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Original Article

Molecular Characterization of Hospital-Acquired Carbapenem-Resistant Gram-Negative Pathogenic Bacteria From a Tertiary Hospital in Lagos State, Nigeria

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Abstract

Background: Carbapenem-resistant gram-negative bacteria are major challenges in antibiotic therapy globally due to their innate ability to cause life-threatening diseases, leading to treatment failure, high morbidity and mortality, and an increase in hospital-acquired infections. Nonetheless, limited studies exist on their molecular characterization in Nigeria. Accordingly, this study aimed to identify the distinct strains of hospital-acquired carbapenem-resistant gram-negative bacteria from a tertiary hospital in Lagos, Nigeria, using molecular characterization.

Methods: Bacteria isolates from the cultures of in-patients' samples were identified phenotypically by selective/differential diagnostic media and biochemical tests based on standard microbiological protocols. Carbapenem resistance was determined using the Kirby-Bauer disc diffusion method. The isolates of carbapenem-resistant bacteria were characterized using 16S rRNA nucleotide sequencing. Their nucleotide sequences were analyzed using the Basic Local Alignment Search Tool, and accession numbers were assigned to them from the NCBI GenBank.

Results: A total of 162 gram-negative bacteria were isolated based on the results. Among them, 13 (8%) were carbapenem-resistant, and 10/13 of the carbapenem-resistant bacteria were molecularly characterized. The molecularly characterized carbapenem-resistant bacteria had 99.38%, 99%, and 98.85% gene similarity indices to *Escherichia coli* (PP808947), *Klebsiella pneumoniae* (PP808950), and *Pseudomonas aeruginosa* (PP808949), respectively. They also showed 98.71%, 98.53%, 98.26%, and 97.80% gene similarity indices to *Enterobacter hormaechei* (PP808946), *E. hormaechei* (PP808945), *Alcaligenes faecalis* (PP808944), and *Providencia stuartii* (PP808942), respectively. *E. coli* was the most predominant molecularly characterized hospital-acquired carbapenem-resistant gram-negative strain.

Conclusion: This study provided knowledge of the phenotypes and genotypes of hospital-acquired carbapenem-resistant gram-negative Enterobacterales in the study population. It is recommended that these molecularly characterized gram-negative bacteria be monitored in hospitals through effective infection control programs to mitigate their disease outbreak.

Keywords: Nosocomial, Meropenem, Imipenem, Nucleotide sequences, Phenotypes, Genotypes

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Introduction

Antibiotic resistance is a major challenge in hospital-acquired bacterial infections, and this has led to a failure in the treatment of such infections, especially in developing countries (1). Carbapenems are reserve antibiotics used for the treatment of multidrug-resistant, life-threatening diseases. Carbapenem-resistant gram-negative bacteria are a major challenge in antibiotic resistance globally due to their innate ability to cause life-threatening diseases. In addition, they are among the top three drug-resistant bacteria in dire need of new antibiotics (2). Carbapenem-resistant Enterobacterales destroy antibiotics that were initially highly active against gram-negative organisms, limiting antibiotic options to new drugs with reduced effectiveness and older ones with high toxicity. Carbapenem resistance in gram-negative bacteria remains an emerging and significant public health threat, especially in low-income and medium-income countries, including Nigeria (3). In Nigeria, there is a paucity of records on carbapenem resistance in hospital-acquired gram-negative bacteria; antibiotic prescription is high, with only 1/2 of prescriptions based on proper therapeutic

reasons and one-third of prescriptions having a broad-spectrum antibiotic (4). The molecular characterization of these carbapenem-resistant gram-negative bacteria strains is essential for monitoring and controlling their disease outbreak. Therefore, this study aims to identify specific strains of hospital-acquired carbapenem-resistant gram-negative bacteria using molecular techniques.

Materials and Methods

Study Design and Setting

This study was performed at the Lagos State University Teaching hospital, Ikeja, Lagos, from February 2023 to April 2024. Lagos State University Teaching Hospital is a state-owned tertiary hospital located in southwestern Nigeria and serves as a referral centre for patients in Lagos and its environs. The molecular characterization of isolated bacteria was performed at the Nigeria Institute of Medical Research, Lagos.

Study Criteria

Inclusion Criteria

Gram-negative bacterial isolates obtained from the urine and wound cultures of patients admitted to the hospital for 48 hours or more, showing clinical symptoms for nosocomial infections and having no evidence of this infection on admission, were included in this study.

Exclusion Criteria

Bacterial isolates from the cultures of patients admitted within less than 48 hours or patients not demonstrating clinical symptoms for nosocomial infections were not included in this study.

Study Population

Overall, 162 gram-negative bacteria were isolated from the wound and urine cultures of patients suspected of having hospital-acquired infections.

Collection and Identification of Bacterial Isolates

One hundred and sixty-two non-duplicate clinical isolates of gram-negative bacteria from the cultures of urine and wound samples of inpatients submitted to the Medical Microbiology and Parasitology Unit Laboratory of Lagos State University Teaching Hospital were collected in Nutrient Agar broth. Then, they were phenotypically identified using selective/differential diagnostic media and biochemical tests according to standard microbiological protocols (5).

Carbapenem Susceptibility Screening Tests

Phenotypic screening for carbapenem resistance was performed by the Kirby-Bauer disc diffusion method using meropenem (10 µg) and imipenem (10 µg) discs. Standard inoculum, standardized by comparing it with 0.5 McFarland standards, was uniformly spread on the entire surface area of sterilized Mueller-Hinton agar plates using a cotton swab stick. The carbapenem discs were placed on the surface of the inoculated agar plates using sterile forceps, and the plates were incubated at 37 °C for 24 hours. The zones of inhibition were measured, and the results were interpreted based on standard reference values of the Clinical and Laboratory Standards Institute guidelines (6). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains for the *Enterobacteriaceae* and non-*Enterobacteriaceae*, respectively. The isolates were recorded as carbapenem-resistant if they showed resistance to both meropenem and imipenem (7). Pure colonies of the selected bacterial isolates were stored in 15% glycerol stock at -20 °C for further studies.

Molecular Characterization of the Isolates of Carbapenem-Resistant Gram-Negative Bacteria

Molecular characterization of target bacterial isolates was performed based on 16S ribosomal RNA conserved gene sequences. The DNA of carbapenem-resistant bacteria isolates was extracted using the Nigerian Institute of Medical Research genomic DNA extraction kit (a spin column-based DNA purification kit for the isolation of genomic DNA from bacterial cells). The extracted DNA was amplified using a thermocycler (Thermo Fisher Scientific Inc.) for the polymerase chain reaction (PCR). The FIREPol Master Mix (Solis BioDyne) ready to load is a concentrated, ready-to-use solution containing all reagents required for PCR (except DNA template, primers, and water), additional compounds for direct loading onto agarose gel, and two tracking dyes (blue and yellow) to monitor progress during electrophoresis. The 16S ribosomal RNA (16S rRNA) sequence of the selected bacterial isolates was amplified using 1.4 kb bacterial primers 16S rRNA27F (5^{1}-AGAGTTTGATCCTGGCTCAG-3^{1}) and 16S rRNA1329R (5^{1}-GGTTACCTTGTTACGACTT-3^{1}) in a 20 µL PCR reaction mixture. Based on the manufacturer's instructions, a negative control made up of the reaction mixture with water instead of DNA was included in each run. The 16S rRNA PCR 20 µL reaction mix cocktail consists of master mix (4 µL), forward primer (0.6 µL), reverse primer (0.6 µL), *nuclease*-free water (12.8 µL), and bacterial DNA (2 µL). The DNA mix was placed into the thermal cycler, and the PCR cycling protocol is presented in Table 1. The amplified fragment sizes were confirmed and visualized using a 1 kb DNA ladder via gel electrophoresis on 1.5% (w/v) agarose gel in 0.5 x TAE buffer at a constant voltage of 100 V for 30 minutes.

The amplicons were sequenced using the ABI 3500XL Genetic Analyzer (Thermo Fisher Scientific Inc.), and the retrieved nucleotide sequence data were analyzed using Chromas bioinformatics software. The resulting bacterial nucleotide sequences underwent further analysis by the Basic Local Alignment Search Tool on the National Centre for Biotechnology Information website. The nucleotide sequences were submitted to GenBank, and accession numbers were assigned to them.

Table 1. 16S rRNA, PCR Cycling Protocol (30 Cycles Each)

Operation	Temperature (°C)	Time
Initial denaturation	95°C	5 minutes
Denaturation	95°C	30 seconds
Annealing	51°C	40 seconds
Elongation	72°C	1 minute and 40 seconds
Elongation	72°C	10 minutes
Hold	4°C	30 minutes

Note. PCR: Polymerase chain reaction.

Results

In general, 106 and 56 isolates of gram-negative bacteria were obtained from urine and wound cultures, respectively. Among the 162 gram-negative isolates, 13 (8%) were carbapenem-resistant (resistant to both meropenem and imipenem). The carbapenem-resistant gram-negative bacteria were from urine cultures, except for *Klebsiella pneumoniae*, which was from the wound. The molecularly characterized carbapenem-resistant nosocomial pathogens are *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Enterobacter hormaechei*, *Alcaligenes faecalis*, and *Providencia stuartii*.

The carbapenem-resistant gram-negative bacteria nucleotide sequences (10/13) showed significant gene sequence similarity. The bacterial strain *NAF* had the highest gene similarity index (99.38%) to *E. coli*. Bacterial strains *Y*, X_2, J_3, I_3, C_2, B_2, T_2, Z_2, and *C* had 99%, 98.85%, 98.71%, 98.53%, 98.26%, 97.80%, 97.40%, 97.18%, and 90.49% gene similarity indices to *K. pneumoniae*, *P. aeruginosa*, *E. hormaechei*, *E. hormaechei*, *A. faecalis*, *P. stuartii*, *P. aeruginosa*, *E. coli*, and *E. coli*, respectively. The strains of hospital-acquired gram-negative bacterial pathogens, identified by molecular characterization, had percentage identity ranging from 90.49% to 99.38% with GenBank accession numbers for their nucleotide sequences from PP808942 to PP808951. Table 2 provides the sequence similarity value of bacterial strains and their accession numbers from the National Centre for Biotechnology Information GenBank.

Table 2. Percentage Similarity, Identity, and Accession Numbers of Molecularly Characterized Carbapenem-Resistant Bacteria Isolates

S/N	Bacteria Strain ID	Bacteria Specie	Identity (%)	Accession No.
1	NAF-907R	*Escherichia coli*	99.38	PP808947
2	Y-27F	*Klebsiella pneumonia*	99	PP808950
3	X_2-27F	*Pseudomonas aeruginosa*	98.85	PP808949
4	J_3-TEM-R	*Enterobacter hormaechei*	98.71	PP808946
5	I_3-27R	*Enterobacter hormaechei*	98.53	PP808945
6	C_2-907R	*Alcaligenes faecalis*	98.26	PP808944
7	B-27R	*Providencia stuartii*	97.80	PP808942
8	T_2-27F	*Pseudomonas aeruginosa*	97.40	PP808948
9	Z_2-907R	*Escherichia coli*	97.18	PP808951
10	C-907R	*Escherichia coli*	90.49	PP808943

Discussion

Hospital-acquired carbapenem-resistant gram-negative bacteria strains were molecularly characterized. The susceptibility tests performed in this research for carbapenem resistance were strictly based on the resistance of bacterial isolates to both imipenem and meropenem (7,8). Previous studies demonstrated that carbapenem-resistant Enterobacterales are generally resistant to cephalosporins, penicillins, and other antibiotic classes (8,9). *Providencia stuartii* is not a common opportunistic organism but has been implicated in healthcare-associated infections, which is in line with the results of this study. Some studies reported that hospital-acquired pathogens cause polymicrobial catheter-associated urinary tract infections (10) and outbreaks in nursing homes and intensive care units (11). However, in Nigeria, there is a lack of information on hospital-acquired carbapenem-resistant *P. Stuartii* infections. Carbapenem-resistant *P. Stuartii* from urine was identified in this study, confirming the presence of extensively drug-resistant strains of these bacteria in Lagos State. Extensively drug-resistant *P. stuartii* is a major problem in North Africa and the Southern Mediterranean, as reported in another study on *P. stuartii* infections (12). Carbapenem-resistant *E. hormaechei* is a clinically important hospital-acquired pathogen detected in this study, which conforms to the results of other studies, mainly in Asia, where carbapenem resistance in *E. hormaechei* had been reported (13,14). Nonetheless, there is a lack of information on the molecular epidemiology of carbapenem-resistant hospital-acquired *E. hormaechei* infections in Nigeria; this could be due to the paucity of research in this area and lack of appropriate molecular diagnostic tools. A multidrug-resistant *P. stuartii* strain associated with urine nosocomial infection was identified in a similar study conducted in Greece (15).

E. coli was the most prevalent carbapenem-resistant hospital-acquired pathogen in this study, which corroborates the findings of other similar works on carbapenem-resistant bacteria (16-18). The hospital-acquired carbapenem-resistant *A. faecalis* strain demonstrated the hospital transmission of these strains in the population of this study, which is comparable to the results of a similar study in Asia, which showed extensive drug resistance by *A. faecalis* (19). In Nigeria, there is a lack of information on carbapenem resistance by *A. faecalis*, *E. hormaechei*, and *P. stuartii* in hospital-acquired infections (20), implying that these pathogens are emerging hospital-acquired pathogens in Lagos State, Nigeria.

Conclusion

Carbapenem-resistant Enterobacteriaceae are a global health threat in antibiotic therapy, resulting in the explosion of hospital-acquired infections. The most common molecularly characterized gram-negative hospital-acquired carbapenem-resistant bacteria in this study were *E. coli*, *P. stuartii*, *A. faecalis*, and *E.*

hormaechei, respectively. These molecularly characterized gram-negative carbapenem-resistant hospital-acquired bacteria pathogens are emerging nosocomial pathogens in Lagos State, Nigeria. This study has provided knowledge of phenotypes and genotypes of carbapenem-resistant gram-negative nosocomial Enterobacterales in the study population, which will further aid in understanding emerging resistance determinants. The clinical monitoring of the changing epidemiology of these carbapenem-resistant gram-negative bacteria acquired from the hospital environment is essential in preventing disease outbreaks by these bacteria. This could be achieved by effective antibiotic stewardship programs and improved infection control strategies in the hospital.

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Authors' Contribution

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study was approved by the Ethics Committee of Lagos State University Teaching Hospital (ethical No. LREC/06/10/2012). Informed consent was obtained from the participants.

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