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Bioinformatics analysis of clinical isolates of *Escherichia coli* from Nigeria and other countries for quinolone and fluoroquinolone resistance

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Abstract

Quinolones were the drugs of choice for the treatment of bacterial infections, especially, infections caused by Gram negative bacteria. Unfortunately, these drugs have been resisted by the microbial agents including *Escherichia coli*, known to be the leading cause of Urinary Tract Infection (UTI) globally. This study therefore was aimed at detecting all the genes involved in Quinolone resistance by the *E. coli* pathogen isolated from Nigeria and from other geographical regions, using robust techniques. Twenty-three sequence data files of *Escherichia coli* from various countries of the world were retrieved from the National Centre for biotechnology Information (NCBI) database and sent for genome assembly for processing of the short reads into long reads. The outcome was config. fasta files which were comprehensively annotated and characterized for genetic functions and mechanisms. A total of 208 antibiotic resistance genes were detected, out of which 27(13.0%) were linked to quinolone resistance and 14(6.7%) to multi-drug resistance. The result of this study significantly implicated many genes in quinolone resistance; notably were the efflux pump genes and their high percentage abundance. We recommend in-depth study of the genes for their expression capabilities, also the structure and features of the efflux pump genes to enable proper redesigning of drugs by integrating anti efflux pump substances that will selectively prevent the expression of the genes for antibiotic resistance, without any harm to the host, or that can destabilize the positive regulation of the operon for antibiotic resistance.

Keywords: Quinolone Resistance; Bacterial Infections; *Escherichia coli.* Antibiotic Efflux; Antimicrobial; Bioinformatics.

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1. Introduction

Antimicrobial agents are great treasures in Medicine owing to their invaluable roles in combating pathogenic micro-organisms and preventing initiation or spread of infections. Over the years, antibiotics have lost their potency to the awesome wits of the bacterial agents, which keep devising means of surviving the deleterious effects of antibiotics. Quinolone drugs which have been the most effective and reliable drugs in treating infections, especially those caused by the Gram-negative bacteria have been also resisted. The problem of antibiotic resistance is multiple, affecting every sphere of the universe, with untold disaster which includes prolonged sickness and prolonged hospital stay, high cost of treatment, morbidity and mortality[1–3]. Over the years, there have been many reports on antibiotic resistance, and scientists all over the world are concerned. Quinolones are chemosynthetically produced drugs known to have broad spectrum action against varieties of infectious microorganisms. Quinolone drugs most commonly used for the treatment of Infections include, Nalidixic acid, Ciprofloxacin, Levofloxacin, Moxifloxacin, Ofloxacin, Pefloxacin and Spafloxacin[4]. The resistance attack on the first generation quinolones especially, Nalidixic acid, led to the production of the more potent and efficient second, third and fourth generation members, the fluoroquinolones[5]. The fluoroquinolones have a slight structural modification from the other quinolones that enhances their efficacy against Gram negative pathogens, as well as widens the scope of activity against Gram positive pathogens[6]; they possess fluorine atoms at position C-6 of the quinolone structure and by substitution or addition of molecules at the other C-positions in the quinolone structure other members were obtained[7].

The quinolones exert their bactericidal action directed on the nucleic acid enzymes, the topoisomerase 11, also known as the DNA gyrase (encoded by GyrA and GyrB) and topoisomerase IV (encoded by ParE and ParC), that play vital roles in the cell synthesis. The topoisomerase enzymes work by introducing negative coiling when the DNA molecules supercoil during replication; they do that by effecting a temporal break on the chromosomes (which is quickly resealed), to enable the smooth replicative action of the DNA polymerase on the bacterial DNA molecule[8]. The fluoroquinolones not only eliminate enzyme function but also trap the enzymes and make it impossible for them to join back the DNA fragments, which results in the fragmentation of the chromosome. The action subsequently results to cell death[8,9]. The resistance of the quinolone and fluoroquinolone drugs by the Enterobacteriaceae family especially, *Escherichia coli* has been widely reported over the years, in the different Regions, States, Nations and continents of the world[10–15]. *Escherichia coli* among some members of the Enterobacteriaceae has received great attention owing to their leading position in causing UTI, recording 80% to 90% prevalence globally[16]; also, their notoriety in many varied and significant diarrheal diseases in humans and animals, and other diseases[17,18]. UTI alone is one infection that enables much prescription and consumption of antibiotics, leading to its abuse and consequently, induction of antimicrobial resistance, among many other factors. It is estimated that about 150 million UTI cases occur globally every year[16]; this implies high rate of antibiotic consumption. High intake of antibiotics has been found to correlate positively with increased antibiotic resistance[14].

Many studies have been able to account for molecular mechanism of antibiotic resistance of the *Enterobacteriaceae* family. However, not much has been known about the molecular mechanism of *E coli* of clinical origin, as most studies are centered on other species especially Salmonella spp[12] and *E coli* from the environment, mainly water, and livestock [4]. Again, many of the molecular mechanisms used are based on PCR techniques[10,11,19] These employ specific primers to amplify the already known regions, which are only a segment of the entire organism. Nevertheless, it is known that quinolone resistance in *E coli* is due to plasmid and chromosomal gene mediation [8,20]. The following genes so far, have been reported in clinical isolates of *E coli*: Qnr A, Qnr B, QnrS, qnrC, qnD, the efflux pumps genes, OqxB, QepA , OqxA and OqxB [8,10](and the aminoglycosyl acetyltransferase genes, Aac (6′) Ib-cr [8,10,11]. In Nigeria and many other developing countries, molecular mechanisms of bacterial resistance are yet to be comprehensively explored and employed appreciably. Most account of bacterial resistance in these areas are determined phenotypically. This entails the rigorous steps of initial culturing of the bacteria and proceeding to measure either the minimum inhibitory concentration (MIC) which is the gold standard, or the diameter of the zone of inhibition in disk diffusion testing. Phenotypic method provides basic and accurate information based on correct identification of bacterial isolates and the particular drug resisted; it does not identify the genes responsible for the resistance, neither does it show the mutations on the genes, resulting to resistance[21].

Sequencing and Bioinformatics with its diverse tools have been recently found extremely useful in detecting and mapping out genes for different phenotypic attributes, making a robust technique for detecting, studying and manipulating antibacterial genes[22,23]. Whole Genome Sequencing (WGS) is a molecular testing involving the analysis of the entire genomic DNA sequence of a cell in a single time, thereby comprehensively characterizing the genome[21,24]. Genome sequencing involves several steps culminating in the extraction of molecules of interest which can be DNA or RNA. These molecules are usually stored in the Genbanks in raw reed files to enable retrieval and use at any time. Bioinformatics involves the use of computer and biological software to store, archive, retrieve and analyze these

molecular data. Bioinformatics offers a lot of in silico analysis and manipulations which are current in our time for direct, precise and reliable results[25]. Mapping is employed to align the sequence data (e.g. DNA) to a reference sequence in order to detect the true location of each read from a potentially large quantity of reference data while distinguishing between technical sequencing errors and true genetic variation within the sample[26]. So many bioinformatics software, databases and genbanks abound, and many can be accessed over the net freely. Databases store a lot of molecular information which can be in the form of nucleic acids, amino acids, or proteins. Such databases include the National Centre for Biotechnology Information (NCBI) database, DNA Database of Japan (DDJ), European Nucleotide Archive (ENA), the PATRIC software tool (currently known as the Bacteria and Virus Bioinformatics Resource Centre (BV-BRC), the Comprehensive antibiotic Resistance Database (CARD) and National Database of Antibiotic Resistance Organisms (NDARO), Universal Protein Resource (UniProt), Swissprot and many others. Many of these Databases contain curated molecular data and many contain tools for analysis. However, there are software such as the Generous software devoted to all kinds of bioinformatics analysis. Such software analytical tools are customized for giving high quality analysis and reliable results. They have in-built quality control and assessment measures and many different methods and algorithms for all kinds of analysis. Some other tools, such as LOOKER STUDIO and CLUSTVIS are mainly for visualization and inferential statistics. Over 60 bioinformatic tools currently exist[26] with at least 47 accessible over the net[23].

In the growing emergence and re-emergence of antimicrobial resistance especially with regards to the Quinolone/Fluoroquinolone (QF) drugs, it is important to employ robust molecular methods to explore all the genes responsible for QF resistance by *Escherichia coli.* The best way to achieve this in our current technological advanced knowledge is to use Whole Genome Sequencing which ensures large amount of sequence data and bioinformatic tools to enable gathering of high quality data from curated databases for more reliable and direct in silico analysis[22]. Therefore, the goal of this study is to use WGS and bioinformatics to detect all the genes involved in QF resistance. This study will also determine the genetic relatedness of *E coli* isolates from Nigeria with those of other regions regarding quinolone resistance.

2. Materials and Methods

2.1. Sequence Data

A total of 23 Whole genome Sequence (WGS) files of clinical isolates of *Escherichia coli,* from 13 different countries were obtained from the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database. Table 1 Shows the information details of the data files.

2.2. Genome assembly

Using the Bacterial and Viral Bioinformatics resource Centre pipeline (PATRIC) pipeline (v3.6.9), the accession number of the sequence data files were keyed in for Genome assembly. The processing of the reads was done using several tools to ensure good quality control and accuracy. The tools include the unicycler assembler (v.0.4.8) which employed the SPAdes (v3.12.0) to assemble small reads from the Illumina sequence reads, the Bandage (v0.8.1) a graphical user interface(GUI) program to enable interaction with the assembler designs; the minimap2(2.17-r974-dirty), which helped to align the nucleotide sequences; pilon (v1.23), quality assessment tool QUAST(V5.2.0) and Sequence alignment/Map tool, Samtools (v1.17)for proofreading, corrections and rating the quality of the assemblies[27–29]. The long assembled contig output was in a file format as contig. fasta file. All statistics were based on contigs of size greater than (>) 300bp.

2.3. Comprehensive Genome analysis and Detection of Quinolone Genes

The assembly contains all the sequence data obtained from Genome assembly were submitted to the PATRIC pipeline (v3.6.9), in their assigned unique genome identifiers, for annotation, prediction of the genes and for functional categorization. The annotation was done using the Rapid Annotation Subsystem Technology (RAST) toolkit (RASTtk)[30], and bacteria as the taxonomic target domain. The functional features of the strains were determined using the genetic codon for most bacteria. The KEGG database was employed to integrate the comprehensive genetic information with metabolic pathways to enable better prediction of functions[31].

To identify as well as quantify the resistome profiles of the clinical WGS data, the Blast-like alignment tool (BLAT) and the K-mer- based AMR genes detection was employed. This utilized curated databases of PATRIC and of others such as the Comprehensive antibiotic Resistance Database (CARD) and National Database of Antibiotic Resistance Organisms (NDARO) to provide drug resistance and classification of all the detected Antibiotic Resistance Genes, (ARGs) into various categories, functions and mechanisms[32].

The entire antibiotic resistance genes were analyzed for their frequencies and distribution in the different strains from the different geographical Regions. All the genes linked to QF resistance were determined according to their relative abundance and in the strains and in the different geographical Regions.

2.4. Phylogenetic Analysis and Average Nucleotide Identity

The PATRIC web tool (v3.6.9), was also employed for this service. The codon method was employed to analyze aligned coding DNA using predefined PATRIC global protein families(PGFams)[33]. The contig. Fasta file were submitted and the alignment was done using the program, RAxML and the Muscle method[34]. The result of the analysis was given in the form of scaled vector graphics (SVG) image and as downloadable Newick file[32]. The Newick file was viewed, annotated, and designed using the Interactive tree of Life (ITOL) (v6.8) software tool.

2.5. Visualization and Analysis of Data

The online software tools, LOOKER STUDIO (https://lookerstudio.google.com) and CLUSTVIS (https://biit.cs.ut.ee/clustvis/), and EXCEL Microsoft were employed to arrange and visualize the results of the antibiotic resistance genes. The heatmap was done using CLUSTVIS[35] software tool. The Average nucleotide identity based on blast was determined using JSPECIES[36]. Jamovi software (v2.4.6) was employed for statistical test for Association between the Genes and the *Escherichia coli* strains; also, for spearman's correlation between the *Escherichia coli* strains based on the presence of the QF antibiotic genes. Graphics were designed using Inskape (v1.1.2).

3. Results

3.1. Data Quality Control and Quality assessment.

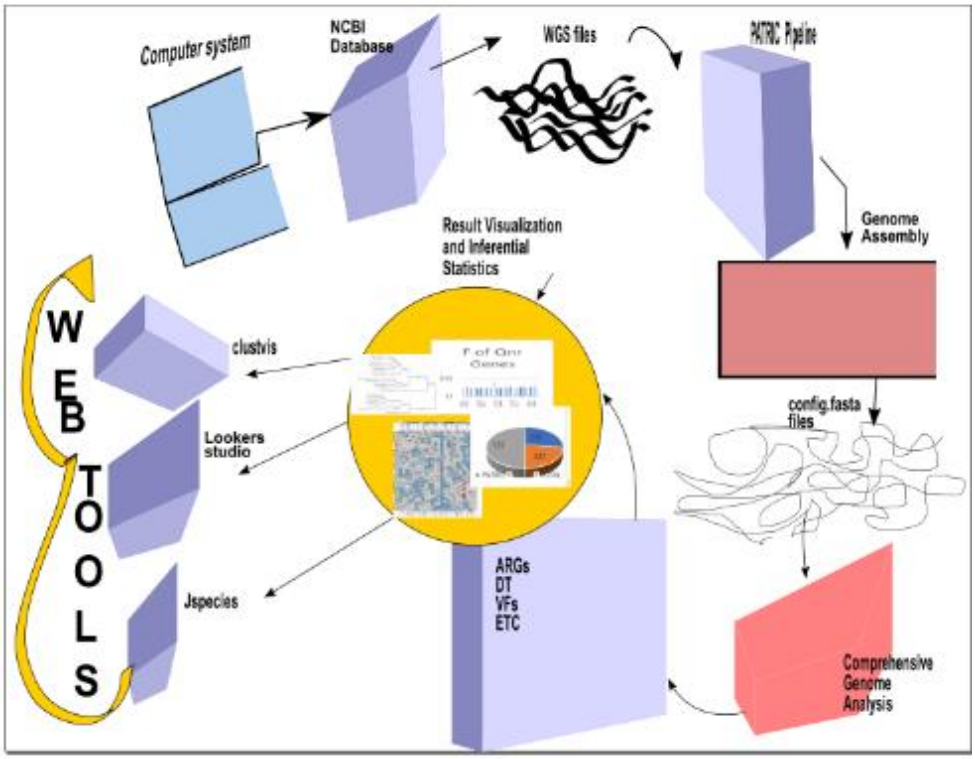

Figure 1 Methodological figure showing the workflow

The WGS data used for this study were carefully selected, to ensure all were of good size, none was below300bp; 91.7% were above 400bp. The assembly statistics was given based on samples greater than 300pb in size. The Check completeness was thorough while the consistency, both fine and coarse, were rated high. Quality assessments indicated possibilities of obtaining rich annotated features including Antibiotic resistance genes (ARGs). The assembly processed outcome was rated good. Table 2 shows the nature and content of the analyzed sequence data. The work process as shown in figure 1, started with searching for the appropriate whole genome sequence data of clinical isolates of

Escherichia coli in the Gene database. The sequence data files were uploaded for genome assembly and comprehensive genome analysis using PATRIC, an internet tool for bioinformatic analysis. Using in-built methods and algorithms, the WGS data files were analyzed to give the antibiotic genes. These genes were explored, visualized, and computed using looker studio, clustvis, excel Microsoft ware and Jamovi (v2.4).

3.2. Identification of the Q/F Resistance Genes

Figures 1 illustrates the strategies used in the detection of the quinolone resistance genes in the sequence data files of clinical isolates of *Escherichia coli.*

A total of 208 ARGS with a total frequency of 3775 were mapped out from 3 different sources, CARD, NDARO and PATRIC databases. Twenty-seven (27) of these genes with the frequency of 735(19.5%) were directly linked to the QF drugs alone or in combination with one or more other antibiotics classes such as aminoglycosides, carbapenems, colistin, sulphanamides, cephalosporins, tetracyclines and many others. Fourteen (14) of these genes with the frequency of 318(8.4%) were linked to multi -drug resistance, while the greater majority, 166 were linked to resistance of drugs other than the QF. The frequency of these genes and their source database are shown in figures 2a, 2b and 2c.

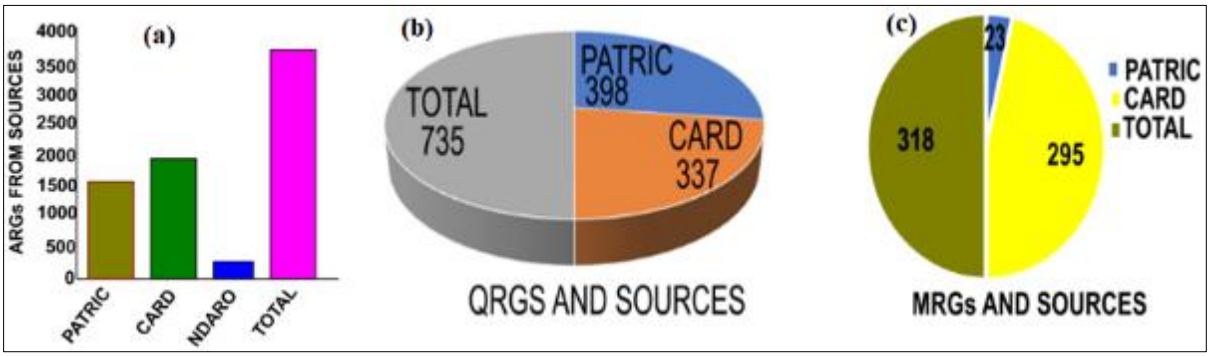

Figure 2 Sources of the Antibiotic Resistant Genes (ARGs) and their Sources. (A) All ARGs; (B) Quinolone resistance Genes (QRGs) and (C) Multi-drug Resistance Genes (MRGs)

3.3. Relative Abundance of the Quinolone resistance Genes

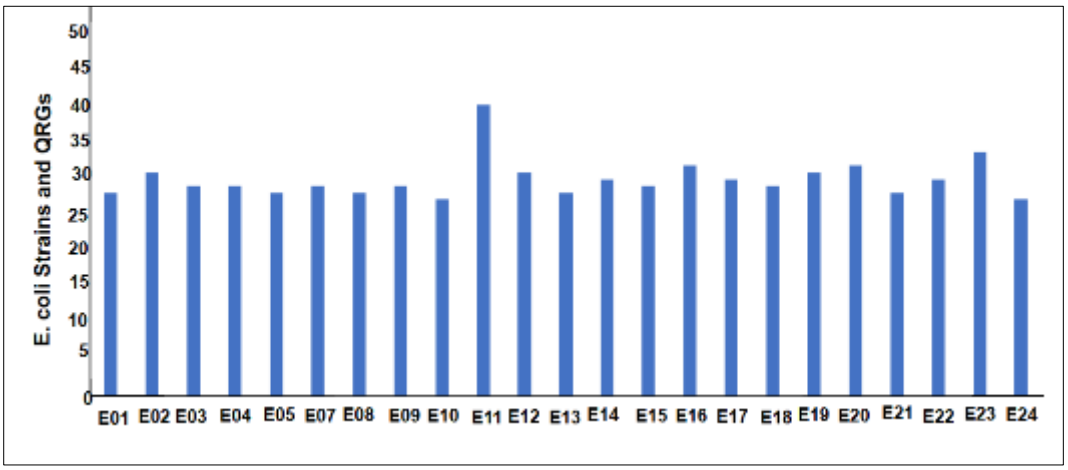

Figure 3a The frequency of the Quinolone Resistance Genes on the *E coli* strains.

Figure 3a shows the abundance of the QF Resistance Genes in the various *Escherichia coli* strains. E11 (Bel_Strain US03) from Belgium, had the highest quinolone resistance gene frequency 43(5.9%). This is followed by E23 (NIG_MA_246) at 36(4.9%). The lowest was E10 (BEL_LtABU36) and E24 (PAK_PH141) at 29(3.9%). Every other strain fell between 34(4.6%) and 29(3.9%). Similarly, Belgium had the highest impact of the quinolone resistant genes at 36(5%), followed by Nigeria at 34(5%). All the other Regions had the same percentage prevalence of 4% (figure 3b). A statistic of correlation on the Strains using spearman's rho indicated (0.873>P=0.05), showing that there is no statistical difference between the strains based on the abundance of the Q/F resistance genes.

The Efflux pump genes were the most frequent, with AcrAB-TolC and MdtEF-TolC occurring highest at 74(10%) and 70(10%) respectively. H-NS occurred at 55(7%), gyrA, gyrB, AcrEF-TolC, and MdtEF-TolC occurred at 48(7%), mdtF at 26(4%), tolC at 25(3%), emrA, mfd and msbA at 24 (3%), GadE, emrB, parE and parC at 23(3%), AcrZ and mdtE at 22 (3%), MdtM at 19 (3%), MexAB-OprM at 7 (1%), AAC(6')-Ib-cr and QnrB family at 5 (1%) . QnrA1, QnrS1, QnrS2 and QnrB2 have the least occurring frequency of (I) each (figure 3c).

E11 portrayed the highest frequency of 43(5.8%).

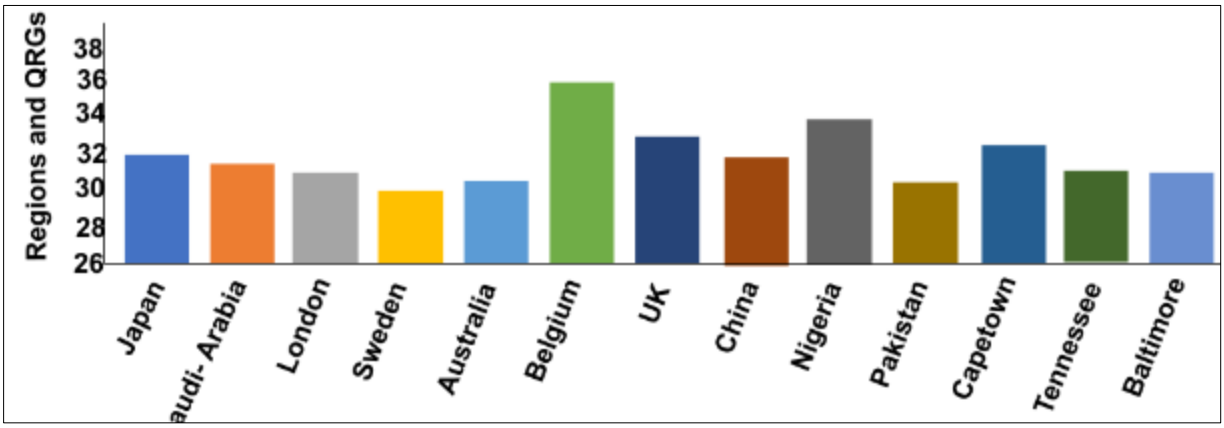

Figure 3b The Various Locations and the Relative Abundance of the Quinolone Resistance Genes

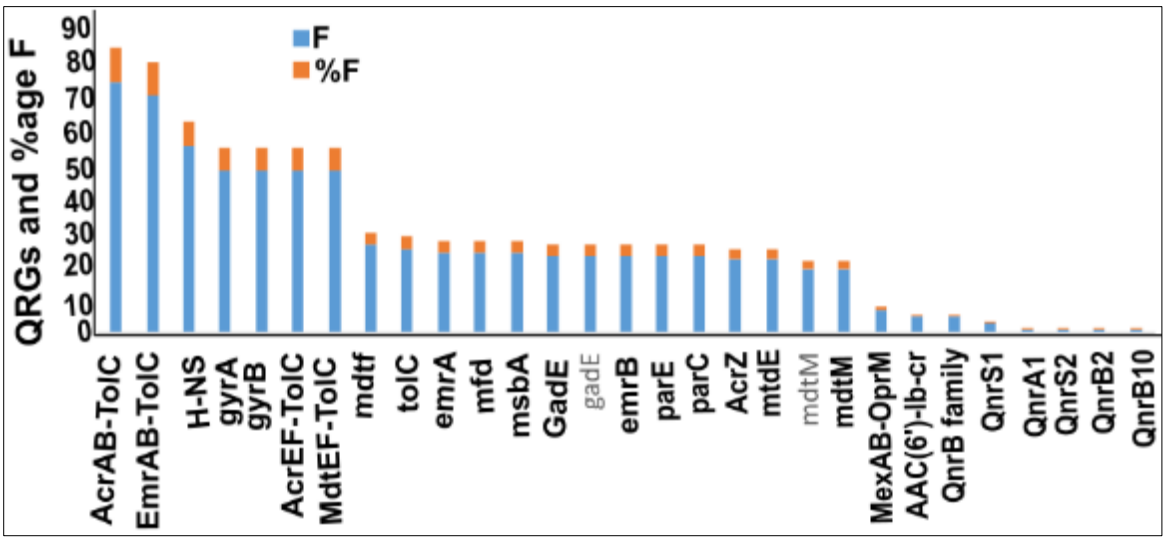

Figure 3c The frequency and the Percentage Frequency of the Quinolone Resistance Genes.

3.4. Classification of the Q/F Resistance Genes

Generally, the mechanisms portrayed for Q/F resistance in this study can be summarized into 5 main groups; the Antibiotic Efflux pump which has the highest frequency of 61.3%; Antibiotic target gene mutation (19.2%), Antibiotic target protein protection (4.9%), Antibiotic inactivation (0.7%) and Regulations which modulate ARG expressions (14%) (figure 4a).

The genes involved in Efflux pump Q/F resistance include AcrEF-Tolc, H-NS, MexAB-OprM, AcrZ, tolc, EmrAB-Tolc, msbA, MdtEF-TolC, emrB, emrA, mdtM, mdtF and gad E, majority of the genes in efflux pump mechanism also partook in regulations which modulate antibiotic resistance gene expression. The genes involved in target gene mutation include ParC, gyrA, parE and GyrB, while Mfd, QnrB10 QnrB2 QnrS1 QnrS2, QnrA1 and QnrB family were involved in target protein protection. AAC (6')-Ib-cr mediated resistance by antibiotic modification and inactivation. EmrAB-Tolc, AcrAB-TolC, H-NS and Gad E were in the 5th class of regulator modulating expression of antibiotic Resistance genes. The mechanisms employed by the strains were highest at 5.8% in E11 and least in E24 at 3.7%. The other isolates scored between 4.6% and 4.1%. (figure 4a).

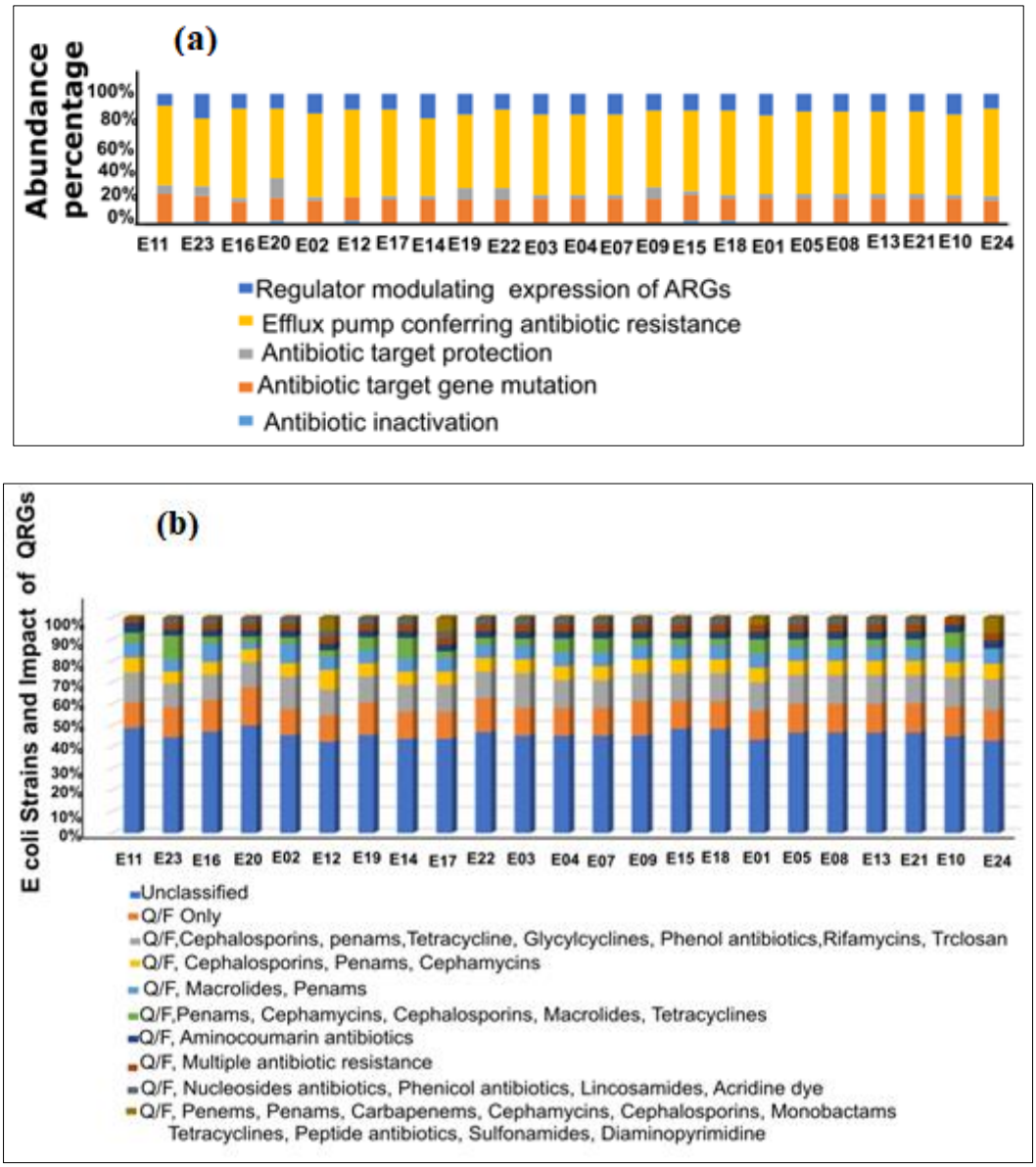

Figure 4 *E coli* Strains and Q/F Resistance Genes Frequency (a) According to mechanism of action and (b) According to the antibiotic class

The Q/F resistance genes were grouped into 10 classes depending on their resistance to other classes of antibiotics (figure 4b). Group 1 includes genes specifically linked to quinolones/ fluoroquinolone. Group 1 and group 2 occurred highest at the frequency of 13.6% and 13.1% respectively while the group 9 controlling many other classes of antibiotics (Penems, Penams, Carbapenems, Cephamycins, Cephalosporins, Monobactams, Tetracyclines, Peptide antibiotics, Sulfonamides, Diaminopyrimidine) occurred less frequently at 1.0%. There is an unclassified group which showed the highest prevalence of 45.9%. The different classes of the genes operated in the strains E11 from Belgium, E23, from Nigeria and E12 from Pakistan s at 3%, 2.7 and 2.6% respectively more than the other strains. However, they were lowest in the greater majority, at 2.2%.

3.5. Relative Abundance and Impact of the Q/F resistance genes

Overall, the following 12 genes had much influence in the isolates as indicated by the intense colour (+4): AcrAB-TolC, EmrAB-TolC, gyrA, gyrB, tolC, emrA, mfd, msbA, QnrS2, QnrA1, QnrB2 and QnrB10; Five genes: H-NS, QnrB family, QnrS1, AcrEF-Tol and AAC (6')-Ib-cr had moderate impact (+3). On the other hand, the following 3 genes had little or no impact: AcrZ, mdtE and MdtM (-4 to 0) (figure 5).

The greatest influence of Q/F resistance genes was seen in E11, from Belgium which highlights ten genes, among which six: Gyr B, GyrA, tolc, msbA, AcrAB-TolC and mfd exert powerful effects, as depicted by the intense colour index of the chart (+4). AcrEF-TolC had mild effect while MdtEF-TolC and mdtF had milder effect (+2). The other isolates that portrayed significant Q/F resistance gene impact include E16 (CAP_Eco04522) from South Africa, E23 (MA_246) from Nigeria, E20 (CHI_8076) from China and to a lesser extent, E19 (UK_IMP51), from United Kingdom. However, the resistance influences resulted from different genes. E16 was influenced moderately by MdtEF-TolC and mdtF, and intensely by EmrAB-TolC and emrA; The influence of quinolone resistance on E23 was due to QnrB2, QnrB10(high impact) and H-NS, AAC (6')-Ib-cr (moderate impact); E20 portrayed the high prevalence of QnrS2, moderate QnrB family, and mild QnrS1 and AAC (6')-Ib-cr, while E19 was significantly controlled by QnrA1, to a lesser degree by QnrB family and less significantly by H-NS. G21(AAC(6')-Ib-cr) showed widest area of coverage across the strains (affected 5 isolates: E12, E20, E23, E15 and E18), followed by G23(QnrS1) affecting 3 isolates: E20, E22 and E09; G08(mdtF) and G07(MdtEF-TolC) affecting E11, E16 and E02; and G20(MexAB-OprM) affecting E24, E17 and E12.

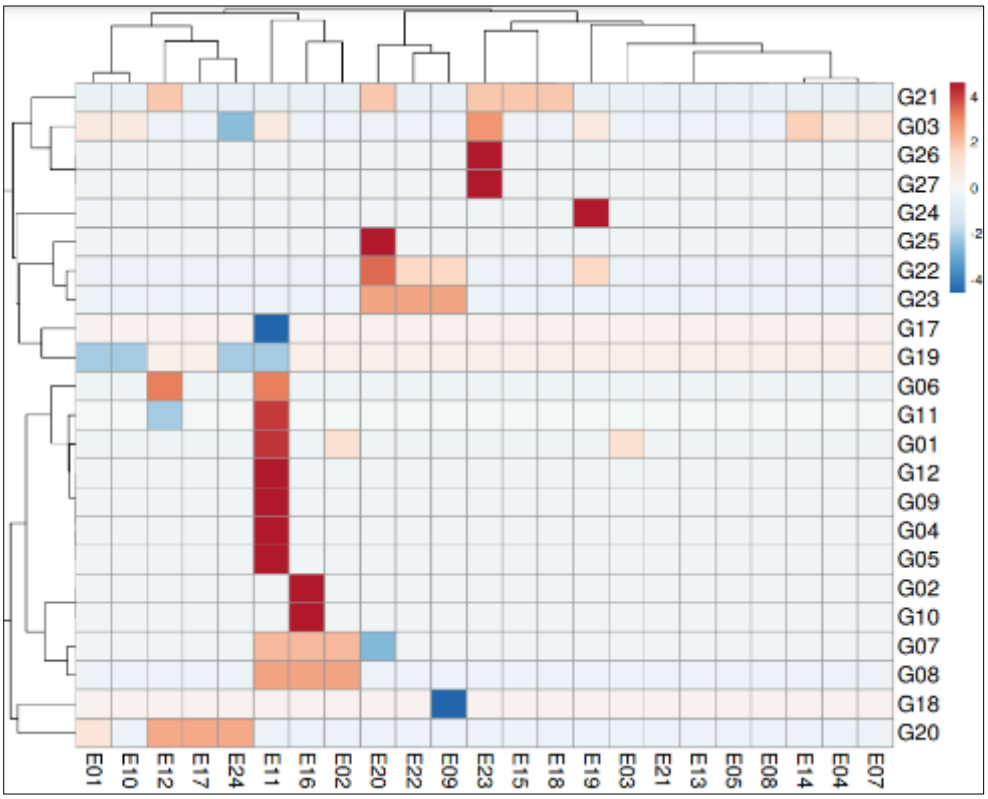

Figure 5 Clustered heatmap of Quinolone Resistance Genes. The colour range -4 to 4 denoted by their increasing intensity depicts the impact of the genes. G01 (AcrAB-TolC); G02(EmrAB-TolC); G03(H-NS); G04(gyrA); G05(gyrB); G06(AcrEF-TolC); G07(MdtEF-TolC); G08(mdtF); G09(tolC); G10 (emrA); G11(mfd); G12(msbA); G17(AcrZ); G18(mdtE); G19(MdtM); G20(MexAB-OprM); G21(AAC(6')-Ib-cr); G22(QnrB family); G23(QnrS1); G24(QnrA1); G25(QnrS2): G26(QnrB2) and G27(QnrB10). G13 (gadE); G14(emrB); G15 (parE) and G16(parC) were omitted by the analysing tool.

However, E11 of all the strains showed the highest expression of the multi-drug resistance genes. Figure 5 highlights the heatmap of the correlation between the various antibiotics and the genes. An Independent sample X2 test of association confirms the strong association between the strains and the quinolone resistance genes.

Generally, genetic resistance influence was predominant on the 1st and 2nd generation Quinolones which include, Nalidixic acid, norfloxacin and Ciprofloxacin respectively. Ciprofloxacin had the highest impact of the quinolone resistance genes at 24.6%, followed by norfloxacin and nalidixic acid at 15.7% and 15.6% respectively (figure 6).

14 genes were mapped out as multi -drug resistant genes. These are implicated in quinolone resistance. They include acrB, acrF, emrD, mdtG, acre, mdtH, mdtL, mdtP, MdtL, emrR, acrA, marA and marR. AcrB has the highest prevalence of 8.2% and the highest impact on E11. AcrF showed moderate prevalence on E11and on E12 (figure 7). Generally, the impact of the multi- resistance genes was found much on the Q/F drugs in this study.

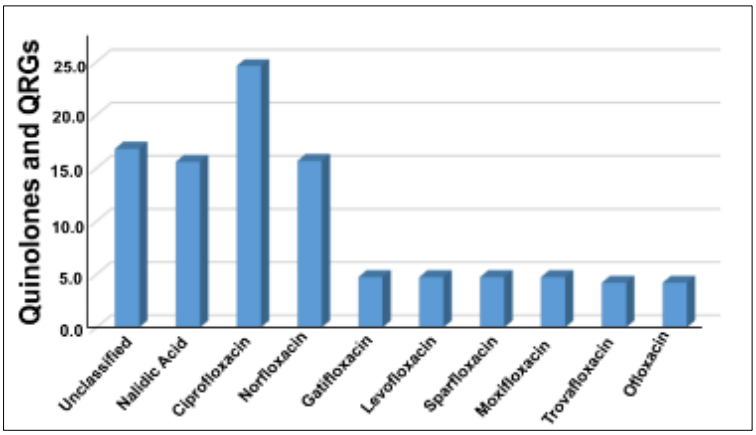

Figure 6 Prevalence of the quinolone resistance genes on the various quinolone drugs.

4. Discussion

Quinolone resistance by the microbial pathogen has been one area of great concern currently, more so as the more improved later generations, commonly and globally used to cure common infections are being seriously resisted. The need to understand the molecular mechanism of quinolone resistance drove this study. The use of bioinformatics enabled reaching out to different locations of the world for the common organism known to cause regular infections for which quinolones are prescribed, and which has been noted as a superbug, a member of ESCAPE. Exploring the genetic properties of the clinical *Escherichia coli* from the various regions of the world has given a holistic and better understanding of the overall mechanisms employed by this specie of bacteria to overcome the therapeutic effects of the quinolone drugs. The use of Whole Genome sequencing technology made the study more robust, giving opportunities to explore more the genetic involvement in antimicrobial resistance.

A total of 208 genes were found to be utilized by clinical *Escherichia coli* bacterial agent to fight against the efficacy of all types of drugs. This is awesome. A total of 27 genes were found to be involved in the resistance of the quinolone family. These genes used various mechanisms which were summarized into five. The Efflux pump mechanism had the most pronounced effects and was more prevalent. The many efflux pump genes detected in this study have rarely been reported in previous studies aimed at detecting quinolone resistance genes; only 3 efflux pump genes were constantly reported, and these include: OqxAB, QepA1 and QepA2[8] or OqxB, OqxA and QepA[10]. Surprisingly, none of these genes were detected in this study. That implies that many more genes involved in quinolone resistance in *Escherichia coli* have not been exhausted. The Efflux pump genes regulate the porins to ensure decreased concentration of the quinolone drugs in the cell, either by minimizing the amount of drug taken into the cell or by reducing the drug concentration in the cell by pumping out[37].

Two efflux pump genes were outstandingly prevalent at 10%: AcrAB-TolC,74(10%) and EmrAB-TolC, 70(10 %), and these are genes coding for efflux pumps which have been described as tripartite protein complex characterized by 3 major protein parts which include: the inner membrane protein(IMP), the outer membrane protein (OMP) and the periplasmic adapter protein(PAP)[38]. The IMP, recognizes, binds and translocates substrate, the OMP channel enables the easy transportation of the substrate from the cell while the PAP, links the IMP and the OMP[38]. AcrAB-TolC and EmrAB-TolC efflux pumps have been studied and described in *E. coli,* and have been associated the resistance of antibiotics including quinolone/fluoroquinolone in *Escherichia coli*[39]. The other tripartite efflux pump genes detected include AcrEF-Tolc, MdtEF-TolC and MexAB-OprM. All the efflux pumps genes work as opera: over expression resulting from their activation or regulation or even mutations, can lead to drug resistance[38]. These have been studied and reported to be involved in quinolone resistance in *E coli*. The efflux pump genes, EmrAB-Tolc, AcrAB-ToIC, H-NS and Gad E were also tagged with another mechanism of regulating and modulating the expression of quinolone resistance genes, also other drugs.

The genes, ParC, gyrA, parE and GyrB were found to use target protein mutation mechanism and constitute the Gyrase and the topoisomerase IV components of the Topoisomerase II which are the targets of the quinolone drugs. These genes are involved in point mutation in some amino acid contents of the molecules, making it difficult for quinolone lethal activity on the bacteria. These genes had been earlier described[8,43,44], and considered very frequent in occurrence as well as very significant[8]. Gyr B was also shown to mediate resistance in aminocouramine antibiotics whose mode of action is on the GyrB molecule of Gyrase, and on clofazimne which directs its action on the DNA.

The Genes QnrB10 QnrB2 QnrS1 QnrS2, QnrA1, QnrB family and Mfd, were involved in target protein protection mechanism. QnrA, QnrB, and QnrS, qnr C and qnrD, qnr VC had been described in earlier studies as mediating quinolone resistance in mainly Gram- negative organsims[45]. However, in *E coli*, all except qnrVc have been detected at different prevalences in different regions[10,11,16,46], using the multiplex PCR technique. In this study qnr D and qnr C and qnr VC were not detected. The qnr genes code for proteins of the pentapeptide repeat family that protect the topoisomerase enzymes from the lethal effect of the quinolone antibiotic[45]. The frequency of the qnr genes was low in this study. The Protein target protection genes were shown to be more specific and associated with the Q/F(group1) drugs by the independent sample X^2 test of Association (P= 1.000>(P=0.05). The AAC (6')-Ib-cr gene employed the mechanism of drug inactivation. It is active on aminoglycosides but also able to modify fluoroquinolones such as ciprofloxacin and norfloxacin by acetylation thereby decreasing their drug activity[8]. It was shown to have moderate impact in this study.

Belgium had the highest number of quinolone resistance genes. However, in real scenario, the sample size per region in this study could be too small to detect countries with the highest impact of quinolone resistance. There was no statistical difference between the various strains based on the abundance of the quinolone resistance genes. However, many countries across the globe have significantly reported quinolone resistance.

The first-generation quinolones (Nalidixic acid) and the second-generation fluoroquinolones (ciprofloxacin and Norfloxacin) were found to portray the highest frequency of the Q/F resistance genes. By implication these drugs are the most resisted. Ciprofloxacin showed the highest level of resistance. This is not surprising as Ciprofloxacin is the most common quinolone used across the globe to treat the commonest infection even without adequate prescription by the medical experts[47–49] And so, misuse and other abuses may be co-founding factors for quinolone resistance. The other quinolones, sparfloxacin, ofloxacin, gatifloxacin, moxifloxacin and travofloxacin are rarely prescribed and used.

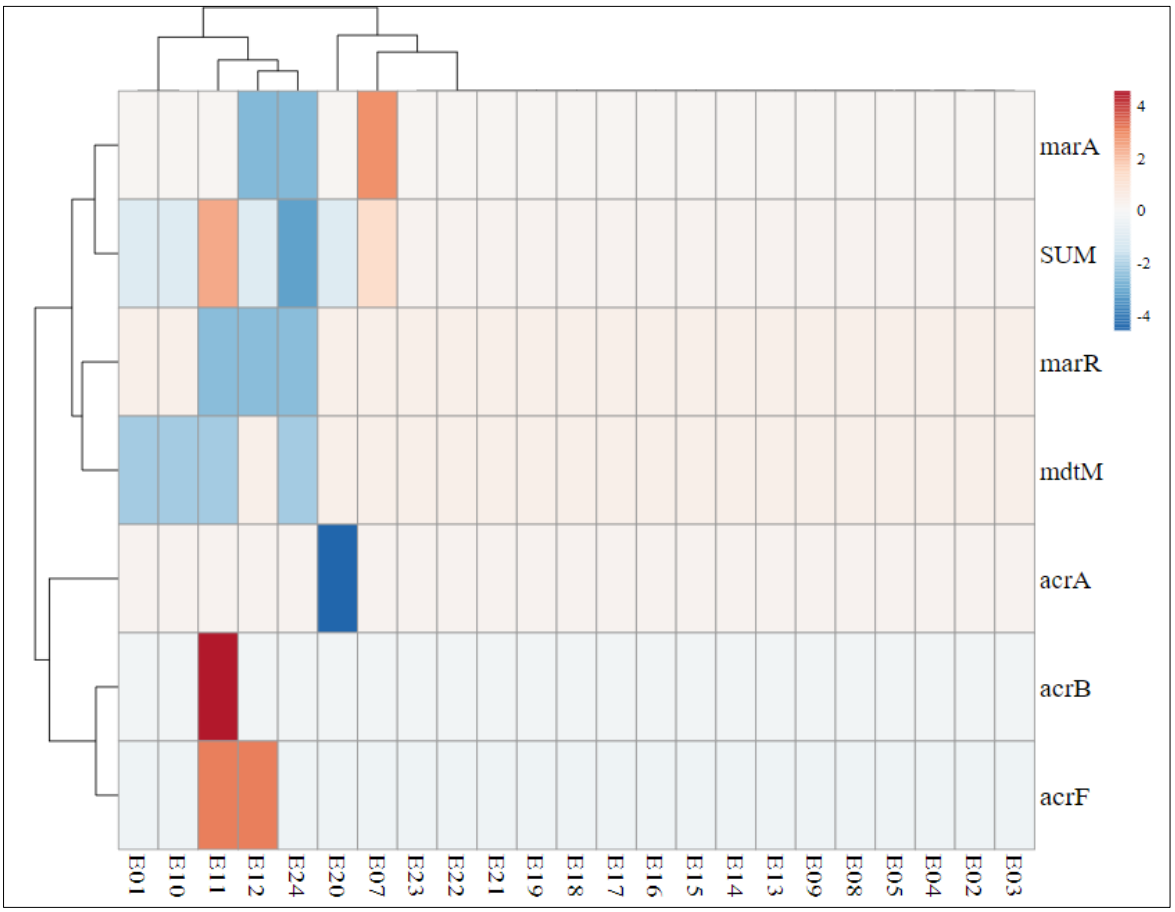

Figure 7 Impact of the multi- drug resistance genes on the *Escherichia coli* strains.

The average nucleotide Identity (ANI) and the phylogenetic analysis indicated that the strains were very closely related. By implication, they should have similar genetic functions including antimicrobial resistance mechanism, virulent factors, and drug targets among many other properties.

AcrB has high influence on E11. AcrF has moderate effect on E11 and on E12 while MAR has a moderate effect also but on E07.

4.1. Phylogeny and Average Nucleotide Identity

The Average Nucleotide Identity (ANI) showed homogeneity of the strains with scores of relatedness between the percentage of 95.18 and 99.9 (figure 8).

The phylogenetic Tree has 16 branches and 3 clades (figure 9). E23 (NIG_MA_246), E21(CHI_BW25113), E17 (SAU_MOD1-EC66350, E22 (NIG_MA_029), E09 (AUS_M82751_ill), E14 (BAL-P0298942.11) and E13 (BAL_P0298942.9) are all from one clade and are 100% related to the immediate and past generation ancestors; likewise the strains, E19 (UK_IMP5), E18 (JAP_NCGM 78), E20 (CHI_8076), E16 (CAP_Eco04522) and E24 (PAK_PH141). In the 3rd clade, E15 (CAP_Eco04518), E12 (PAK_PH101-2) and E07 (TEN_VUTI483) are 85% related to their immediate ancestor. E10 (BEL_LtABU36) and E11 (BEL-US03) share a very common immediate ancestor at 100% relatedness. However, the second branch of the ancestral lineage of the 3rd clade has a bootstrap value of 19, which is quite low and strange. Every other branch has a bootstrap value of 100 showing the high similarity and genetic relatedness.

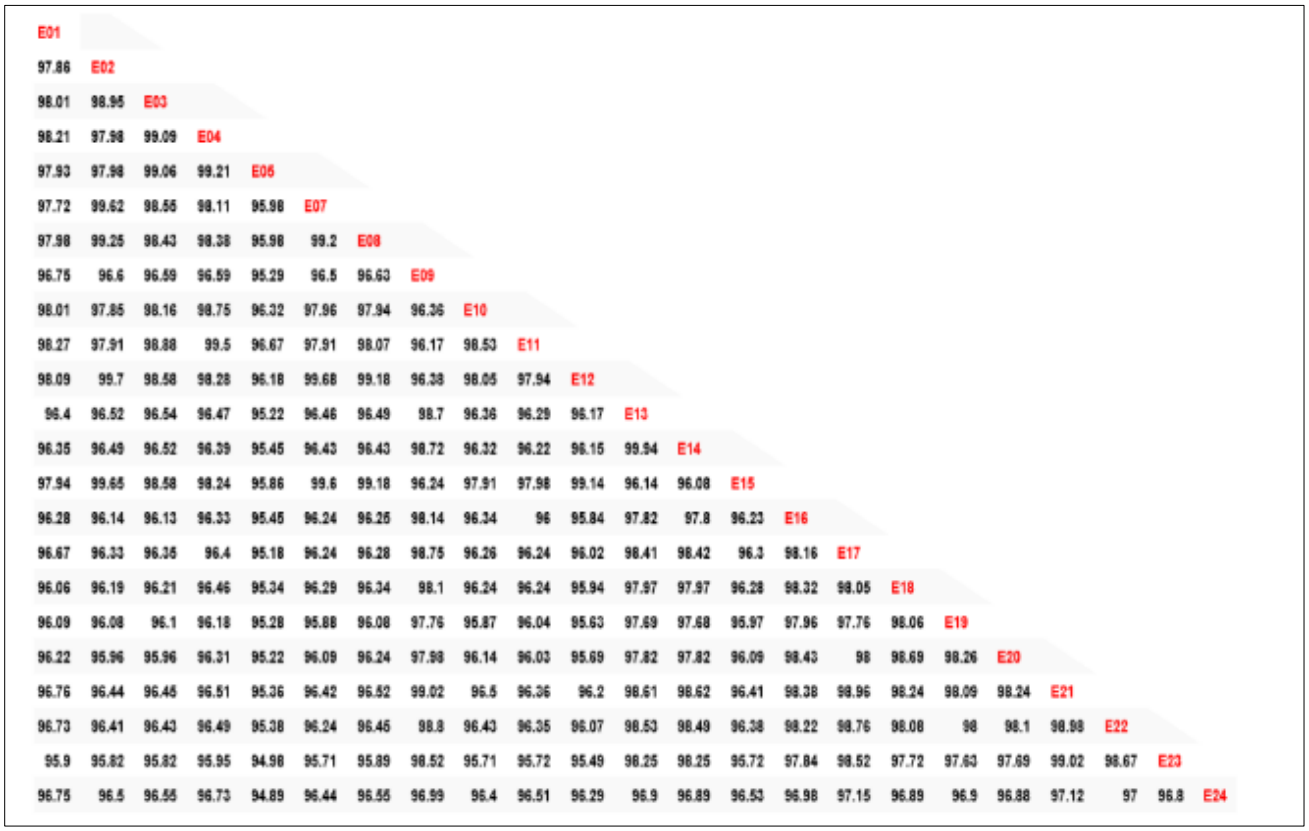

Figure 8 Average Nucleotide Identity using blast (ANIb). Showing the percentage relatedness or identity of the *Escherichia coli* Strains which sequence data were used in this study.

Table 1 Details of the WGS clinical samples of Escherichia coli obtained from NCBI SRA

ID	BIOPROJECT	BIOSAMPLE	`RUN ACCESSION	STRAIN	SIZE (mb)	LOCATION	SOURCE	SUBMITTER	YEAR	SEQUENCING PLATFORM
E1	PRJNA819016	SAMN36399219	SRR25229479	TEN_VUTI484	458.3	Tennessee	Urine	Vanderbilt University Medical Center,	2023	ILLUMINA (NextSeq 2000)
E2	PRJNA976817	SAMN35442869	SRR24751079	JAP_CMZE100	406.4	Japan	Urine	Kyoto University Graduate School of Medicine	2023	ILLUMINA (NextSeq 1000) run
E3	PRJNA939963	SAMN33549805	SRR23652003	SAU_O6:H1	496.3	Saudi Arabia	Food	Saudi food and drug authority	2023	ILLUMINA (Illumina NovaSeq 6000) run:
E4	PRJEB51925	SAMEA112356746	ERR10799306	LON_ST131	401.5	London	Urine	university of west London	2023	ILLUMINA (Illumina HiSeq 1500) run
E5	PRJNA914629	SAMN32539345	SRR23033115	SWE_M5_KL_1	422.1	Sweden	stool	University of Gothenburg, Astrid von Mentzer	2023	BGISEQ (DNBSEQ-G400) run
E7	PRJNA819016	SAMN36399218	SRR25229480	TEN_VUTI483	418	Tennessee	Urine	Vanderbilt University Medical Centre,	2023	ILLUMINA (NextSeq 2000)
E8	PRJNA797179	SAMN28668821	SRR19561918	AUS_M14304_ill	436.7	Australia	blood	University of Queensland	2022	1 ILLUMINA (NextSeq 500) run
E9	PRJNA797179	SAMN28669037	SRR19561566	AUS_M82751_ill	481.3	Australia	rectal swab	University of Queensland	2022	1 ILLUMINA (NextSeq 500) run
E10	PRJNA592372	SAMN13422941	SRR10568376	BEL_LtABU36	405.7	Belgium	urine	LMM, University of Antwerp	2020	1 ILLUMINA (Illumina MiSeq) run
E11	PRJNA592372	SAMN13423049	SRR10568402	BEL-US03	408.2	Belgium	blood	LMM, University of Antwerp	2020	1 PACBIO_SMRT (Sequel) run
E12	PRJNA261540	SAMN03074764	SRR1610042	PAK_PH101-2	443.1	Pakistan	clinical	Washington University	2015	1 ILLUMINA (Illumina HiSeq 2500) run
E13	PRJNA77529	SAMN00829349	SRR446894	BAL_P0298942.9	427.8	Baltimore	clinical	University of Maryland Institute for	2012	1 ILLUMINA (Illumina HiSeq 2000) run

								Genome Sciences (UMIGS)		
E14	PRJNA77533	SAMN00829341	SRR446886	BAL-P0298942.11	442.9	Baltimore	clinical	University of Maryland Institute for Genome Sciences (UMIGS)	2012	2 ILLUMINA (Illumina HiSeq 2000) run
E15	PRJEB46655	SAMEA8997801	ERR6384856	CAP_Eco04518	457.4	Capetown	clinical	Universitaetsklinikum Freiburg	2021	1 ILLUMINA (Illumina MiSeq) run
E16	PRJEB46655	SAMEA8997805	ERR6384860	CAP_Eco04522	416.1	Capetown	clinical	Universitaetsklinikum Freiburg	2021	2 ILLUMINA (Illumina MiSeq) run
E17	PRJNA230969	SAMN04992465	SRR6727474	SAU_MOD1-EC6635	391.2	Saudi-Arabia	faeces	FDA Centre for Food Safety and Applied Nutrition (CFSAN)	2018	1 ILLUMINA (Illumina MiSeq) run
E18	PRJDB3087	SAMD00019975	DRR022998	JAP_NCGM 78	418.7	Japan	blood	NCGM	2016	ILLUMINA (Illumina MiSeq) run
E19	PRJEB38818	SAMEA6990780	ERR4280194	UK_IMP51	357	UK	clinical	IMPERIAL COLLEGE LONDON	2022	1 ILLUMINA (Illumina HiSeq 4000) run
E20	PRJNA903069	SAMN31781979	SRR25500926	CHI_8076	401.3	China	Skin tissue	Nanjing Drum Tower Hospital	2023	ILLUMINA (Illumina NovaSeq 6000) run
E21	PRJNA816422	SAMN35060766	SRR24524030	CHI_BW25113	438.6	China	clinical	The Second Affiliated Hospital, University of South China	2023	1 ILLUMINA (Illumina HiSeq 2000) run
E22	PRJNA293225	SAMN12525617	SRR9917504	NIG_MA_029	399	Nigeria	faeces	FDA Centre for Food Safety and Applied Nutrition (CFSAN)	2018	1 ILLUMINA (Illumina MiSeq) run
E23	PRJNA293225	SAMN13245798	SRR10420703	NIG_MA_246	334.9	Nigeria	faeces	FDA Centre for Food Safety and Applied Nutrition (CFSAN)	2019	1 ILLUMINA (Illumina MiSeq) run

E24	PRJNA261540	SAMN03074771	SRR1610049	PAK_PH141	430.9	Pakistan	clinical	Washington University	2015	1 ILLUMINA (Illumina HiSeq 2500) run

Table 2 Analytical information of the Various WGS of the Strains

ID	STRAIN	PLASMID	GENOME LENGTH(BP)	GC CONTENT (%)	(CDS) OPEN READING FRAME	ANNOTATED PROTEINS	HYPOTHETICAL PROTEINS	RNA GENES	CRISPR REPEATS	GENES WITH PGFM DOMAINS	CONTIG N50	CONTIGS
E01	TEN_VUTI484	0	5,208,274	50.56	5,250	4678	665	84	0	5,102	210287	97
E02	JAP_CMZE100	0	5,094,498	50.79	5064	4477	587	81	0	4943	181,620	115
E03	SAU_O6:H1	0	5048809	50.78	5023	4457	559	79	0	4903	181,582	115
E04	LON_ST131	0	5,129,008	50.39	5,133	4,493	640	81	0	4,998	231,696	121
E05	SWE_M5_KL_1	0	4,837,115	50.54	4,891	4,434	457	83	20	4,777	298,271	86
E07	TEN_VUTI483	0	5,293,485	50.72	5,471	4,761	710	81	0	5,324	159,709	195
E08	AUS_M14304_ill	0	5,050,333	50.82	5,037	4,509	528	82	0	4,908	123,913	170
E09	AUS_M82751_ill	0	4,733,662	50.65	4,710	4,265	445	78	21	4,626	163,952	96
E10	BEL_LtABU36	0	5,181,197	50.52	5,164	4,563	601	77	21	5,029	203,072	119
E11	BEL-US03	0	5,216,558	50.59	6,800	6,105	695	111	109rp rg	6,592	3,114,476	8
E12	PAK_PH101-2	0	4,244,406	51.66	4,199	3,782	417	39	0	4,125	45,779	214

E13	BAL_P0298942.9	0	4,687,728	51.25	4,750	4,324	426	63	12	4,654	29,541	340
E14	BAL-P0298942.11	0	4,931,681	50.46	5,042	4,464	578	50	12	4,916	74,650	239
E15	CAP_Eco04518	0	5,216,244	50.81	5,332	4,696	636	80	0	5,190	144,855	196
E16	CAP_Eco04522	0	5,138,071	50.6	5,275	4,678	597	80	12	5,164	103,407	261
E17	SAU_MOD1-EC6635	0	4,889,712	50.64	4,877	4,434	443	81	19	4,786	133,847	116
E18	JAP_NCGM 78	0	5,070,078	50.74	5,117	4,499	618	84	30	4,975	139,616	131
E19	UK_IMP51	0	5,156,792	50.61	5,270	4,532	738	49	9	5,139	68,574	317
E20	CHI_8076	0	5,104,795	50.65	5,166	4,603	563	83	36	5,035	169,133	163
E21	CHI_BW25113	0	4,548,394	50.74	4,467	4,211	256	80	21	4,408	173,974	84
E22	NIG_MA_029	0	4,630,241	50.63	4,619	4,222	397	80	32	4,545	109,438	128
E23	NIG_MA_246	0	5,465,295	50.41	5,620	4,872	748	83	12	5,462	127,826	228

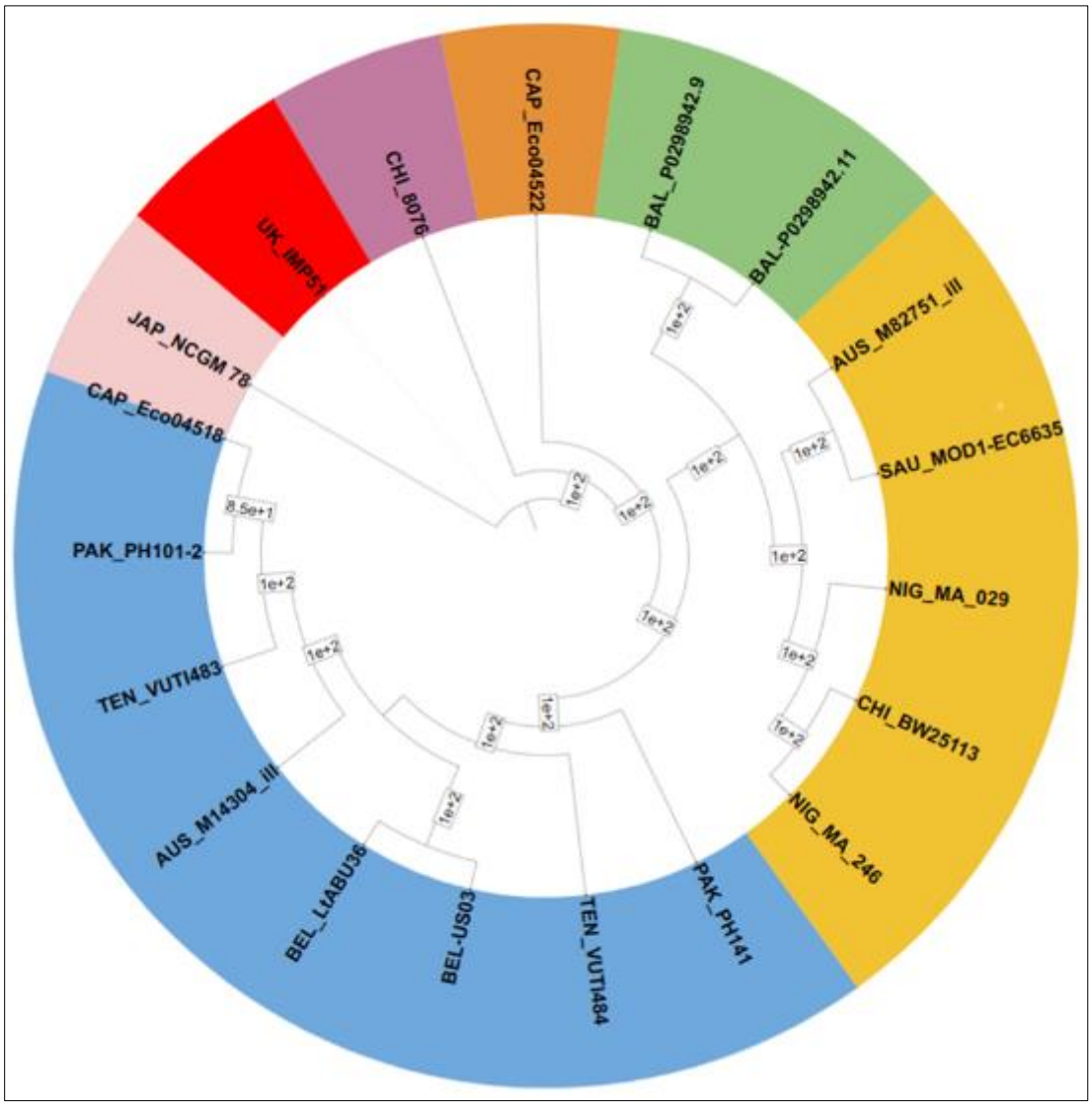

Figure 9 Phylogenetic Tree of the *Escherichia coli* strains.

5. Conclusion

In conclusion, bioinformatics has demonstrated robustness in characterizing the *E coli* strains based on the quinolone genes and genetic relatedness. We recommend adequate investigations into the efflux pump and genes, to understand their structure and how they receive or dislodge substrates in and out of the cells, and how the genes are expressed. This will lead to the production of adequate anti- efflux pump substrates that will specifically combine with the bacterial efflux pumps without any harm to the host and antagonize the drug resistance action of the expressed genes, or negatively control the operon so that the resistance product is not expressed at all. With this, the quinolone drugs that have been abused by resistance genes can be modified of redesigned. The knowledge of the efflux pumps and gene structure will also guide the production of new drugs. We also recommend unfolding the resistance genes and mechanisms of all the drugs so far resisted and apply the same investigations for new drug re-designing.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author contributions

Udochi Anayo Ugo designed the study, collected data, and did analysis with Ebele Onuigbo, Lilian Ngozi Chukwuma and Chukwunonso Mabel Eduzor. Mohammed provided guidance and graphics while Miriam Goodness, Gonesh, Iniekong and Nosa Aima did the editing. All the Authors proofread and approved the manuscript.

Data Availability

All raw data used for this study can be found online in repositories using the website: (https:// www. ncbi. nlm. nih. gov/)

Declarations

This study was not involved with experiment with any human or animals and so ethical approval was not sought.

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