

Characterisation of 16S rRNA gene, phylogenetic analysis, and multidrug resistance profiling of hospital-associated *Myroides* and *Bacillus* strains from Anambra State, Nigeria

Victor Chukwusom Godwin

vc.godwin@unizik.edu.ng

Nnamdi Azikiwe University

Short Report

Keywords: Multidrug resistance, 16S rRNA gene sequencing, Healthcare-associated infections, Plasmid profiling, *Myroides* sp. strain Awka-1, *Bacillus* sp. strain Awka-1, Anambra State

Posted Date: August 31st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10569552/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Characterisation of 16S rRNA gene, phylogenetic analysis, and multidrug resistance profiling of hospital-associated *Myroides* and *Bacillus* strains from Anambra State, Nigeria

Victor Chukwusom Godwin^{1*}

^{1*}Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Enugu-Onitsha Expressway, Awka, 420007, Anambra, Nigeria.

Corresponding author(s). E-mail(s): vc.godwin@unizik.edu.ng;

Abstract

Objective: Hospital indoor microenvironments host multidrug-resistant (MDR) opportunistic bacteria that contribute significantly to healthcare-associated infections (HAIs). This study evaluated the phenotypic multidrug resistance profiles, extrachromosomal plasmid carriage, 16S rRNA gene sequence resources, and phylogenetic topologies of two MDR opportunistic pathogens—*Myroides* sp. strain Awka-1 (PZ744397) and *Bacillus* sp. strain Awka-1 (PZ744398)—isolated from hospital environments in Awka, Anambra State, Nigeria.

Results: Target MDR isolates were recovered from hospital air (*Myroides* sp. strain Awka-1 PZ744397) and healthcare staff attire (*Bacillus* sp. strain Awka-1 PZ744398). Antimicrobial susceptibility testing revealed high-level multidrug resistance, with Multidrug Resistance Index (MRI) values of 0.5 (*Myroides* sp. strain Awka-1) and 0.4 (*Bacillus* sp. strain Awka-1). Plasmid profiling demonstrated extrachromosomal plasmid carriage in both strains. Evolutionary trees constructed via Maximum Likelihood method placed *Myroides* sp. strain Awka-1 PZ744397 in a high-confidence clade alongside *Myroides odoratimimus* subsp. *xuanwuensis* strain M2S3B6 and *Myroides odoratimimus* strain WRBC, whilst *Bacillus* sp. strain Awka-1 PZ744398 clustered firmly within the *Bacillus cereus* species complex (alongside *Bacillus cereus* strain NOORBSRJ12 and *Bacillus*

cereus ATCC 14579). These GenBank sequence deposits provide baseline molecular markers for tracking environmental reservoirs of MDR pathogens in regional healthcare settings.

Keywords: Multidrug resistance, 16S rRNA gene sequencing, Healthcare-associated infections, Plasmid profiling, *Myroides* sp. strain Awka-1, *Bacillus* sp. strain Awka-1, Anambra State

1 Introduction

The persistence of multidrug-resistant (MDR) opportunistic bacteria on inanimate surfaces and in ambient hospital air poses severe public health risks, particularly in developing healthcare systems [1, 2]. Hospital air currents and contaminated personnel clothing frequently act as vectors for non-fermenting Gram-negative bacteria and Gram-positive bacilli-forming spores [3, 4], which have the intrinsic potential of causing severe opportunistic infections in patients with immunodeficiency [5, 6].

Establishing definitive molecular identification and surveillance mechanisms for environmental bacteria is essential for infection control [7]. Although conventional phenotypic characterisation provides foundational data, 16S rRNA gene sequencing remains the gold standard for unambiguous identification and phylogenetic tracking of clinical and environmental isolates [8, 9].

To establish molecular baseline data for sub-Saharan Africa, this study aimed to perform 16S rRNA gene characterisation, phylogenetic mapping, plasmid extraction, and antimicrobial resistance profiling of two MDR strains—*Myroides* sp. strain Awka-1 (GenBank: PZ744397) and *Bacillus* sp. strain Awka-1 (GenBank: PZ744398)—recovered from healthcare facilities in Awka, Anambra State, Nigeria.

2 Methods

2.1 Isolation, Antibigram and Plasmid Extraction

Isolates were obtained from hospital air and personnel clothing using standard microbiological culture techniques [3, 4]. Kirby-Bauer disc diffusion method on Mueller-Hinton agar was employed in the antibiotic susceptibility testing and this was done according to standard criteria [23]. The Multidrug Resistance Index (MRI) was calculated using the equation (a/b) , where a refers to the number of antibiotics resisted and b refers to the total number of antibiotics tested [24]. Plasmid DNA was extracted from overnight bacterial cultures using the ZymoPURE™ Plasmid Miniprep Kit and visualised on 2% agarose gel that was already stained with ethidium bromide [11].

2.2 16S rRNA Amplification, Sequencing and Phylogenetics

Using the Zymo Quick-DNA BioFluid/Cell Kit, genomic DNA was extracted. The amplification of the 16S rRNA gene was carried out using universal bacterial primers [25]:

- 27F: 5'-AGAGTTTGATCMTGGCTCAG-3'
- 1492R: 5'-TACGGYTACCTTGTTACGACTT-3'

PCR amplification was executed in 25 μ L volumes containing 12.5 μ L OneTaq 2 $\times$ Master Mix, 1 μ L of each primer (10 μ M), 2 μ L DNA template, and 8.5 μ L nuclease-free water. Cycle sequencing was conducted using the BrilliantDye™ Terminator Kit v3.1 on an ABI 3510 Genetic Analyser. Sequences were queried against GenBank using BLASTn [26]. Alignments were generated with MUSCLE and phylogenetic trees were created using MEGA software via Maximum Likelihood models with 1,000 bootstrap iterations [27].

3 Results

3.1 Antimicrobial Susceptibility and Plasmid Profiles

Antimicrobial susceptibility testing demonstrated multidrug resistance in multiple clinically vital antibiotic classes [10]. *Myroides* sp. strain Awka-1 PZ744397, isolated from airborne settle plates in a specialist hospital ward, resisted penicillins (AMP), cephalosporins (CEP), aminoglycosides (GEN, STR), and quinolones (NAL), yielding a Multidrug Resistance Index (MRI) of 0.5. *Bacillus* sp. strain Awka-1 PZ744398, isolated from healthcare personnel attire, exhibited resistance to fluoroquinolones (NOR), penicillins/aminopenicillins (AMX, AXC) and phenicols (CHL), with an MRI of 0.4 (Table 1). Agarose gel electrophoresis of plasmid DNA preparations showed intense DNA signals retained close to the loading wells in both isolates, with diffuse migration into the gel. The negative control showed no distinct bands. However, discrete plasmid bands were not clearly resolved under the electrophoretic conditions used (Figure B2). The presence of plasmid shows that horizontal gene transfer of resistance factors is a potential risk within the hospital environment [11, 12].

Table 1 Summary of Isolate Profiles, Accession Numbers, and Data Sources				
Label	Resource Name	GenBank Accession	Biological Source	MDR Profile / Resistant Classes
Data File 1	<i>Myroides</i> sp. strain Awka-1 16S rRNA	PZ744397	Indoor Hospital Air	AMP ^R , CEP ^R , GEN ^R , STR ^R , NAL ^R
Data File 2	<i>Bacillus</i> sp. strain Awka-1 16S rRNA	PZ744398	Staff Attire / Clothing	NOR ^R , AMX ^R , CHL ^R , AXC ^R
Figure 1	<i>Myroides</i> Evolutionary Tree	Figure 1 (embedded)	Bioinferred Alignment	Phylogenetic placement relative to Flavobacteriaceae
Figure 2	<i>Bacillus</i> Evolutionary Tree	Figure 2 (embedded)	Bioinferred Alignment	Phylogenetic placement within Bacillaceae
Note: Resistance index profiles indicate multidrug-resistant characteristics across distinct bacterial isolates.				
¹ AMP = Ampicillin, CEP = Cephalosporins, GEN = Gentamicin, STR = Streptomycin, NAL = Nalidixic acid.				
² NOR = Norfloxacin, AMX = Amoxicillin, CHL = Chloramphenicol, AXC = Amoxicillin-clavulanic acid.				

3.2 16S rRNA Sequencing and Molecular Phylogenetics

Sequencing of the amplified 16S rRNA gene established precise identification and evolutionary relationships for both isolates.

1. ***Myroides* sp. strain Awka-1 (PZ744397)**: As illustrated in Figure 1, phylogenetic reconstruction placed the isolate within the *Myroides odoratimimus* lineage. It formed a distinct sub-clade alongside *Myroides odoratimimus* subsp. *xuanwuensis* strain M2S3B6 and *Myroides odoratimimus* strain WRBC (bootstrap support = 73%/48%). Outgroup rooting with *Flavobacterium aquatile* strain DSM 1132 and *Flavobacterium columnare* NBRC 100251 confirmed clear genus boundaries.

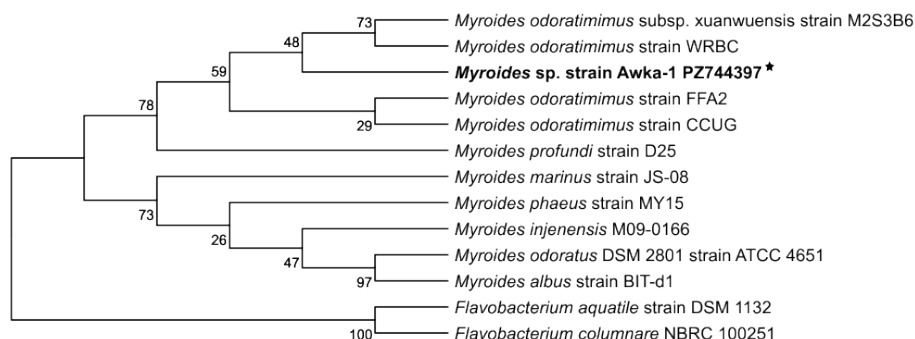


Fig. 1 Molecular phylogenetic tree of *Myroides* sp. strain Awka-1 (GenBank: PZ744397). This was created using the method of Maximum Likelihood, based on 16S rRNA gene sequences which shows the position of *Myroides* sp. strain Awka-1 (GenBank: PZ744397) relative to reference Flavobacteriaceae strains. Bootstrap values (1,000 replicates) are presented at branch nodes.

2. ***Bacillus* sp. strain Awka-1 (PZ744398)**: Phylogenetic topology (Figure 2) situated the strain squarely within the *Bacillus cereus* sensu lato complex. The isolate shared a tight monophyletic node (bootstrap value = 83%) with *Bacillus cereus* strain NOORBSRJ12 and reference strain *Bacillus cereus* ATCC 14579, with *Paenibacillus polymyxa* IAM 13419 establishing outgroup separation.

Sampling Scope: Sampling was restricted to three healthcare facilities within Awka, Anambra State; a broader multicenter surveillance in Nigeria will provide comprehensive regional prevalence data [22].

Supplementary information.

- Additional file 1: Figure A1. Ethical approval and strain collection permissions. Documentation of institutional and ethical approval for the collection of bacterial cultures.
- Additional file 2: Figure B2. Plasmid profiling gel electrophoresis. Photo of agarose gel electrophoresis showing the plasmid profiles of two bacterial strains.
- Additional file 3: Figure C3. Results of the antibiotic susceptibility test. Photographs showing the antibiotic susceptibility test patterns for the two bacterial strains.
- Additional file 4: Table D1. Drugs used for Antibiotic Sensitivity Test, their Category and Concentration.

Declarations

- **Funding:** This research received no external funding and was entirely self-funded by the author.
- **Conflict of interest/Competing interests:** The author declares that they have no competing interests.
- **Ethics approval and consent to participate:** Ethical approval for bacterial culture collection was obtained from [Regina Caeli Specialist Hospital Ethics Committee (Approval No. 2022/009)]. Consent to participate is Not applicable.
- **Consent for publication:** Not applicable.
- **Data availability:** The datasets generated and/or analysed during this study are available in the NCBI GenBank repository, with accession numbers; PZ744397 and PZ744398.
- **Materials availability:** Not applicable
- **Code availability:** Not applicable.
- **Author contribution:** [V.C.G] is the sole author and was responsible for all aspects of study design, data collection, analysis, and manuscript writing.

Appendix A Ethical approval and strain collection permission

REGINA CAELI SPECIALIST HOSPITAL AWKA	
email-reginacaelihospit <i>k</i> @Yahoo.com	
P.O.Box 52 AWKA	Tel: 08108414851
Our Ref. _____	
Your Ref. RC/SH/Ethics.Cmtel/2022/009	21 Feb, 2022

NOTIFICATION OF FULL APPROVAL AFTER COMMITTEE REVIEW

TOPIC OF RESEARCH ISOLATION AND CHARACTERIZATION OF
MULTIDRUG - RESISTANT BACTERIAL PATHOGENS PREVALENT
IN HOSPITAL ENVIRONMENT WITHIN AWKA METROPOLIS

NAME OF PRINCIPAL INVESTIGATOR GODWIN VICTOR CHUKHUSOM

ADDRESS OF PRINCIPAL INVESTIGATOR DEPARTMENT OF APPLIED MICROBIOLOGY
AND BREWING, NNAMDI AZIKWE UNIVERSITY, AWKA

DATE OF RECEIPT OF VALID APPLICATION 14TH FEB, 2022

This is to inform you that the research described in your Proposal and its Consent forms and Methodology have been reviewed and given full approval by the HEALTH RESEARCH ETHICS COMMITTEE of Regina Caeli Specialist Hospital, Awka.

DATE OF APPROVAL 21 FEB, 2022

DATE OF EXPIRATION OF APPROVAL 20 FEB, 2023

You are to note that this Committee can conduct a compliance visit to your research site(s) without notifying you. You also have to abide strictly by the Study Methodology presented to this Committee. Any change if necessary will require approval by the HEALTH RESEARCH ETHICS COMMITTEE. This approval lapses at the end of the approval period.

Congratulations and God bless.



Dr. Arinze Ikediloha (MBBS, FMCOG)
For: HEALTH RESEARCH ETHICS
COMMITTEE, RCSH, Awka

Fig. A1 Ethical approval and strain collection permission. Documentation of ethical approval and institutional permission for bacterial culture collection.

Appendix B Plasmid profiling gel electrophoresis

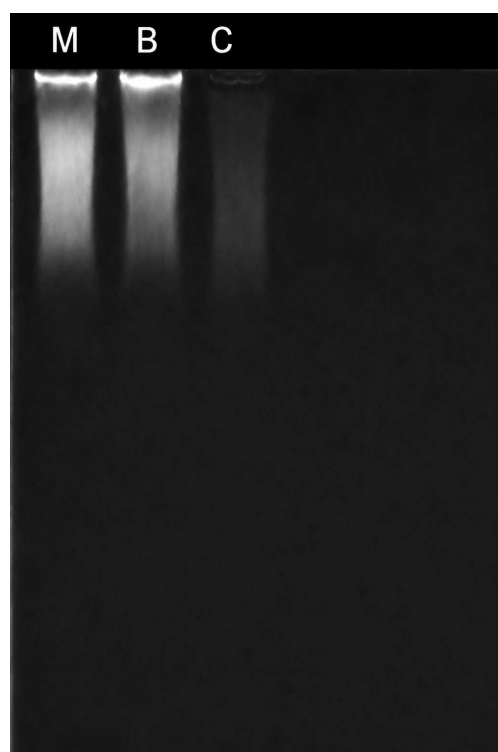


Fig. B2 Plasmid profiling gel electrophoresis. Agarose gel electrophoresis photo displaying the plasmid profiles of two bacterial strains. M=*Myroides* sp. strain Awka-1 (PZ744397); B=*Bacillus* sp. strain Awka-1 (PZ744398); C = Negative Control

Appendix C Antibiotic susceptibility test results



Fig. C3 Antibiotic susceptibility test results. Photographs showing the antibiotic

Appendix D Antibiotic Reference Data

Table D1 Antibiotic Abbreviations, Categories, and Concentrations

Abbreviation	Names	Category	Concentration (μg)
OFX	Ofloxacin	Quinolone	10
PEF	Pefloxacin	Quinolone	10
AU	Augmentin	Penicillin + β -lactamase	30
CPX	Ciprofloxacin	Quinolone	10
CN	Gentamicin	Aminoglycosides	10
S	Streptomycin	Aminoglycosides	30
CEP	Ceporex	Cephalosporin	10
NA	Nalidixic acid	Quinolone	30
SXT	Septrin	Sulfonamides	30
PN	Ampicillin	Penicillin	30
NB	Norfloxacin	Quinolone	10
AMX	Amoxicillin	Penicillin	20
RD	Rifampicin	Rifampin	20
E	Erythromycin	Macrolide	30
CH	Chloramphenicol	Chloramphenicol	30
APX	Ampiclox	Penicillin	20
LEV	Levofloxacin	Quinolone	20

References

- [1] Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *The Lancet*. 2011;377(9761):228–241.
- [2] Bonadonna L, Briancesco R, Coccia AM. Microorganisms in hospital environments: ambient air and surfaces. *Annali dell’Istituto Superiore di Sanità*. 2017;53(2):144–152.
- [3] D’Alessandro D, Giammanco G, Sozzi G. Environmental control in operating rooms: air microbial sampling. *Journal of Preventive Medicine and Hygiene*. 2016;57(1):E36–E41.
- [4] Bearman G, Bryant K, Leekha S, Mayer J, Munoz-Price LS, Murthy R, et al. Healthcare personnel attire in non-operating-room settings. *Infection Control & Hospital Epidemiology*. 2014;35(2):107–121.
- [5] Mitchell BG, Dancer SJ, Anderson M, Dehn E. Risk of organism acquisition from prior room occupants: a systematic review and meta-analysis. *Journal of Hospital Infection*. 2015;91(3):211–217.
- [6] World Health Organization. Global report on infection prevention and control 2022. Geneva: World Health Organization; 2022.
- [7] Dancer SJ. Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination. *Clinical Microbiology Reviews*. 2014;27(4):665–690.
- [8] Clarridge JE. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews*. 2004;17(4):840–862.
- [9] Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Journal of Medical Microbiology*. 2008;57(8):908–934.
- [10] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. 2012;18(3):268–281.
- [11] Carattoli A. Plasmids and the spread of resistance. *International Journal of Medical Microbiology*. 2013;303(6-7):298–304.
- [12] Bennett PM. Plasmid-encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*.

2008;153(S1):S347–S357.

- [13] Licker M, Sorescu T, Rus M, Cirismariu A, Horhat F, Jurescu W, et al. Extensively drug-resistant *Myroides odoratimimus* isolated from a urinary tract infection in an immunocompromised patient. *Infection and Drug Resistance*. 2018;11:2421–2425.
- [14] Hu S, Jiang T, Zhou Y, Fu Y, Zhang Z, Liu Y, et al. Whole-genome sequencing analysis of multidrug-resistant *Myroides odoratimimus* clinical isolates. *Frontiers in Microbiology*. 2021;12:738602.
- [15] Endicott-Yazdani TR, Dhiman N, Benavides R, Spak CW. *Myroides odoratimimus* bacteremia in a diabetic patient. *Proceedings (Baylor University Medical Center)*. 2015;28(3):342–343.
- [16] Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, et al. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLOS ONE*. 2015;10(2):e0117617.
- [17] Bottone EJ. *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology Reviews*. 2010;23(2):382–398.
- [18] Mbanga J, Dube S, Munyanduki H. Environmental contamination and antimicrobial resistance patterns of bacteria isolated from hospital surfaces. *African Journal of Microbiology Research*. 2018;12(14):320–328.
- [19] Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, et al. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*. 2022;50(D1):D20–D26.
- [20] Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, et al. GenBank. *Nucleic Acids Research*. 2018;46(D1):D41–D47.
- [21] Behzadi P, Garcia-Perdomo HA, Karpiński TM. Metagenomics and whole-genome sequencing in surveillance of antimicrobial resistance. *Infection and Drug Resistance*. 2021;14:4205–4216.
- [22] Ojulari SL, Bilal S, Salami AK, Olarinoye JK, Oloyede T, Agbede OO. Antimicrobial resistance patterns and environmental contamination in tertiary health facilities in Nigeria. *Journal of Global Antimicrobial Resistance*. 2020;22:156–162.
- [23] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.
- [24] Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and*

Environmental Microbiology. 1983;46(1):165–170.

- [25] Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*. 1991;173(2):697–703.
- [26] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *Journal of Molecular Biology*. 1990;215(3):403–410.
- [27] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*. 2013;30(12):2725–2729.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Rawplasmid.png](#)