Diversity, Resistance Pattern and Level of Antibiotic Resistance Determinants Associated with Bacteria Isolated from Different Water Sources in Southwest Nigeria

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Abstract

Safe water is an essential resource for the existence and sustenance of life. The unavailability of safe water is a significant cause of water-related diseases, especially in developing countries like Nigeria. A spike in the incidence of waterborne diseases has become a public health concern. Hence, this study was designed to evaluate the diversity of bacteria, resistance pattern and level of antibiotic resistance determinants associated with bacteria isolated from different water sources in South-West Nigeria. A total of 120 water samples were collected across South-Western Nigeria from which bacteria were isolated, and identification was achieved by sequencing its partial 16S rRNA. Disc diffusion and multiplex PCR were used to determine antibiotic susceptibility and resistance determinants. A total of 50 bacterial isolates were identified, consisting of *Proteus* spp. (2), *Bacillus* spp. (8), *Enterobacter* spp. (8), *Klebsiella* spp. (9), *Escherichia coli* (7), *Citrobacter* spp. (6), *Pseudomonas* spp. (4), and *Serratia marcescens* (6). All the isolates were resistant to ceftazidime, cefuroxime, cefixime and amoxicillin/clavulanate, whereas 72%, 60%, 54% and 44% were resistant to nitrofurantoin, gentamicin, ciprofloxacin and ofloxacin respectively. Quinolone-resistant genes (*qnrB, qnrA* and *qnrS*), and tetracycline-resistant genes (tetA and tetB) were found in the isolates. The beta-lactamase gene and class 1 integron were also detected in the isolates, with the class 1 integron genes occurring the least. The results revealed that isolates were highly resistant to antibiotics used, as evident in the presence of antibiotic genes. This reveals the unsanitary states of water consumed by the general public, and the ingestion of these antibiotic-resistant bacteria is responsible for the birth of untreatable diseases.

Keywords: Diversity, Antibiotic resistance, Resistance pattern, Resistance genes.

Introduction

Water is a critical resource for daily life's existence, sustenance, and continuance (do Nascimento *et al.*, 2020). It is used in various domestic activities such as drinking, bathing, cooking, and industrial processes. Although water is a universally important solvent, its role in disease transmission has been reported (Sharma & Sharma, 2022). Safe water consumption is critical for maintaining and promoting good health (Raimi *et al.*, 2019). The unavailability of water has become a dire issue in various rural areas, especially in developing countries, due to, but not limited to, factors like overpopulation (Ghaderi *et al.*, 2022), unsanitary waste disposal processes (David *et al.*, 2022), and insufficient water treatment facilities (Sharma & Sharma, 2022).
2020), wars and population displacement (You et al., 2020) and open defecation (Kookana et al., 2020).

Primary sources of these water contaminants and pathogens are the discharge of domestic and industrial effluent wastes, leakage from septic tanks, marine dumping, radioactive waste and atmospheric deposition into the water. Uddin et al. (2019) say that over 5 million people die yearly from untreated water and bad hygiene habits. However, most people living near water sources in Nigeria continue to drink from rivers, streams, and other water bodies, regardless of their state or treatment (Raimi et al., 2019).

Several pathogens have been identified as waterborne, including Proteus spp., Bacillus spp., Enterobacter spp., Klebsiella spp., Escherichia coli, Citrobacter spp., Pseudomonas spp. and Serratia marcescens (Barrantes et al., 2022). A limited supply of potable water in developing nations like Nigeria is a significant reason for the occurrence of some waterborne diseases like typhoid, cholera, traveller's diarrhoea, or shigellosis, which are associated with significant morbidity and mortality (Okpasuo et al., 2020), especially in this era of bacterial multidrug resistance.

Antibiotic resistance is a global concern that is wreaking havoc on the economy and inflicting financial hardship on patients (Ventola, 2015). Several studies have connected antibiotic abuse and the presence of resistance genes to the development of antibiotic resistance (Cepas et al., 2019; Richard et al., 2022). Antibiotic-resistant microorganisms have caused enormous death and morbidity worldwide (Murray et al., 2022). It was recorded that 671,689 illnesses were caused by bacterial antibiotic resistance (Innes et al., 2020), and 495 million fatalities were caused by bacterial antibiotic resistance in 2019 (Murray et al., 2022). The six most prevalent pathogens responsible for mortality due to antibiotic resistance in 2019 were Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, accounting for 929,000 deaths (Murray et al., 2022). Acquisition of resistance determinants by bacteria can potentially equip them to resist as many as nine different antibiotic classes (He et al., 2020). Antibiotics, biocides, metals, pharmaceuticals, and personal care items released into the environment can promote antimicrobial resistance, thereby increasing selective pressure on bacteria in the aquatic environment, either directly or indirectly (Heß et al., 2018). With the increasing unavailability of safe drinking water in Nigeria and the threat of antibiotic resistance in microorganisms globally, it becomes imperative to determine the diversity, resistance pattern and level of antibiotic determinants associated with microorganisms isolated from different water sources in South-West Nigeria. This study was designed to expose the increasing danger of consuming unsafe water and the implication of ingesting these resistant organisms.

Materials And Methods

Sample Collection

A total of 120 water samples were collected from the six states in southwest Nigeria; Lagos state {Badagry (2), Ojota (2), Ketu (1), Epe (3), Ikorodu (2), Lekki (2), Ikotun (2), Egbada (2), Surulere (2) and Ikeja (2)}, Ogun state {Abeokuta (2), Ikeja (2), Ikenne (3), Sagamu (3), Ifo (2), Ijebu-ode (2), Mowe (2), Ijebu-Igbo (2) and Ilaro (2)}, Oyo state {Shaki (2), Iseyin (3), Egbeda (2), Ido (3), Ibadan (3), Asipa (2), Orelupe (3) and Ibarapa (2)}, Osun state {Gbongan (3), Ise (2), Iwo (3), Osogbo (2), Ejigbo (3), Iledun (2), Odo Otin (3) and Modakeke (2)}, Ondo state {Ikare (2), Owo (2), Okitipupa (2), Ilaje (2), Ile Oluji (2), Akure (3), Ondo (2), Idoani (2) and Idanre (3)} and Ekiti state {Ado-Ekiti (3), Ikere (2), Efon (3), Gbonyin (2), Ido-Osi (3), Iwaraja (2), Ilawe (3) and Ilorin (2)}. A litre of water samples was collected from each sampling point consisting of wells (30), taps (65) and rivers (25). The samples were aseptically inoculated into an enrichment medium (Buffered peptone water, Oxoid LTD, Basingstoke, Hampshire, England) and transported to the Microbiology laboratory and processed within 24 hours using standard microbiological procedures on different agar media (Eosin methylene blue agar, Centrimee agar, MacConkey agar and Thiosulfate-Citrate-Bile Salts-Sucrose). Purification of isolates was
achieved using nutrient agar. After 24 hours post-inoculation, several colonies of interest were isolated and kept in a 40% glycerol broth at −20°C for subsequent analysis.

**DNA Extraction & Polymerase Chain Reaction**

The genomic DNA was extracted according to the manufacturer’s instructions using the Quick-DNA mini prep plus kit (Zymo Research, Biolab, USA). Exactly 200 µl of a physiologically young culture of the isolates (16-24 hours old) were inoculated into Eppendorf tubes, and an equivalent volume of biofluid cell buffer was added, and the contents of the tubes were vortexed for 10-15 seconds before being incubated for 10 minutes at 55°C. After digestion, a volume of Genomic binding buffer (420µl) was added and vortexed for 10-15 seconds. The mixtures were transferred into a Zymo-Spin collection tube and centrifuged for one minute at 12000 rpm. The spin columns were pipetted with exactly 700µl of gDNA wash buffer and spun at 12000 rpm for 1 minute; the collecting tubes with the flow-through were discarded. The spin columns were transferred into clean Eppendorf tubes, and 50µl of DNA elution buffer was poured directly onto the matrix. It was incubated for 5 minutes at room temperature before being centrifuged for 1 minute at a speed of 12000 rpm to elute the DNA. The eluted DNA was immediately used for Polymerase Chain Reaction (PCR).

**Gene Detection by Multiplex PCR**

A PCR mixture consisting of 15 µl of OneTaqQuick load 2X Master Mix Buffer (New England Biolabs), 0.5 µl of 10 mM of both the forward and reverse sequence selected primer (qnrA, qnrB, qnrS, tetA, tetB, SHV, HEP) was added to each PCR tube containing 3.0 µl of DNA and the reaction mixture was rounded up to 30 µl with sterile water. DNA amplification was done with miniPCR (USA) with thermal cycling profiles appropriate for the primer combinations employed (Table 1). The amplified DNA was verified by gel electrophoresis; the gel mixture was prepared according to the manufacturer’s instruction, heated and poured into the gel tank, and allowed to solidify after insertion of the comb to create wells. The bands were viewed using a trans-illuminator.

**Species Barcoding**

Isolate representatives (19) were chosen for sequencing. Prior to sequencing, Extracted PCR amplified DNA of selected representatives; PCR mixture consisting of 12.5 µl of OneTaq PCR load 2X Master Mix Buffer (New England Biolabs), 0.5µl of 16SrRNA primers; 341F: 5’-CCTACGGAGGCAGCAG3' and R806: 5’GGACTACHVGGGTWTCTAAT-3’, and an aliquot of 2.0 µl of DNA suspension were added to sterile PCR tubes. With sterile water, the reaction mixture was rounded up to 25 µl. The following thermal cycling profile was used for DNA amplification using miniPCR (USA): Initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation (30 seconds at 95°C), annealing (1 minute at 48°C), and extension (the 30s at 68°C) and a final extension at 68°C for 5 minutes. Standard techniques were used to generate unidirectional sequence reads combined using the Bioedit sequence tool. Molecular evolutionary genetics analysis (MEGA 11) was used to undertake evolutionary analysis.

**Susceptibility Testing**

The Kirby-Bauer disc diffusion method was used for the antibiotic susceptibility test, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021), to test for the sensitivity or resistance of isolates to the antibiotics. In a test tube containing 1ml of nutrient broth (Oxoid, UK), a single colony of the pure isolate was inoculated and cultured overnight at 37°C. The overnight broth was standardised to match the McFarland standard of 0.5. A sterile swab stick was dipped in the standardised suspension and spread across the surface of Mueller Hilton agar plates (Oxoid LTD, Basingstoke, Hamshire, England) that had already been prepared. Antimicrobial susceptibility discs (Abtek Biologicals Ltd.) containing different antibiotics: ceftazidime (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), cefixime (CXM, 5 µg), ofloxacin (OFL, 5 µg), amoxicillin/clavulanate (AUG, 30µg), nitrofurantoin (NIT, 300 µg), ciprofloxacin (CPR, 5 µg) were used for the disc diffusion test according to clinical laboratory standard institute (2021).
Table 1: Sequence of primers used in this study, annealing temperatures and their molecular weights.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Oligonucleotide Sequence; 5'-3'</th>
<th>Amplicon size (bp)</th>
<th>Annealing Temperature (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hep58</td>
<td>TCATCGCTTGTTATGACTGT</td>
<td>856</td>
<td>47</td>
<td>Barns et al., 2021</td>
</tr>
<tr>
<td>Hep59</td>
<td>GTAGGGCTATATGAGCAACG</td>
<td>660</td>
<td>57</td>
<td>Doosti et al., 2015</td>
</tr>
<tr>
<td>SHV F</td>
<td>TCGGGGCCGCTAGGCAATG</td>
<td>543</td>
<td>49</td>
<td>Abdel-Rahman et al., 2021</td>
</tr>
<tr>
<td>SHV R</td>
<td>AGCGAGGGCAATAATCCCG</td>
<td>360</td>
<td>51</td>
<td>Abdel-Rahman et al., 2021</td>
</tr>
<tr>
<td>QNRA F</td>
<td>GATAAAGTTTTTCAGCAAGGG</td>
<td>599</td>
<td>46</td>
<td>Abdel-Rhman et al., 2021</td>
</tr>
<tr>
<td>QNRAR F</td>
<td>ATCCAGATCGGCAAGGTTA</td>
<td>494</td>
<td>49</td>
<td>Yu et al., 2005</td>
</tr>
<tr>
<td>QNRB F</td>
<td>GCGATATCGCCAGTCCGA</td>
<td>571</td>
<td>45</td>
<td>Yu et al., 2005</td>
</tr>
<tr>
<td>QNRS R</td>
<td>TGCAATTTTGATAATCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TETA F</td>
<td>TTGGCATTCTGACCTCAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TETA R</td>
<td>GTATTGCGGCCAGGTTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TETB F</td>
<td>CAGTGTGTGTGTCTAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TETB R</td>
<td>GCTTGGAAATACCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bp: base pair, Tm: Temperature, °C: degree centigrade.

Results

Species Distribution and Diversity

Fifty (50) bacterial isolates were obtained from the total of 120 samples collected. Different species were identified; tap water sample (15), well water (16) and river water (19) consisting of *Proteus* spp. (2), *Bacillus* spp. (8), *Enterobacter* spp. (8), *Klebsiella* spp. (9), *Escherichia coli* (7), *Citrobacter* spp. (6), *Pseudomonas* spp. (4), and *Serratia marcescens* (6). *Escherichia coli* strains were 46%, 36%, 14% and 11% in Lagos, Ogun, Ekiti, and Oyo states, respectively. In Lagos, Ondo and Ekiti states, there was 27%, 33% and 14% occurrence of *Enterobacter* spp., while *Klebsiella* spp. occurred in Lagos state (9%), Ogun state (18%), Oyo state (22%), Osun State (29%) and Ekiti State (30%). *Pseudomonas* spp. and *Citrobacter* spp. were isolated in Osun (14% and 43%, respectively), *Citrobacter* spp. in Oyo State (22%), *Pseudomonas* spp. in Ogun State (9%) and in Ondo State (33%). In Ekiti, *Proteus* spp. and *Bacillus* spp., occurred at 14% each. In Ondo State, *Bacillus* spp. was found at a frequency of 17% and 45% in Oyo State. The isolates sequenced were deposited in the gene bank under the accession number ON360948 to ON360966. All isolates were grouped into 9 clusters using the Molecular Evolutionary Genetics Analysis (MEGA) (Figure 1). In Cluster 1: *Enterobacter mori* strain LA13, *Bacillus cereus* strain ON20; Cluster 2: *Enterobacter mori* strain LA07; Cluster 3: *Bacillus thuringiensis* strain OY06, *Citrobacter amalonaticus* strain OG14, *Enterobacter cloacae* strain OS20, *Serratia marcescens* strain OG02; Cluster 4: *Klebsiella* sp. strain OG14, *Bacillus* sp. (in: Bacteria) strain LA05 Cluster 5: *Escherichia coli* strain LA04, *Proteus hauseri* strain EK01; Cluster 6: *Pseudomonas* sp. strain OG09; Cluster 7: *Citrobacter* sp. strain OS06, *Enterobacter cancerogenus* strain LA18; Cluster 8: *Klebsiella pneumoniae* strain OY15, *Klebsiella pneumoniae* strain LA06; Cluster 9: *Enterobacter hormaechei* strain ON07. The evolutionary history was inferred using the Neighbour-Joining method, and distances were computed using the Jukes-Cantor method. All isolates were grouped into 9 clusters.
Figure 1: Phylogenetic tree illustrating the relationship between isolates identified and their close relatives in NCBI.
Table 2: Bacteria isolates’ identification, accession number and its close relatives in NCBI

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Ascension Number</th>
<th>Location</th>
<th>Similarity to close relatives %</th>
<th>Close relatives in NCBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter mori strain</td>
<td>ON360948</td>
<td>Lagos</td>
<td>100%</td>
<td>Enterobacter mori strain MGP7</td>
</tr>
<tr>
<td>Klebsiella sp. strain</td>
<td>ON360949</td>
<td>Ogun</td>
<td>83%</td>
<td>Klebsiella sp. PG1</td>
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<tr>
<td>Enterobacter mori strain</td>
<td>ON360950</td>
<td>Lagos</td>
<td>89%</td>
<td>Enterobacter mori strain MGP7</td>
</tr>
<tr>
<td>Escherichia coli strain</td>
<td>ON360951</td>
<td>Lagos</td>
<td>92%</td>
<td>Escherichia coli strain DP01</td>
</tr>
<tr>
<td>Citrobacter sp. strain</td>
<td>ON360952</td>
<td>Osun</td>
<td>84%</td>
<td>Citrobacter sp. strain MS5</td>
</tr>
<tr>
<td>Enterobacter cancerogenus strain</td>
<td>ON360953</td>
<td>Lagos</td>
<td>83%</td>
<td>Enterobacter cancerogenus strain JXG4</td>
</tr>
<tr>
<td>Serratia marcescens strain</td>
<td>ON360954</td>
<td>Ogun</td>
<td>88%</td>
<td>Serratia marcescens strain HYRSM1</td>
</tr>
<tr>
<td>Pseudomonas sp. strain</td>
<td>ON360955</td>
<td>Ogun</td>
<td>88%</td>
<td>Pseudomonas sp. OSG41</td>
</tr>
<tr>
<td>Citrobacter amalonaticus strain</td>
<td>ON360956</td>
<td>Ogun</td>
<td>90%</td>
<td>Citrobacter amalonaticus strain OPNF5.11</td>
</tr>
<tr>
<td>Proteus hauseri strain</td>
<td>ON360957</td>
<td>Ekiti</td>
<td>89%</td>
<td>Proteus hauseri strain KY072916.1</td>
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<tr>
<td>Klebsiella pneumoniae strain</td>
<td>ON360958</td>
<td>Oyo</td>
<td>93%</td>
<td>Klebsiella pneumoniae subsp. ozaenae strain UTIKN1</td>
</tr>
<tr>
<td>Bacterium strain</td>
<td>ON360959</td>
<td>Ekiti</td>
<td>92%</td>
<td>Bacterium strain IF35</td>
</tr>
<tr>
<td>Bacillus thuringiensis strain</td>
<td>ON360960</td>
<td>Oyo</td>
<td>86%</td>
<td>Bacillus thuringiensis strain FDAARGOS_794</td>
</tr>
<tr>
<td>Klebsiella pneumoniae strain</td>
<td>ON360961</td>
<td>Oyo</td>
<td>98%</td>
<td>Klebsiella pneumoniae strain A_27-F_B11_05</td>
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<td>Enterobacter cloacae strain</td>
<td>ON360962</td>
<td>Osun</td>
<td>98%</td>
<td>Enterobacter cloacae strain HS-6</td>
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<td>Klebsiella pneumoniae strain</td>
<td>ON360963</td>
<td>Lagos</td>
<td>100%</td>
<td>Klebsiella pneumoniae strain A_27-F_B11_05</td>
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<td>Enterobacter hormaechei strain</td>
<td>ON360964</td>
<td>Ondo</td>
<td>98%</td>
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<td>Bacillus cereus strain</td>
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<td>95%</td>
<td>Bacillus cereus strain DS-2</td>
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<td>Bacillus sp.</td>
<td>ON360966</td>
<td>Lagos</td>
<td>84%</td>
<td>Bacillus sp. 11AN2</td>
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</table>
Antibiotic Susceptibility Test

The resistance profiling of the bacteria isolates showed that all (100%) were resistant to ceftazidime (CAZ), cefuroxime (CRX), cefixime (CXM) and amoxicillin-clavulanic Acid (AUG) across the States. In Ekiti State, 71% of the isolates were resistant to nitrofurantoin (NIT), while 43% were resistant to ofloxacin (OFL), gentamicin (GEN) and ciprofloxacin (CPR). However, in Lagos State, 82%, 36% and 18% of the isolates were resistant to NIT, OFL, GEN and CPR, respectively. In Ogun State, 90%, 60%, 50% and 40% of the isolates were resistant to the NIT, GEN, CPR, and OFL in that order. Likewise, in Ondo State, 83% of the isolates were resistant to NIT, while 67% were resistant to GEN and CPR. In Osun State, 100% of the isolates were resistant to GEN, whereas 63%, 50% and 38% of the isolates were resistant to NIT, OFL and CPR, respectively. In Oyo State, 78%, 56%, 44% and 33% of the isolates were resistant to CPR, GEN, OFL and NIT (Figure 2). Overall, all the isolates were resistant to CAZ, CRX, CXM and AUG, whereas 72%, 60%, 54% and 44% were resistant to the NIT, GEN, CPR and OFL, respectively. Intermediate resistance was only observed in three antibiotics; OFL (2%), GEN (6%) and CPR (34%).

Resistant Gene Detection

Quinolones resistant genes (qnrB, qnrA and qnrS) were found in the isolates at a frequency of 36% (18), 24% (12) and 16% (8), respectively, whereas tetracycline-resistant genes (tetA and tetB were found 10% (5) and 6% (3) of isolates respectively. In that order, the beta-lactamase gene and class 1 integron were detected in 40% (20) and 52% (26) of the isolates. The distribution of the genes across the Southwestern states is represented in Figure 3.

Figure 2: Antibiotic susceptibility profile of bacteria isolates across states in South-West Nigeria

Figure 3: Resistant gene profile of bacteria isolates across states in South-Western Nigeria
qnr: quinolone-resistant genes; tet: tetracycline-resistant genes; SHV: sulfhydryl reagent variable; HEP: Integron class 1 gene.

Figure 4: Gel Electrophoregram of Multiplex PCR for resistant gene detection
Lane M: Ladder; Lane 2, 4, 6, 7, 8, 9, 10, 11, 12: tetB (571bp); Lane 3, 4, 5, 9, 10, 14: SHV(660bp); Lane 2, 4, 6, 7, 8, 9, 11, 12: HEP (856).
Figure 5: Gel Electrophoregram of Multiplex PCR for resistant gene detection
Lane M: Ladder; Lane 1, 2, 5, 6, 7, 8, 9, 12: qnrA (543); Lane 4, 11, 12, 14: qnrB (360bp)

Discussion

The bacteria species distribution in this study was similar to the results of Sirisha et al. (2017). The abundance of microorganisms in the water samples can be attributed to a variety of factors, including faulty or inadequate tube-well structures, proximity to sources of contamination (dump sites and toilet facilities, bathing in rivers), unsanitary practices and activities near the source (indiscriminate use and poor handling of fetchers used for the wells) (Agbabiaka & Olofintoye, 2019). In this study, most isolated bacteria belong to the worrisome family Enterobacteriaceae. This is due to its pathogenic potential in healthcare and community settings, especially multidrug resistance. The Enterobacteriaceae family is the most common cause of urinary tract infection, ellulitis, wound infections, septicemia, ear infections, meningitis, abscess, pneumonia, bacteremia, endocarditis, sinusitis, sore throat, gastroenteritis, necrotic enteritis and poisoning and diarrhoea (Babalola et al., 2021; Cristina et al., 2019; Otorkpa, 2019; Paudyal et al., 2017; Manhique et al., 2020). Bacillus spp. is infamous for causing food poisoning and eye infections; nevertheless, in hospitals, Bacillus spp. is usually treated as an environmental pollutant by doctors (Ikeda et al., 2015). Pseudomonas spp. is a pathogen that causes bacteremia, ventilator-associated pneumonia, urinary tract infections, and skin and soft-tissue infections; it is also known for developing drug resistance that affects practically all antibiotic classes (Bassetti et al., 2018).

It was observed from the phylogenetic relationship that there is an evolutionary similarity among isolates from Lagos, Osun, Oyo and Ogun State. This similarity could be attributed to the proximity in geographical areas, frequent interstate trade and the confluence of shared water bodies. The study by Ezeamagu et al. (2021) reported that the isolates from farther locations showed more similarities than isolates from closer locations as opposed to the result from this study. This study indicated that the evolutionary relationship might not be a distance function.

Isolates in this study were found to have high levels of resistance to ceftazidime, cefuroxime,
cefixime, amoxicillin/clavulanate, ciprofloxacin, and gentamicin. Iliyasu et al. (2018) and Barns et al. (2021) found similar results, possibly due to the proximity of sampling locations in the studies. This study showed a significant resistance level to the antibiotics utilised, possibly due to antibiotic pressure in the environment. While the overuse and misuse of antibiotics in humans are undoubtedly a primary driving force for this severe problem, antibiotic-resistant genes have also been recognised to play an essential role in the emergence and spread of antibiotic resistance (Bengtsson-Palme et al., 2018). Efflux pumps, bypass of antibiotic-inhibited metabolic pathways, alteration of target sites, and, most critically, degradation of antibiotics by microbial enzymes have all been found to allow bacteria to survive antibiotic therapy and develop resistance (Munita & Arias, 2016).

Quinolone-resistant genes (qnrA, qnrB, and qnrS), tetracycline resistance genes (tetA and tetB), and ESBL genes (SHV, TEM) have all become a global public health concern (Sharma et al., 2016). In this investigation, the qnrA, qnrB, and tetA genes were found in 24 per cent, 16 per cent, and 10% of the isolates, respectively. The results were similar to the study by Hossain et al. (2018), who reported that qnrB (26.15%) and tetA (16.92%) were present in their study except for qnrA, which was absent. The absence of qnrA could result from the difference in geographical location. The slight variation in gene prevalence could also result from the difference in sample size. In this study, SHV-resistant genes were detected in 40% of the isolates, similar to the study by Haller et al. (2018), with 41.1% occurrence. Extended-spectrum beta-lactamases (ESBLs) are enzymes widely employed by Gram-negative bacteria, particularly the Enterobacteriaceae, and are responsible for carbapenem resistance in most antibiotic classes (Coque et al., 2008; Pitout & Laupland, 2008; Abdelhady et al., 2016). Temoneira (TEM), sulphydryl-variable (SHV), and cefotaximase (CTX-M) genes are found on mobile genetic elements (plasmids), which can be passed from one bacterial strain to another. Although over 150 ESBLs were detected worldwide, class A beta-lactamases have received the most attention (Rupp & Fey, 2003).

The high level of integron gene pollution is evident in this study and studies by Ghazalibina et al. (2019), McKinney et al. (2018) and Pormohammad et al. (2019), where significant levels of integrons were detected in clinical samples tallied with the high occurrence of Class 1 integron genes in this study. This has a significant health implication because it poses a threat to consumers of unsafe water as these genes can be transferred between organisms by horizontal gene transfer, thus increasing the risk of treatment failure in the event of infection. It is more likely that integrons Class 1 are frequently detected among clinical isolates than environmental isolates in Nigeria.

Conclusion and recommendation

The results revealed that the bacteria isolated were highly resistant to antibiotics due to prevailing associated antibiotic resistance determinants, which negatively affect treatment outcomes in the event of infection. Therefore, the urgent need for a safe water supply to various communities, awareness of the proper use of antibiotics and an antibiotic surveillance system is necessary to curtail the spread of antibiotic-resistant bacteria in our community.

References


