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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.*,

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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), Egbeda (2), Surulere (2) and Ikeja (2)}, Ogun state {Abeokuta (2), Ikenne (2), Ilishan (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), Orelope (3) and Ibarapa (2)}, Osun state {Gbongan (3), Ife (2), Iwo (3), Osogbo (2), Ejigbo (3), Ifelodun (2), Odo Otin (3) and Modakeke (2)}, Ondo state {Ikare (2), Owo (2), Okitipupa (2), Ilaje (2), Ile Oluji (2), Akure (3), Ifon (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 Ilejemeje (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, Centrimide 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 Quick load 2X Master Mix Buffer (New England Biolabs), 0.5µl of 16SrRNA primers; 341F: 5'-CCTACGGGAGGCAGCAG3' 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.

Primers	Oligonucleotide Sequence; 5'-3'	Amplicon size (bp)	Annealing Temperature (°C)	References
Hep58	TCATCGCTTGTTATGACTGT	856	47	Barns <i>et al.</i> , 2021
Hep59	GTAGGGCTTATTATGCACGC			
SHV F	TCGGGCCGCGTAGGCATGAT	660	57	Doosti <i>et al.</i> , 2015
SHV R	AGCAGGGCGACAATCCCGCG			
QNRA F	GATAAAGTTTTTCAGCAAGAGG	543	49	Abdel-Rahman <i>et al.</i> , 2021
QNRAR	ATCCAGATCGGCAAAGGTTA			
QNRB F	GGCATTGAAATTCGCCACTG	360	51	Abdel-Rahman <i>et al.</i> , 2021
QNRB R	TTTGCTGCTCGCCAGTCGA			
QNRS F	TGGAACCTACAATCATACA	599	46	Abdel-Rhman <i>et al.</i> , 2021
QNRS R	TGCAATTTTGATACCTGATG			
TETA F	TTGGCATTCTGCATTCATC	494	49	Yu <i>et al.</i> , 2005
TETA R	GTATAGCTTGCCGGAAGTCG			
TETB F	CAGTGCTGTTGTGTCATTAA	571	45	Yu <i>et al.</i> , 2005
TETB R	GCTTGGAATACTGAGTGAA			

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

Isolates	Ascension Number	Location	Similarity to close relatives %	Close relatives in NCBI
<i>Enterobacter mori</i> strain	ON360948	Lagos	100%	<i>Enterobacter mori</i> strain MGP7
<i>Klebsiella</i> sp. strain	ON360949	Ogun	83%	<i>Klebsiella</i> sp. PG1
<i>Enterobacter mori</i> strain	ON360950	Lagos	89%	<i>Enterobacter mori</i> strain MGP7
<i>Escherichia coli</i> strain	ON360951	Lagos	92%	<i>Escherichia coli</i> strain DP01
<i>Citrobacter</i> sp. strain	ON360952	Osun	84%	<i>Citrobacter</i> sp. strain MS5
<i>Enterobacter cancerogenus</i> strain	ON360953	Lagos	83%	<i>Enterobacter cancerogenus</i> strain JXG4
<i>Serratia marcescens</i> strain	ON360954	Ogun	88%	<i>Serratia marcescens</i> strain HYRSM1
<i>Pseudomonas</i> sp. strain	ON360955	Ogun	88%	<i>Pseudomonas</i> sp. OSG41
<i>Citrobacter amalonaticus</i> strain	ON360956	Ogun	90%	<i>Citrobacter amalonaticus</i> strain OPNF5.11
<i>Proteus hauseri</i> strain	ON360957	Ekiti	89%	<i>Proteus hauseri</i> strain KY072916.1
<i>Klebsiella pneumoniae</i> strain	ON360958	Oyo	93%	<i>Klebsiella pneumoniae</i> subsp. ozaenae strain UTIKN1
<i>Bacterium</i> strain	ON360959	Ekiti	92%	<i>Bacterium</i> strain IF35
<i>Bacillus thuringiensis</i> strain	ON360960	Oyo	86%	<i>Bacillus thuringiensis</i> strain FDAARGOS_794
<i>Klebsiella pneumoniae</i> strain	ON360961	Oyo	98%	<i>Klebsiella pneumoniae</i> strain A_27-F_B11_05
<i>Enterobacter cloacae</i> strain	ON360962	Osun	98%	<i>Enterobacter cloacae</i> strain HS-6
<i>Klebsiella pneumoniae</i> strain	ON360963	Lagos	100%	<i>Klebsiella pneumoniae</i> strain A_27-F_B11_05
<i>Enterobacter hormaechei</i> strain	ON360964	Ondo	98%	<i>Enterobacter hormaechei</i> strain Houri Alizadeh-4
<i>Bacillus cereus</i> strain	ON360965	Ondo	95%	<i>Bacillus cereus</i> strain DS-2
<i>Bacillus</i> sp.	ON360966	Lagos	84%	<i>Bacillus</i> sp. 11AN2

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.

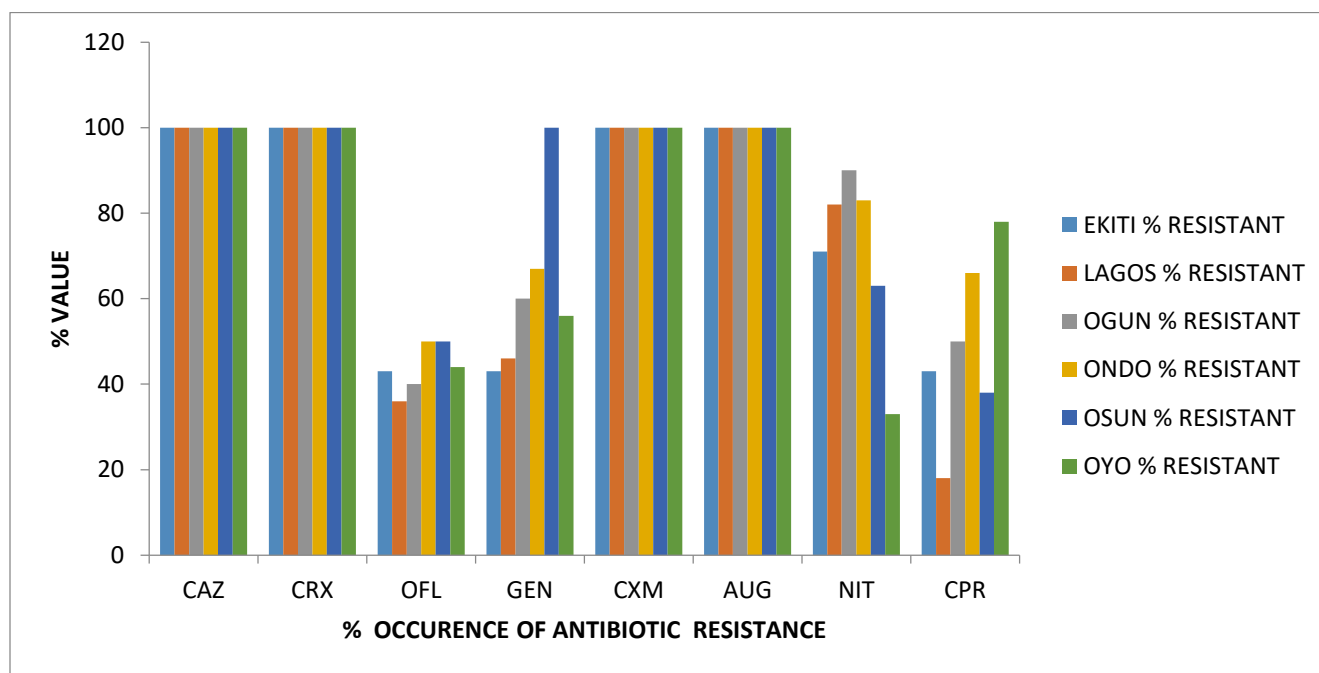


Figure 2: Antibiotic susceptibility profile of bacteria isolates across states in South-West Nigeria

CAZ: ceftazidime, CRX: ceftriaxone, OFL: ofloxacin, GEN: gentamicin, CXM: cefixime, AUG: amoxicillin-clavulanic Acid, NIT: nitrofurantoin, CPR: ciprofloxacin

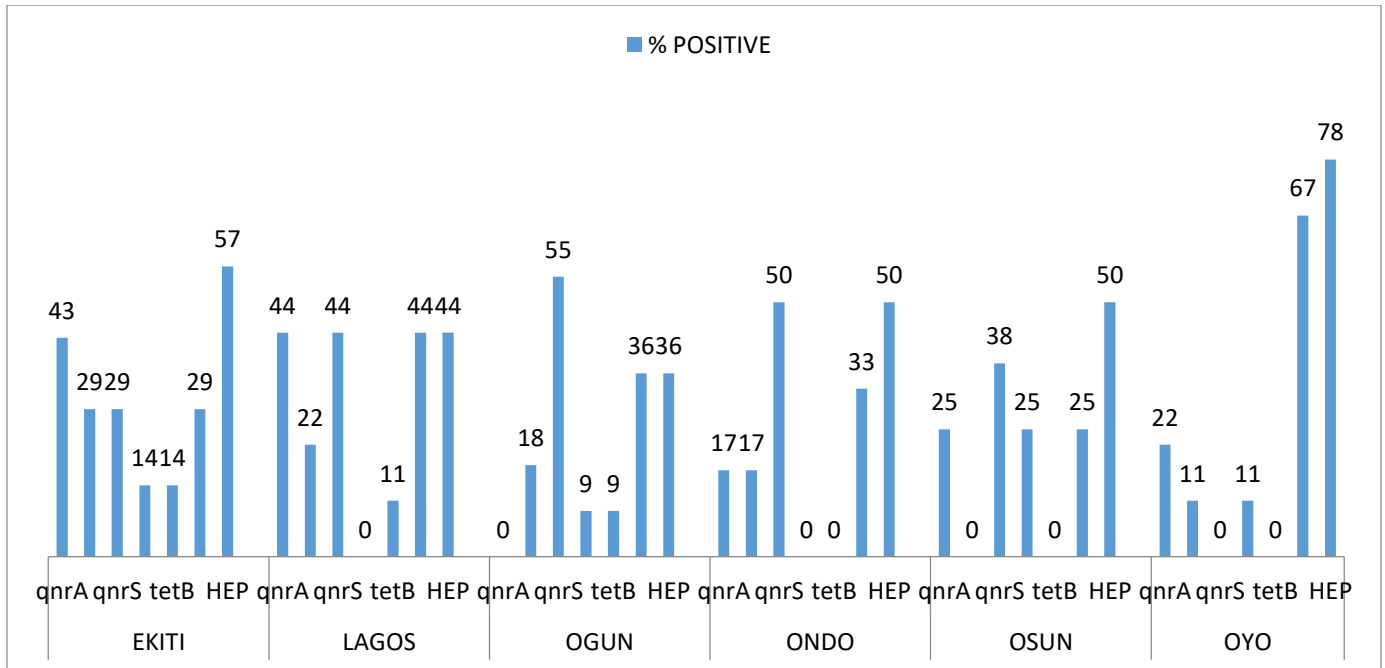


Figure 3: Resistant gene profile of bacteria isolates across states in South-Western Nigeria
qnr: quinolone-resistant genes; tet: tetracycline-resistant genes; SHV: sulphydryl reagent variable;
HEP: Integron class 1 gene.

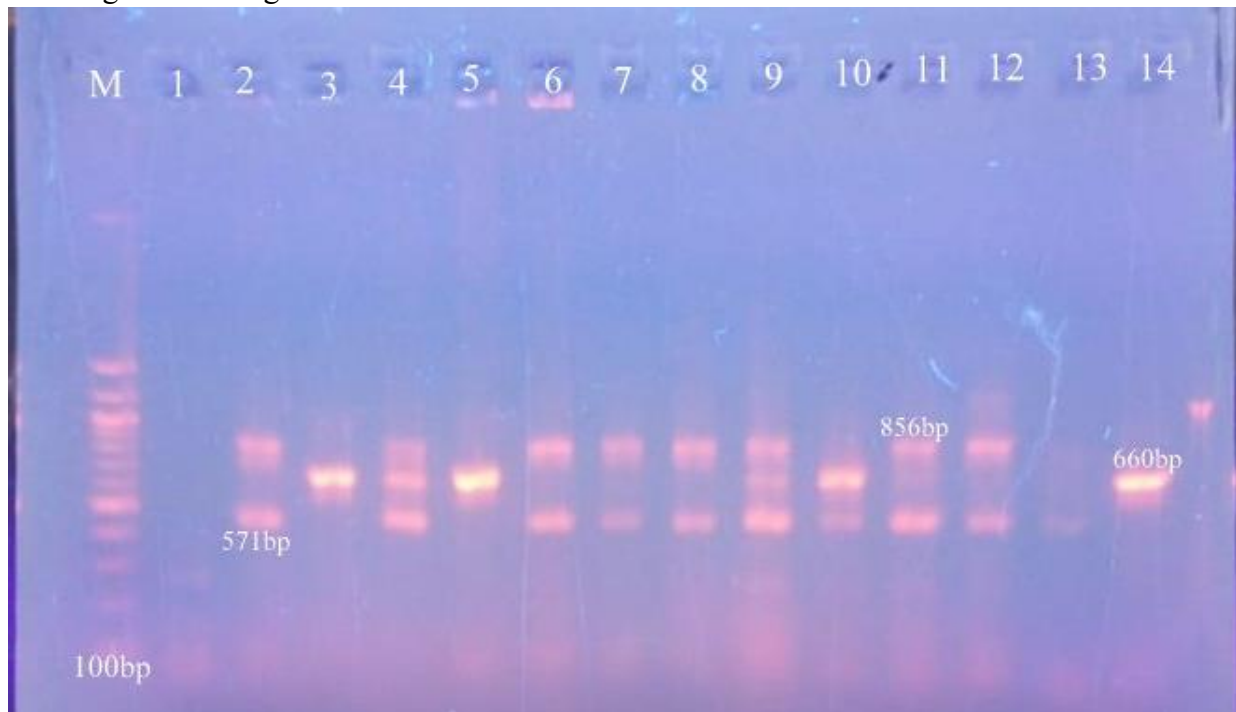


Figure 4: Gel Electrophoregram of Multiplex PCR for resistant gene detection
Lane M: Ladder; Lane 2, 4, 6, 7, 8, 9, 10, 11, 12: tetB (571bp); Lane 3, 4, 5, 9, 10, 14: SHV(660bp);
Lane 2, 4, 6, 7, 8, 9, 11, 12: HEP (856).

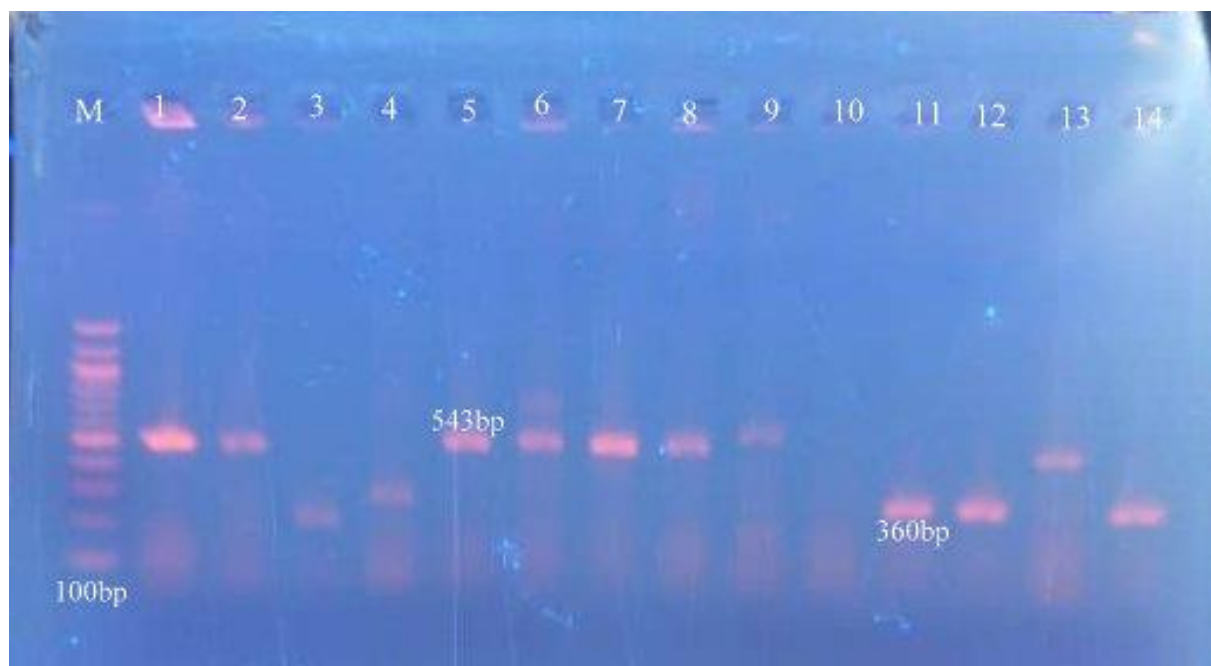


Figure 5: Gel Electrophoregram of Multiplex PCR for resistant gene detection
Lane M: Ladder; Lane 1, 2, 5, 6 7, 8, 9, 12: qnrA(543); Lane 4, 11, 12, 14: qnrB (360bp)

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, cellulitis, 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. Ilyasu *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), sulfhydryl-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

- Abdelhady, S. I., Elsheshtawy, N. M. & Hamdi, N. A. (2016). Rapid detection of extended spectrum β -lactamases production in *Escherichia coli*. *International Journal of Applied Microbiology and Biotechnology Research* 4, 109–116.
- Abdel-Rhman, S. H., Elbargisy, R. M. & Rizk, D. E. (2021). Characterisation of Integrons and Quinolone Resistance in Clinical *Escherichia coli* Isolates in Mansoura City, Egypt. *International Journal of Microbiology*, 2021, 6468942. <https://doi.org/10.1155/2021/6468942>
- Agbabiaka, T. O. & Olofintoye, B. O. (2019). Microbial Diversity in Water and Biofilm Samples from Well Sources in Ilorin Metropolis, Nigeria. *NotulaeScientiaBiologicae*, 11(1), 56–62.
- Babalola, T. F., Olowomofe, T. O., Omodara, T. R. & Ogunyemi, T. Y. (2021). Antibiotic

- Resistance Pattern and Plasmid Profile of Bacteria Isolates from Household Water Distribution Tanks in Ado-Ekiti. *Journal of Pure and Applied Microbiology*, 15(3), 1697-1705.
- Barrantes, K., Chacón, L., Morales, E., Rivera-Montero, L., Pino, M., Jiménez, A. G., Mora, D. C., Jiménez, P. S., Silva, B. & Romero-Esquivel, L. G. (2022). Occurrence of pathogenic microorganisms in small drinking-water systems in Costa Rica. *Journal of Water and Health*, 20(2), 344–355.
- Barns, J. N., Ezeamagu, C. O., Nkemjika, M. E., & Akindele, T. S. (2021). Prevalence of integrons in Enterobacteriaceae obtained from clinical samples. *Journal of Microbiology and Antimicrobials*, 13(1), 1-10.
- Bassetti, M., Vena, A., Croxatto, A., Righi, E., & Guery, B. (2018). How to manage *Pseudomonas aeruginosa* infections. *Drugs in context*, 7, 212527. <https://doi.org/10.7573/dic.212527>
- Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews*, 42(1).
- Cepas, V., López, Y., Muñoz, E., Rolo, D., Ardanuy, C., Martí, S. & Soto, S. M. (2019). Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. *Microbial Drug Resistance*, 25(1), 72-79.
- Coque, T. M., Baquero, F. & Canton, R. (2008). Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Eurosurveillance*. 13(47), 5437-5453.
- Cristina, M. L., Sartini, M. & Spagnolo, A. M. (2019). *Serratia marcescens* Infections in Neonatal Intensive Care Units (NICUs). *International Journal of Environmental Research and Public Health*, 16(4), 610. <https://doi.org/10.3390/ijerph16040610>
- David, V. E., John, Y. & Hussain, S. (2020). Rethinking sustainability: a review of Liberia's municipal solid waste management systems, status, and challenges. *Journal of Material Cycles and Waste Management*, 22(5), 1299–1317.
- Doosti, A., Pourabbas, M., Arshi, A., Chehelgerdi, M. & Kabiri, H. (2015). TEM and SHV Genes in Klebsiellapneumoniae Isolated from Cockroaches and Their Antimicrobial Resistance Pattern. *Osong Public Health and Research Perspectives*, 6(1), 3–8. <https://doi.org/10.1016/j.phrp.2014.10.011>
- do Nascimento Vieira, A., Kleinermanns, K., Martin, W. F., & Preiner, M. (2020). The ambivalent role of water at the origins of life. *FEBS Letters*, 594(17), 2717-2733.
- Ezeamagu, C. O., Harry, V. O., Ama, E. & Barns, J. N. (2021) Evaluation of antibiotic resistance of Enterobacteriaceae isolated from edible snails. *Malaysian Journal of Microbiology*. 17(2) 155-164 DOI: <http://dx.doi.org/10.21161/mjm.200925>
- Ghaderi, Z., Hall, M. C. M. & Ryan, C. (2022). Overtourism, residents and Iranian rural villages: Voices from a developing country. *Journal of Outdoor Recreation and Tourism*, 37, 100487.
- Ghazalibina, M., Mortazavi, H., Babadi, M., Rahimi, M., Khaledi, A., Teymouri, M. & Saburi, E. (2019). Prevalence of integrons and antibiotic resistance pattern in *Acinetobacter baumannii* isolated from clinical samples of Iranian patients: a systematic review and meta-analysis. *Ethiopian Journal of Health Sciences*, 29(5).
- Haller, L., Chen, H., Ng, C., Le, T., Koh, T., Barkham, T., Sobsey, M. & Gin, K. (2018). Occurrence and characteristics of extended-spectrum β -lactamase- and carbapenemase-producing bacteria from hospital effluents in Singapore. *Science of the Total Environment*. 615, 1119–1125.
- He, Y., Yuan, Q., Mathieu, J., Stadler, L., Senehi, N., Sun, R. & Alvarez, P. (2020) Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment. *NPJ Clean Water* 3, 4. <https://doi.org/10.1038/s41545-020-0051-0>
- Heß, S., Berendonk, T. U. & Kneis, D. (2018). Antibiotic-resistant bacteria and resistance genes in the bottom sediment of a small stream and the potential impact of remobilisation. *FEMS Microbiology Ecology*, 94(9).

- Ikeda, M., Yagihara, Y., Tatsuno, K., Okazaki, M., Okugawa, S. & Moriya, K. (2015). Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* bloodstream infections. *Annals of Clinical Microbiology and Antimicrobials*, 14(1), 1-7.
- Iliyasu, M. Y., Uba, A. & Agbo, E., B. (2018). Phenotypic detection of multidrug-resistant extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from clinical samples. *African Journal of Cellular Pathology*, 10(2), 25-32.
- Innes, G. K., Randad, P. R., Korinek, A., Davis, M. F., Price, L. B., So, A. & Heaney, C. D. (2020). External societal costs of antimicrobial resistance in humans attributable to antimicrobial use in livestock. *Annual Review of Public Health*, 41, 141–157.
- Kookana, R. S., Drechsel, P., Jamwal, P., & Vanderzalm, J. (2020). Urbanisation and emerging economies: Issues and potential solutions for water and food security. *Science of the Total Environment*, 732, 139057.
- Manhique, G. A., Hessel, C. T., Plessis, E. M. D. U., Lopes, S. M., de Oliveira Elias, S., Tondo, E. C. & Korten, L. (2020). Prevalence of Enterobacteriaceae on ready-to-eat salads, drinking water and surfaces in food markets of Maputo, Mozambique. *Journal of Food and Nutrition Research*, 8(1), 63-73.
- McKinney, C. W., Dungan, R.S., Moore, A. & Leytem, A., B. (2018). Occurrence and abundance of antibiotic resistance genes in agricultural soil receiving dairy manure, *FEMS Microbiology Ecology*. 94(3), 2018, fyy010, <https://doi.org/10.1093/femsec/fyy010>
- Munita, J., M. & Arias, C., A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*. 4(2).
- Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A. & Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*.
- Okpasuo, O., J., Aguzie, I., O., Joy, A., T. & Okafor, F., C. (2020). Risk assessment of waterborne infections in Enugu State, Nigeria: Implications of household water choices, knowledge, and practices. *AIMS Public Health*, 7(3), 634–649. <https://doi.org/10.3934/publichealth.2020050>
- Otokpa, O. J. (2019). Overview of major bacterial contaminants of drinking water in Nigeria: a review. *International Journal of Pathogen Research*. 2, 1-9.
- Paudyal, N., Anihouvi, V., Hounhouigan, J., Matsheka, M. I., Sekwati-Monang, B., Amoa-Awua, W. & Fang, W. (2017). Prevalence of foodborne pathogens in food from selected African countries - A meta-analysis. *International Journal of Food Microbiology*, 249, 35-43.
- Pitout, J., D. & Laupland, K., B. (2008). Extended-spectrum B-lactamase producing Enterobacteriaceae: an emerging public-health concern. *The Lancet Infectious Diseases* 8(3), 159-166.
- Pormohammad, A., Pouriran, R., Azimi, H. & Goudarzi, M. (2019). Prevalence of integron classes in Gram-negative clinical isolated bacteria in Iran: a systematic review and meta-analysis. *Iranian Journal of Basic Medical Sciences*, 22(2), 118–127. <https://doi.org/10.22038/ijbms.2018.32052.7697>
- Raimi, M., O., Omidiji A., Ayibatobira, A., Anu, B., Odipe, O. E. & Deinkuro, N., S. (2019). 'Digging Deeper' Evidence on Water Crisis and Its Solution in Nigeria for Bayelsa State: A Study of Current Scenario (July 18, 2019). *International Journal of Hydrogen* 3(4): 244 – 257, <http://dx.doi.org/10.2139/ssrn.3422483>
- Richard, E., Darracq, B., Loot, C. & Mazel, D. (2022). Unbridled Integrons: A Matter of Host Factors. *Cells*, 11(6), 925. <https://doi.org/10.3390/cells11060925>
- Rupp, M., E. & Fey, P., D. (2003). Extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae. Considerations for diagnosis, prevention and drug treatment. *Drugs* 63(4), 353-65
- Sharma R. & Sharma S.R. (2022) Microbial Aspect in Wastewater Management: Biofilm. In: Roy S., Garg A., Garg S., Tran T.A. (eds) Advanced Industrial Wastewater Treatment and Reclamation of Water. *Environmental Science*

- and Engineering. Springer, Cham. https://doi.org/10.1007/978-3-030-83811-9_4
- Sharma, V. K., Johnson, N., Cizmas, L., McDonald, T. J. & Kim, H. (2016). A review of the influence of treatment strategies on antibiotic-resistant bacteria and antibiotic-resistance genes. *Chemosphere*, 150, 702-714.
- Sirisha, T., Basha, P. & Kavitha, B. (2017) Isolation and Characterisation of Pathogenic Bacteria from Kundu River Water of Nandyal, Kurnool, Andhra Pradesh, India. *Journal of Applied Sciences*, 17: 475-481. <https://doi.org/10.3923/jas.2017.475.481>
- Uddin, M., A., Siddiqua, M., A. & Ahmed, M., S. (2020). Isolation and quantification of indicator and pathogenic microorganisms along with their drug resistance traits from bottled and jar water samples within Dhaka city, Bangladesh. *Stamford Journal of Microbiology*, 9(1), 12–14. <https://doi.org/10.3329/sjm.v9i1.45651>
- Van Boeckel, T., P., Brower, C., Gilbert, M., Grenfell, B., T., Levin, S., A., Robinson, T., P., Teillant, A. & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, 112(18), 5649–5654.
- Ventola, C., L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P&T: A Peer-Reviewed Journal for Formulary Management*, 40(4), 277-283
- You, D., Lindt, N., Allen, R., Hansen, C., Beise, J. & Blume, S. (2020). Migrant and displaced children in the age of COVID-19: How the pandemic is impacting them and what can we do to help. *Migration Policy Practice*, 10(2).
- Yu, Z., Michel Jr, F. C., Hansen, G., Wittum, T. & Morrison, M. (2005). Development and application of real-time PCR assays for quantification of genes encoding tetracycline resistance. *Applied and Environmental Microbiology*, 71(11), 6926-6933.
- Management Practice of Dysmenorrhea among University of Gondar Students, Northwestern Ethiopia: A Cross-Sectional Study. *International Journal of Reproductive Medicine*, (3):1-8
- Nwankwo, T., Aniebue, U., & Aniebue, P., (2010). Menstrual Disorders in Adolescent School Girls in Enugu, Nigeria. *Journal of Paediatrics and Adolescent Gynecology*, 23:358-363.
- Parazzini F, Chiaffarino F, Surace M, Chatenoud L, Cipriani S, Chiantera V, Benzi G, Fedele L., (2004). Selected food intake and risk of endometriosis. *Hum Reproduction* 19(8):1755-1759.
- Sanctis DV., Soliman A, Bernasconi S., (2016). Definition and self-reported pain intensity in adolescents with dysmenorrhea: A debate report. *J Pediatr & Child Health Care*, 1:1006
- Shehata N., Arafa A., El Wahed H., (2018). Epidemiology of Dysmenorrhea among university students in Egypt. *Int J Women's Health Wellness*, 4:2474–353
- UnsalAlaettin, UnalAyranci, Mustafa T., Gul Arslan & ElifCalik (2010). Prevalence of dysmenorrhea and its effect on quality of life among a group of female university students, Upsala. *Journal of Medical Sciences*, 115 (2): 138-145