expansion of vancomycin-resistant *Enterococcus faecium* in an academic tertiary hospital in Southwest Germany: a large-scale whole-genome-based outbreak investigation. *Antimicrob Agents Chemother* 2019; **63**: 1–19. https://doi.org/10.1128/AAC.01978-18

**37** Ozer EA, Allen JP, Hauser AR. Characterization of the core and accessory genomes of *Pseudomonas aeruginosa* using bioinformatic tools spine and AGEnt. *BMC Genom* 2014; **15**: 737. https://doi.org/10.1186/1471-2164-15-737

**38** Arndt D, Grant JR, Marcu A *et al*. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 2016; **44**: 16–21. https://doi.org/10.1093/nar/gkw387

**39** Li H, Handsaker B, Wysoker A *et al*. The sequence alignment/map format and SAMtools. *Bioinform* 2009; **25**: 2078–9. https://doi.org/10.1093/bioinformatics/btp352

**40** Van der Auwera GA, Carneiro MO, Hartl C *et al*. From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. *Curr Prot Bio* 2013; **11**: 1–43.

**41** Nguyen LT, Schmidt HA, von Haeseler A *et al*. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015; **32**: 268–74. https://doi.org/10.1093/molbev/msu300

**42** Hoang DT, Chernomor O, von Haeseler A *et al*. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 2018; **35**: 518–22. https://doi.org/10.1093/molbev/msx281

**43** Kalyaanamoorthy S, Minh BQ, Wong TKF *et al*. Modelfinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 2017; **14**: 587–89. https://doi.org/10.1038/nmeth.4285

**44** Richter M, Rosselló-Móra R, Oliver Glöckner F *et al*. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2015; **32**: 929–31. https://doi.org/10.1093/bioinformatics/btv681

**45** Zankari E, Hasman H, Cosentino S *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; **67**: 2640–44. https://doi.org/10.1093/jac/dks261

**46** Naas T, Oueslati S, Bonnin RA *et al*. Beta-lactamase database (BLDB) - structure and function. *J Enzyme Inhib Med Chem* 2017; **32**: 917–9. https://doi.org/10.1080/14756366.2017.1344235

**47** Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018; **3**: 124. https://doi.org/10.12688/wellcomeopenres.14826.1

**48** Peter S, Bosio M, Gross C *et al*. Tracking of antibiotic resistance transfer and rapid plasmid evolution in a hospital setting by nanopore sequencing. *mSphere* 2020; **5**: 1–16. https://doi.org/10.1128/mSphere.00525-20

**49** Darling AE, Mau B, Perna NT. Progressivemauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS ONE* 2010; **5**: e11147. https://doi.org/10.1371/journal.pone.0011147

**50** Bortolaia V, Kaas RS, Ruppe E *et al*. Resfinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500. https://doi.org/10.1093/jac/dkaa345

**51** Cazares A, Moore MP, Hall JPJ *et al*. A megaplasmid family driving dissemination of multidrug resistance in *Pseudomonas*. *Nat Commun* 2020; **11**: 1370. https://doi.org/10.1038/s41467-020-15081-7

**52** Urbanowicz P, Bitar I, Izdebski R *et al*. Epidemic territorial spread of IncP-2-type VIM-2 carbapenemase-encoding megaplasmids in nosocomial *Pseudomonas aeruginosa* populations. *Antimicrob Agents Chemother* 2021; **65**: e02122-20. https://doi.org/10.1128/AAC.02122-20

**53** Zhang Y, Meng L, Guan C *et al*. Genomic characterisation of clinical *Pseudomonas aeruginosa* isolate PAG5 with a multidrug-resistant megaplasmid from China. *J Glob Antimicrob Resist* 2020; **21**: 130–31. https://doi.org/10.1016/j.jgar.2020.03.012

**54** Kizny Gordon A, Phan HTT, Lipworth SI *et al*. Genomic dynamics of species and mobile genetic elements in a prolonged blaIMP-4-associated carbapenemase outbreak in an Australian hospital. *J Antimicrob Chemother* 2020; **75**: 873–82. https://doi.org/10.1093/jac/dkz526

**55** Dößelmann B, Willmann M, Steglich M *et al*. Rapid and consistent evolution of colistin resistance in extensively drug-resistant *Pseudomonas aeruginosa* during morbidostat culture. *Antimicrob Agents Chemother* 2017; **61**: 1–27. https://doi.org/10.1128/AAC.00043-17

**56** Olusegun FI, Adeola RM, Akanmu IN *et al*. Chronic ulcers in Ikeja-Lagos, Nigeria: an eighteen month review of infecting bacterial agents and susceptibility pattern. *Nig Q J Hosp Med* 2013; **23**: 269–72.

**57** Ogunsola FT, Arewa DG, Akinsete IE *et al*. Aetiology of bacteraemia among adult AIDS patients attending Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. *Niger Postgrad Med J* 2009; **16**: 186–92.

**58** Iwalokun BA, Oluwadun A, Akinsinde KA *et al*. Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria. *J Ophthalmic Inflamm infect* 2011; **1**: 95–103. https://doi.org/10.1007/s12348-011-0024-z

**59** Fadeyibi IO, Raji MA, Ibrahim NA *et al*. Bacteriology of infected burn wounds in the burn wards of a teaching hospital in Southwest Nigeria. *Burns* 2013; **39**: 168–73. https://doi.org/10.1016/j.burns.2012.02.005

**60** Adekunle C, Mustapha A, Odewale G *et al*. Detection of antibiotic resistance genes among multiple drug resistant *Pseudomonas aeruginosa* isolated from clinical sources in selected health institutions in Kwara state. *Infect Disord Drug Targets* 2021; **21**: e170721187999. https://doi.org/10.2174/1871526520666201116103625

**61** Bernabé KJ, Langendorf C, Ford N *et al*. Antimicrobial resistance in West Africa: a systematic review and meta-analysis. *Int J Antimicrob Agents* 2017; **50**: 629–39. https://doi.org/10.1016/j.ijantimicag.2017.07.002

**62** Castanheira M, Deshpande LM, Costello A *et al*. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009-11 in 14 European and Mediterranean countries. *J Antimicrob Chemother* 2014; **69**: 1804–14. https://doi.org/10.1093/jac/dku048

**63** Masuda N, Gotoh N, Ishii C *et al*. Interplay between chromosomal beta-lactamase and the MexAB-OprM efflux system in intrinsic resistance to beta-lactams in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; **43**: 400–2. https://doi.org/10.1128/AAC.43.2.400

**64** Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev* 2009; **22**: 582–610. https://doi.org/10.1128/CMR.00040-09

**65** Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev* 2011; **35**: 736–55. https://doi.org/10.1111/j.1574-6976.2011.00268.x

**66** Yousefi S, Nahaei MR, Farajnia S *et al*. A multiresistant clone of *Pseudomonas aeruginosa* sequence type 773 spreading in a burn unit in Orumieh, Iran. *APMIS* 2013; **121**: 146–52. https://doi.org/10.1111/j.1600-0463.2012.02948.x

**67** Oyelade AA, Adelowo OO, Fagade OE. blaNDM-1-producing *Vibrio parahaemolyticus* and *V. vulnificus* isolated from recreational beaches in Lagos, Nigeria. *Environ Sci Pollut Res* 2018; **25**: 33538–47. https://doi.org/10.1007/s11356-018-3306-2