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Determination of Quinolone Resistance Genes among Clinical Isolates of Pathogenic Enterobacteriaceae: A Case Study

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ABSTRACT: With growing morbidity and mortality, particularly in developing nations like Nigeria, increasing multidrug resistance among *Enterobacteriaceae* has complicated treatment choices. The commonly recommended remedy for infections caused by these agents is Fluoroquinolones (FQs). This study investigates the characteristics of FQs resistance genes within the clinical isolates of Enterobacteriaceae. It also determined the antibiotic resistance pattern of the isolates in the Yola metropolis of Adamawa State, Nigeria. A total of 472 Enterobacteriaceae isolates recovered from the targeted patients tested for antimicrobial susceptibility. Disc diffusion technique was employed to determine antibiotic susceptibility and resistance. Deoxyribonucleic acid (DNA), gyrB, and ParC were confirmed using multiplex PCR. DNA sequencing technique was carried out to identify alterations in the gyrB and ParC. About (27%) of isolates were resistant to quinolones (Perfloxacin), 32 % to Aminoglycosides (Streptomycin) and 20% to sulfonamides (Septrin). Multiple antibiotic-resistant phenotypes with multiple antibiotic-resistant indexes (MAR) ranging between 0.4 to 0.8 indicates similarity to 13 different species of Enterobacteriaceae. Three were shown to be *Salmonella species*, 5 *Citrobacter*, 2 *Enterobacter*, 1 *Aeromonas taiwanensis,* 1 *Kluyvera genomosp* and 1 *Escherichia coli* harbouring one or more gyrB, each indicating various alterations in the genes. While just one gene can explain quinolone resistance, both the gyrB and parC genes are involved in the resistance to fluoroquinolones. High resistance to FQs is conferred by QRDR gene accumulation and mutations. These findings highlight the pressing need for legislation prohibiting the over-the-counter sale of antibiotics, as is the case currently.

Keywords: Antimicrobial susceptibility; enterobacteriaceae; gram-negative bacterial infections; multidrug-resistance; quinolone resistance genes, Nigeria

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INTRODUCTION

Multidrug-resistant gram-negative bacteria (MDR-GNB), especially Carbapenem-resistant Enterobacteriaceae (CRE), have become a concern to medical facilities worldwide. The Centers for Disease Control and Prevention (CDC) acknowledged the CRE's ongoing impact on disease. In 1996, CRE infections became prevalent in certain regions of the United States, Europe, and Africa, particularly Nigeria (Codjoe and Donkor, 2017). Multidrug-resistant (MDR) bacterial infections are more common than ever before, and they are linked to high morbidity and death, protracted hospital stays, and higher healthcare expenses (Khawcharoenporn et al., 2013).

It is becoming more challenging to treat nosocomial infections due to the formation and spread of resistance in Enterobacteriaceae. It poses a serious risk of creating species immune to all currently available medications. According to the CDC, AMR is expected to result in an increase in direct healthcare expenses of up to $20 billion and societal expenditures of up to $35 billion annually in the United States alone (Tang et al., 2021). In intensive care units in the US, 31% of Enterobacter species infections and 20% of Klebsiella pneumonia infections are now caused by strains resistant to third-generation cephalosporins (Padmini et al., 2017). A rod-shaped, Gram-negative, facultatively anaerobic, non-spore-forming bacillus, Enterobacteriaceae (Odeyemi et al 2021). Numerous pathogens exist in the family, including Klebsiella, Enterobacter, Citrobacter, Salmonella, Escherichia coli, Shigela, Proteus, Serratia, and other species (Patel et al., 2014). These bacterial species are essential for maintaining the hosts' regular digestion and immunological systems in humans and companion animals (Olowo-Okere et al., 2020).

Although most of the bacteria in this group are members of the Enterobacteriaceae family, which were once considered harmless, some strains may be capable of causing pathological conditions and diseases. They include diarrhoea, gastroenteritis, urinary tract infections, inflammatory bowel diseases, pyelonephritis, septicemia, pneumonia, peritonitis, and meningitis in humans, companion animals, and infections linked to prosthetic devices like catheters (Rafalskiy et al., 2020).

Carbapenem-resistant Enterobacteriaceae (CRE) is often responsible for severe, life-threatening infections and represents a critical threat to the available antibiotic agents and global health. Antibacterial resistance, particularly in Gram-negative bacteria, challenges the ability to treat common infections and causes the greatest threats to global public health systems. Resistance is worrisome in resource-limited countries such as sub-Saharan Africa, where infections are common and last-resort antimicrobial agents are scarce and/or unaffordable (Ngbede et al., 2021). The definitive diagnosis of MDR Enterobacteriaceae requires the isolation of the organisms from urine and stools. Blood cultures, urine cultures, and marrow cultures have been employed in diagnosing Enterobacteriaceae. Urine and stool samples are inoculated onto the agar plates (Khilnani et al., 2019).

Like any other microorganism, infections with Enterobacteriaceae are treated using β-lactam and fluoroquinolone antibiotics.

These antibiotics include ofloxacin, colistin sulfate, cotrimoxazole, ampicillin, streptomycin, and amoxicillin (De Angelis et al., 2020). These antibiotics bind tightly to the bacterial enzymes (DNA gyrase or topoisomerase IV) and inhibit bacterial growth.

Despite extensive intervention with antibiotics, infections of Enterobacteriaceae continue to occur with the emergence of multi-drug-resistant strains.

The development of MDR Enterobacteriaceae is a subject of global concern. The resistance is due to the production of extended-spectrum β-lactamase (ESBL). Production of ESBL is a vital mechanism causing resistance towards third-generation drugs; cephalosporins, such as ceftazidime, ceftriaxone, and cefotaxime, are used as empirical therapy of antibiotics (Amelia et al., 2016). One of the elements that hastened this occurrence is the increased use of antimicrobial drugs and their improper use. Additionally, the acquisition and dissemination of multidrug-resistant bacteria are significantly influenced by ongoing international travel, tourism, and business travel [Allocati et al., 2013]. However, reports of carbapenem-resistant illnesses, particularly those brought on by Enterobacteriaceae and other gram-negative bacteria, have surfaced, giving rise to legitimate worries that the pipeline for antibiotics may have run dry (Adetoye and Hilda, 2020). In particular, manufacturing extended-spectrum β-lactamases and carbapenemases can result in multidrug resistance patterns that seriously limit treatment options. Additionally, -lactam resistance could lead to increases in drug toxicity, mortality, and medical costs linked to infections caused by Enterobacteriaceae (De Angelis et al., 2020). In most parts of the world, plasmid-mediated quinolone resistance (PMQR) is caused by mutations in chromosomal genes that encode the quinolone targets. Such as DNA gyrase and topoisomerase IV, have been documented more often during the past few decades. Generally speaking, three plasmid-mediated quinolone resistance pathways have been described. They are qnr proteins that protect the quinolone targets, aac (60)-Ib-cr enzyme that acetylates aminoglycosides, ciprofloxacin, and norfloxacin, Efflux pumps associated with QepA, which excretes hydrophobic fluoroquinolones 9Aworh et al., 2019). In the current investigation, we looked into the frequency of the QR determinants, such as gyrB and ParC, in fluoroquinolone-resistant Enterobacteriaceae isolates isolated from clinical samples. Nevertheless, the presence of these factors in clinical isolates from our region hasn't been examined by any prior survey.

MATERIALS AND METHODS

Materials

Some equipment includes a PCR machine, DNA sequencer, Orbital shaker, Heating block, Thermocycler, and Electrophoresis set and power pack, laptops, and Vortex Machine. In contrast, the bacteria isolate from blood and stool samples were collected from patients attending the mapped health facilities. The blood samples were inoculated in a tube without anticoagulant. After clotting, the serum was separated and stored in aliquots of 200 ml at +4°C. Testing was done immediately or stored for a week without affecting the antibody titre.

The serum was frozen at - 20°C for longer-term storage. Stool samples were collected from patients with acute typhoid fever symptoms, and specimens were collected in a sterile wide-mouthed plastic container. They were processed within two hours after collection (Chessbrough, 2010). Figure 1 present the sequential stages involved in succession.

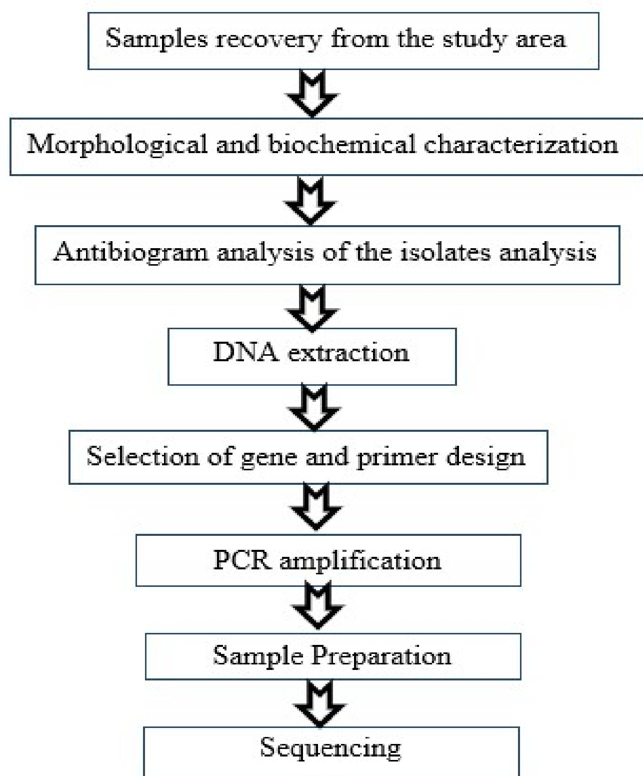

Figure 1: Flow Chart of Research outlining the sequential stages involved in succession.

Methods

A total of 472 Blood and stool samples were collected from primary and secondary health facilities within Yola Metropolis of Adamawa state between May and December 2018 and 2019, respectively. Each serum was accompanied by a predesigned questionnaire with the following information – Age, sex and date of collection.
The samples were processed according to the guidelines provided by Chesbrough (2006) for the laboratory diagnosis of enteric pathogens. Samples collected were primarily inoculated onto S-S agar and, finally, onto Nutrient agar. The colonies were confirmed using biochemical tests for peptone water, Thiosulphate Citrate Bile Salt Sucrose (TCBS) using biochemical parameters (TSI, KIA, SIM, Simon citrate, indole test, Triple sugar iron agar test, Citrate utilization test, Urea test and motility test) by (Chessbrough, 2010).

Antimicrobial susceptibility testing

The antimicromial susceptibility testing was done by disc diffusion method as described by (CLSI (2012). In a nutshell, the antibiotic discs (Oxoid) contained the following medications: Septrin (30 mg), Sparfloxacin (10 mg), Ciprofloxacin (30 mg), Amoxicillin (30 mg), Augmentin (10 mg), Gentamicin (30 mg), Pefloxacin (30 mg), Tarivid (10 mg), and Streptomycin (30 mg). On a plate of susceptibility testing agar that had been uniformly inoculated with the test organism, a disc of blotting paper impregnated with a known volume and suitable concentration of an antibiotic was placed.

The test organism's growth is inhibited at a distance from the disc that is related, among other things, to the organism's antimicrobial susceptibility as it diffuses into the medium. While resistant strains have smaller inhibition zones or grow closer to the disc's edge, susceptible strains are halted at a distance from the disc. Clinical and Laboratory Standards Institute Guidelines are used to interpret the data (Chandrasekaran et al., 2018). All tests are conducted using the reference strain Escherichia coli (ATCC 25922).

Multiple Antibiotic resistance determination

The Multiple antibiotic resistance (MAR) index of the isolates was determined using (Aworh et al., 2019) as:

$$MAR = a/b \tag{1}$$

where:

a = representing antibiotics number to which the test isolates depicted resistance and
b = representing antibiotics total to which the test isolate has been evaluated for susceptibility
The susceptibility index (SI) was determined for each isolate by using the formula:

$$SI = x/y, \tag{2}$$

where x represents the number of antibiotics to which the test was susceptible, and y represents the number of antibiotics to which the test isolate demonstrated resistance (19).

Statistical analysis

SPSS software (version 16) was used to conduct statistical analysis using a chi-square test. The statistical

Table 1: PCR primers used in the current study.

				PCR conditions				
Target	**Printer**	**Nucleotide Sequence (5'-3')**	**Size (bp)**	**Pre-denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**
gyrB	gyrB-F gyrB-R	GCGCTGTCCGAACTGTACCT TGATCAGCGTCGCCACTTCC	181	94°C, 30 sec.°	94°C, 30 sec, 35x	48°C, 30 sec, 35x	68°C, 30 sec., 35x	68°C, 5 min
parC	parC-F parC-R	CTATGCGATGTCAGAGCTGG TAACAGCAGCTCGGCGTATT	270	94°C, 30 secs°	94°C, 30 sec, 35x	48°C, 30 sec, 35x	68°C, 30 sec., 35x	68°C, 5 min

Table 2: Samples collected from the study area

Source	Male	Female	Total	Chi-Square	P-Value
Stool	62	70	132	32.572[a]	.000
Serum	150	190	34		
Total	212	260	472		

Table 3: Distribution of Enterobacteriaceae based on age and sex-wise in the study population.

Age group	Male	Female	Total	Chi-Square	P-Value
□□□ yrs	29	30	59	1.338[b]	0.512
11 – 20	55	72	127	2.091[c]	0.554
21 – 30	80	79	159	.757[d]	0.685
31 – 40	33	51	84	.635[e]	0.728
41 – 50	7	18	25	1.200[f]	0.549
51 – 60	5	8	13	1.667[g]	0.197
61 – 70	2	1	3	3.000[i]	0.083
71 – 80	1	1	2		
Total	**212**	**260**	**472**		

significance level was set at a P value of 60.05.

DNA extraction, PCR amplification and selection of primers

Following the manufacturer's instructions, DNA was extracted using the Zymo Research Kit (Zymo research, Biolab England). By utilizing particular primers (Table 1) from Zymo research, Biolab England, and adhering to the manufacturer's instructions, a multiplex PCR reaction was used to determine the amplification of the QRDR genes (gyrB and parC). Briefly, PCR was performed in a 25 µL reaction mixture containing two µL of purified DNA (approximately 500 ng/L), 12.5 µL of one Taq Quick-Load 2× master Mix with standard Buffer (New England Biolabs inc), 0.5 uL of each primer and 9.5 µL of nuclease-free water. Single PCR reactions were used to amplify the gyrB and parC genes using specific primers (Table 1). PCR products were resolved on 1% agarose gel with ethidium bromide dye, and the gel was visualized under a UV transilluminator (UVP Cambridge, UK).

Sequencing of PCR products

An automated DNA sequencer and data-gathering software from Applied Biosystems, USA, were used for the sequencing process. Using NCBI's BLAST software, the translated nucleotide sequences of the QRDR in the genes gyrB and parC were matched to corresponding reference protein sequences http://www.ncbi.nlm.nih.gov/blast.

DNA sequencing and analysis

The procedure outlined by (Hamzah et al., 2021) was utilized to analyze the outcomes. In a nutshell, the gyrB and parC genes' amplified PCR products were sequenced using the Sanger DNA sequencing technique (incaba biotechTM Africa genomics company). Utilizing the NCBI BLAST program (NCBI, USA), Clustal W Multiple Sequence Alignment Program (Bioedit Sequence Alignment Editor, version 7.2.5), and the Codon Code Sequence Assembly and Alignment Software, mutations in QRDRs were discovered by comparing the sequencing data with those of the Enterobacteriaceae species (GenBank accession no. U00096.3) (Codon Code, Corp, Centerville, MA, USA).

RESULTS

Among the 472 samples collected, 28% and 72% are from stool and serum samples, respectively. Furthermore, 44.9% of the isolates are male subjects, while 55% are females (Table 2). This makes the ratio of male to female 1:1.33. The ages ranged between □10 to 80 years. Further analysis revealed that the difference in the distribution of the enteric pathogens statistically in the study area concerning gender and age was not significant ($p>0.05$) (Table 3). Also, the results indicated that 181 samples

Table 4: Sensitivity of Enterobacteriaceae to different drugs.

Sensitivity	SP	CH	PEF	CN	AU	CPX	SXT	S	AM	OFX
Susceptible	170	162	154	163	167	172	161	149	171	163
Resistance	11	19	27	18	14	9	20	32	10	18
% Susceptible	93.9	89.5	85.1	90.1	92.3	95.0	88.9	82.3	94.5	90.1

SP = Sparfloxacin (10ug), CH = Chloramphenicol (30ug), PEF = Pefloxacin (30 ug), CN = Gentamycin (30 ug), AU =Augmentin (10 ug), CPX = Ciprofloxacin (30ug), SXT = Septrin (30ug), S = Streptomycin (30 ug), AM = Amoxicillin (10ug), OFX = Tarivid (10ug).

were confirmed positive biochemically for gram-negative bacteria.

Antimicrobial susceptibility and resistance

In order to ascertain an antibiotic's susceptibility, the disk diffusion approach produced the following results: 93.9% of the isolates were susceptible to Sparfloxacin, 89.5% to chloramphenicol, 85.08% to Pefloxacin, 90.06% to Gentamycin, 92.27% to Augmentin, 95.03% to ciprofloxacin, 88.95% to Septrin, 82.32% to streptomycin, 94.48% to Amoxicillin (Table 4). There is an 82–95% range in the susceptibility percentage. Streptomycin showed the lowest susceptibility, and ciprofloxacin showed the highest. Additionally, the research area's Enterobacteriaceae resistance profile revealed that Streptomycin, Pefloxacin, and Septrin had the highest rates of resistance (32, 27, and 20 per cent, respectively). Ciprofloxacin and amoxicillin showed low resistance, with 9 and 10%, respectively.

Results for multiple antibiotic resistance determination

The isolates' Multiple Antibiotic Resistance (MAR) index was calculated using (19). The results of this investigation showed that neither the isolates from the primary healthcare facilities in the study area nor their susceptibility to all tested antibiotics showed 100% resistance. The MAR indices for the phenotypic resistance were 0.4 and 0.3, respectively, and ranged from 6 to 7 antibiotics. Two isolates with a MAR of 0.4 showed resistance to six different antibiotics. With an R-Phenotype of Ofx Sxt Cn Cpx Am Au Ch, one isolate showed resistance to seven antibiotics, including Ofloxacin, Septrin, Gentamycin, Ciprofloxacin, Amoxicillin, Augmentin, and chloramphenicol. Ofloxacin, Septrin, Gentamycin, Ciprofloxacin, Amoxicillin, Augmentin, and chloramphenicol are the antibiotics that demonstrated the most resistance (Table 5).

DNA Extraction for Enterobacteriaceae

Random isolates to confirm the presence of extracted DNA showed gel electrophoresis results for the presence of genomic DNA (gDNA) from the isolates. The results confirmed the presence of gDNA in all 27 MDR strains (Figure 2).

PCR Screening for the GyrB and ParC genes

The outcomes demonstrated that all 27 MDR strains contained gDNA (Figure 2). 87.5% of the isolates were positive for the quinolone resistance determining region (QRDR) of gyrB by polymerase chain reaction (Figures 3 and 4). Various types of organisms carrying the gyrB gene were discovered when the sequences of the QRDR were matched using an essential local alignment search tool (BLAST) study with the nucleotide sequence database of gyrB from the NCBI website. The results further revealed that 14 organisms suggest the presence of gyrB, while two returns have no similarities. Of those, 3/16 (18.8%) are *Salmonella species*, 4/16 (25%) are *Citrobacter species*, 2/16 (12.5%) are *Enterobacter species*, and 1/16 (6.25%) and 1/16 (7.1%) are the other species. Figures 5-7 presents PCR patterns and alterations for ParC and gyrB.

Sequencing analysis for GyrB and ParC genes

ParC was found to be present in every one of the isolates after polymerase chain reaction analysis. The presence of 7 distinct groups of Enterobacteriaceae was discovered when the sequences of the QRDR were matched using an essential local alignment search tool (BLAST) analysis with the nucleotide sequence database of gyrB from the NCBI website. Further analysis of the results revealed that 4/24 (16.7%) were Salmonella Species, 12/24 (50%) were Citrobacter Species, 324 (12.5%) were Enterobacter Species, 1/24 (4.2%) were Aeromonas taiwanensis, 1/24 (4.2%) were Kluyvera genosperms, 1/24 (4.2%) were Escherichia coli and 2/24 (8.3%) were Klebsiella (Table 6).

QRDR mutations in gyrA and parC genes

Using PCR and subsequent QRDR sequencing of their gyrB and parC genes, isolates resistant to Q and FQs were examined. In *Salmonella Specie* (1/4, 25%) of Q- resistant isolates, mutations at codons 4 of the gyrB gene (Tyr4Thr) and 31 of the parC gene (Cys31Gly) were found; all

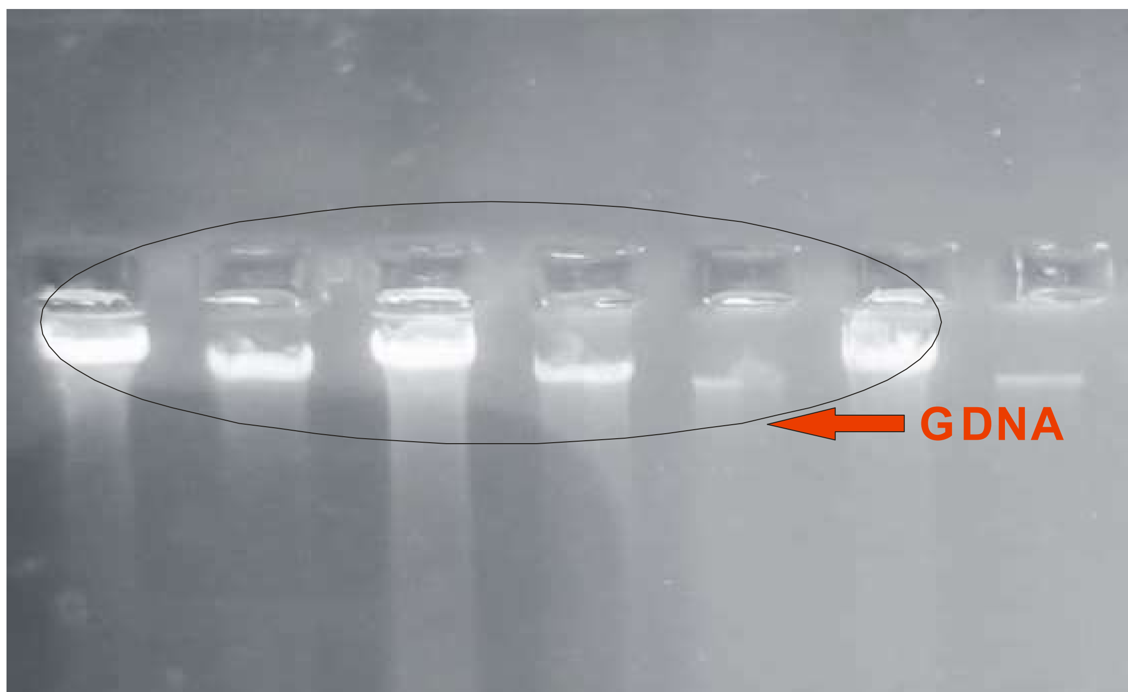

Figure 2: Agarose gel electrophoresis pattern showing genomic DNA; the plate indicates the presence of DNA of the isolates.

Figure 3: Agarose gel electrophoresis pattern showing single PCR products of gene mutation of gyrB from the Enterobacteriaceae (Lane 1 – 7).

belonged to the high resistance phenotype. The parC gene showed deletion at codon 11 and double mutations at codons 16 and 17 (AD16,17WK), as well as eight 8-point mutations at the gyrB genes' positions 7, 46, 55, 135, 145, 148, 139, and 146 (Arg7Gly, Tyr46Thr, Lys55Thr, Arg135Gly, Gly145Ala, Thr148Gly, Cys139Thr, and Cys146Gly) Q and FQs resistant isolates, were studied by PCR and subsequent sequencing of gyrB and parC genes. Mutation at codon 4 of the gyrB gene (Tyr4Thr) and codon 31 of the parC gene (Cys31Gly) was detected in

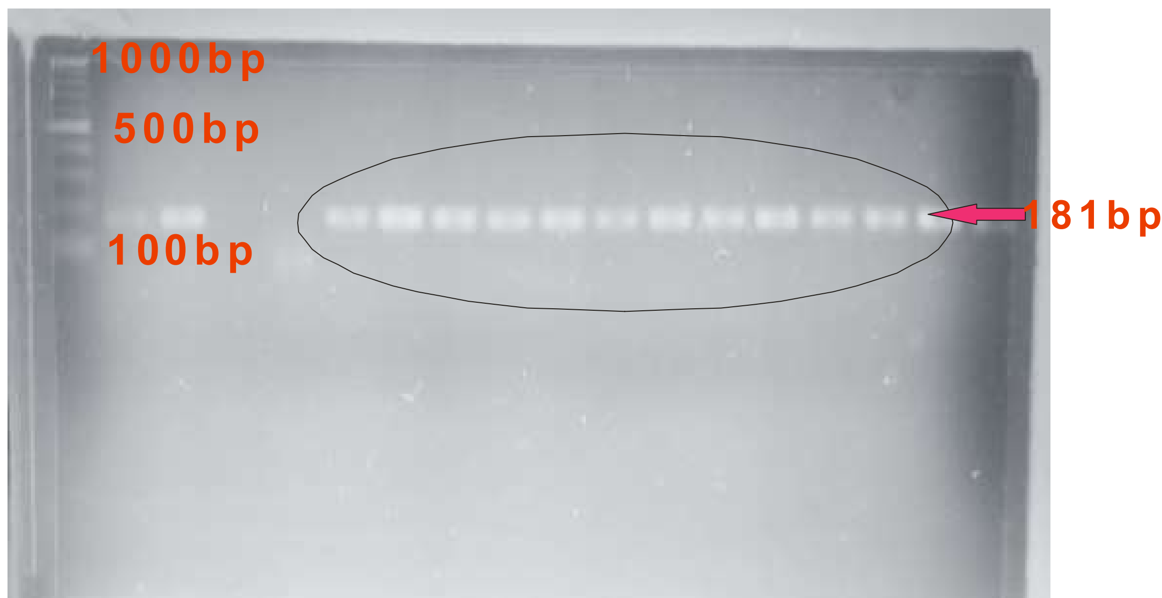

Figure 4: Agarose gel electrophoresis pattern showing single PCR products of gene mutation of gyrB from the Enterobacteriaceae.

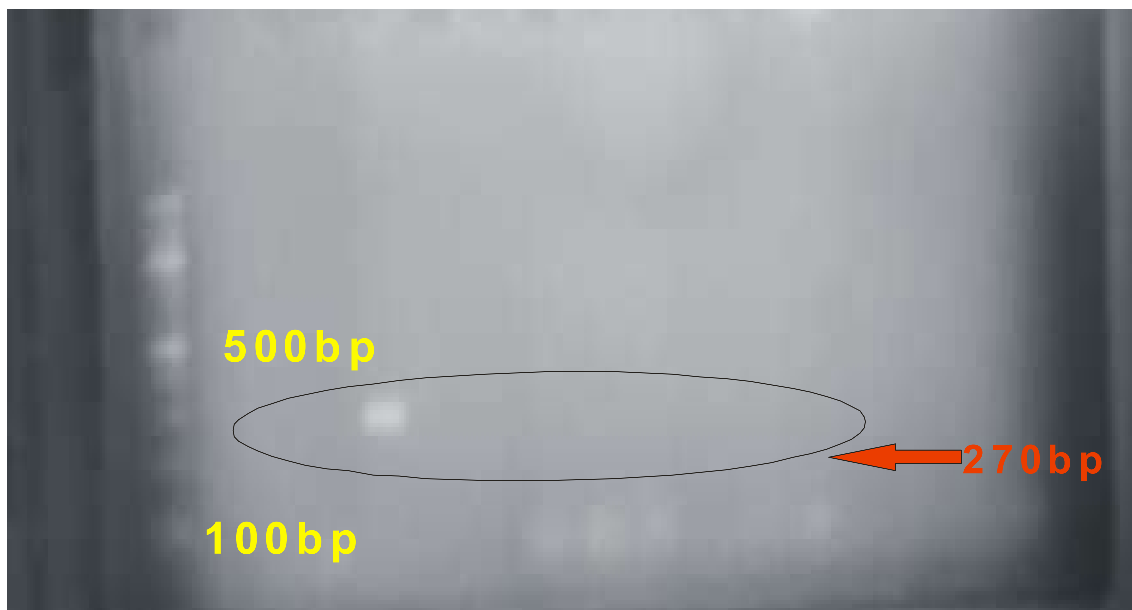

Figure 5: Agarose gel electrophoresis pattern showing single PCR products of gene mutation of ParC from the Enterobacteriaceae (Lane 1-10).

Salmonella Specie (1/4, 25%) of Q resistant isolates; All are belonging to the high resistance phenotype. The deletion was noticed at codon 11, and double mutation was detected at 16 and 17 codons (AD16,17WK) of the parC gene and 8-point mutations at position 7, 46, 55, 135, 145, 148, 139 and 146 codons of gyrB genes (Arg7Gly, Tyr46Thr, Lys55Thr, Arg135Gly, Gly145Ala, Thr148Gly, Cys139Thr and Cys146Gly) in *Citrobacter Species* (7/12, 58%). One deletion was noticed on codon ten and 11-points mutations at codon 11, 13, 19, 28, 34, 40, 43, 67, 82, 91 and 94 (Tyr10 deleted, Thr11Ala, Arg13Gly, Cys19Gly, Tyr28Cys, Tyr34Cys, Lys40Gly, Tyr43Thr, Tyr67Cys, Ser82Gly, Tyr91 and Arg94Gly) in 1/1, 100% in gyrB of *E. Coli*. Two-point mutations were noticed at codons 89, 92 and 103 (Ala89Gly, Cys92Thr, Gly103Ala) in 2/2, 100% of ParC gene of *Klebsiella Species*

Table 5: Resistance Phenotype and Multiple antibiotic-resistant Enterobacteriaceae Isolates from the Study Area.

Health Facilities	Isolate No	R-Phenotype	MAR
FMC	15	Ofx Am Sxt Cpx Ch	0.5
	148	Am Pef Cn Ch Sxt	0.5
	18	Ofx Pef Sxt Cpx Am Au Ch	0.3
	249	Sxt Pef Cn Cpx Am Sp Ch	0.3
	224	Ofx Sxt Cn Cpx Am Au Sp Ch	0.2
	32	Ofx Pef Sxt Cpx Au Sp Ch	0.3
	331	Am Pef Cn Cpx Sp Ch Sxt	0.3
	29	Ofx Am Sp S Sxt Ch	0.4
	33	Pef Cn Cpx Ch Am Au Sp	0.3
	425	Ofx Am Cn Sxt Sp S Ch	0.2
	30	Ch Pef Cpx Sxt S Am Au	0.2
	147	Ofx Pef Cn Cpx Sp Sxt Ch	0.3
	134	Ofx Pef Cn Sxt Am Ch S	0.3
	12A	Ofx Pef Am S Sxt Ch Sp Cpx	0.2
	316	Sxt S Ch Cpx Am Au Sp	0.3
Specialist Hospital	119	Ofx Pef Cn Cpx Ch Am Au Sxt	0.2
	5A	Ofx Pef Ch Sp Cpx Am Au	0.3
	3A	Ofx Pef Cn Sp Cpx Am S Sxt	0.2
	4A	Ofx Am Cn Cpx S Sxt Au	0.2
	8A	Am Pef Cn S Sxt Au Ch Sp	0.3
	44	Ofx Am S Sxt Ch Sp Cpx	0.3
	9A	Ofx Pef Ch Cpx Am Sxt	0.4
	237	Pef Cn Ch Cpx Am S Sxt	0.3
	438	Cn Ch Cpx S Am Au Sxt	0.3
PHCC (Shagari)	17	Ch Pef Am Cpx S Sxt	0.4
PHCC (W/Hausa)	41	Ofx Sxt Cn Cpx Am Au Ch	0.3
Ardo Yahaya PHC	26	Pef Sxt Cpx Sp Am Ch	0.4

Table 6: Prevalence of different species of Enterobacteriaceae isolated from the study area.

Organisms	N	%
Salmonella Spp	4	16.7
Citrobacter Species	12	50
Enterobacter Species	3	12.5
Aeromonas taiwanensis	1	4.2
Kluyvera genomosp	1	4.2
Escherichia coli strain	1	4.2
Klebsiella Pneummoniae	2	8.3

Table 7: Distribution of gyrB and parC among FQs resistance phenotypes of Clinical Enterobacteriaceae isolated from the study.

Species	Type of resistance	MAR	Number	gyrB alterations	ParC alterations
Salmonella Species	High resistance	0.2	4	Tyr4Thr	Cys31Gly
Citrobacter Species	High resistance	0.3	12	Ala11 was deleted, AG16,17WK	Arg7Gly, Tyr46Thr, Lys55Thr, Arg135Gly, Gly145Ala, Thr148Gly, Cys139Thr and Cys146Gly
Enterobacter Species	High resistance	0.3	3	NMD	Cyst32Ser, Gly64Arg, Gly86Ser, Gly124Arg, Ala138Arg, Gly141Thr
Aeromonas taiwanensis	High resistance	0.4	1	Thr76Cys, CysGly93,94GlyCys	NMD
Kluyvera genomosp	High resistance	0.4	1	Ala11 was deleted Tyr28Thr, Tyr73Thr	NMD
Escherichia coli strain	High resistance	0.3	1	Tyr10 deleted, Thr11Ala, Arg13Gly, Cys19Gly, Tyr28Cys,Tyr34Cys, Lys40Gly, Tyr43Thr, Tyr67Cys, Ser82Gly, Tyr91 and Arg94Gly	NMD
Klebsiella Pneumoniae		0.3	2	NMD	Ala89Gly, Cys92Thr, Gly103Ala

NMD, no mutation detected.

(Table 7, Figure 6 and 7).

DISCUSSION

It has become a major clinical and public health issue that Enterobacteriaceae are becoming increasingly resistant to antibiotics (Ranjbar and Farahani, 2019). Due to the efficiency and widespread usage of quinolones and fluoroquinolones, there has been an average global increase in resistance, which is mediated by gene mutations, changes in efflux or cell membranes, and plasmid-conferred resistance (Islam et al., 2018). In this work, the features of quinolone resistance genes were

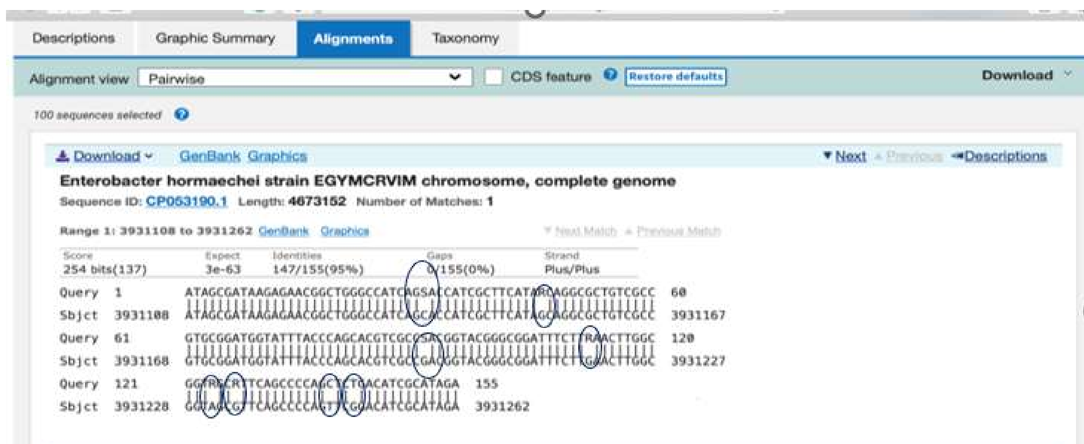

Figure 6: Alteration in parC (codon 32, 64, 86, 124, 138, 141). Nucleotide sequence of par C regions of the Enterobacter Specie FQs-susceptible (Cyst32Ser, Gly64Arg, Gly86Ser, ly124Arg Ala138Arg, Gly141Thr).

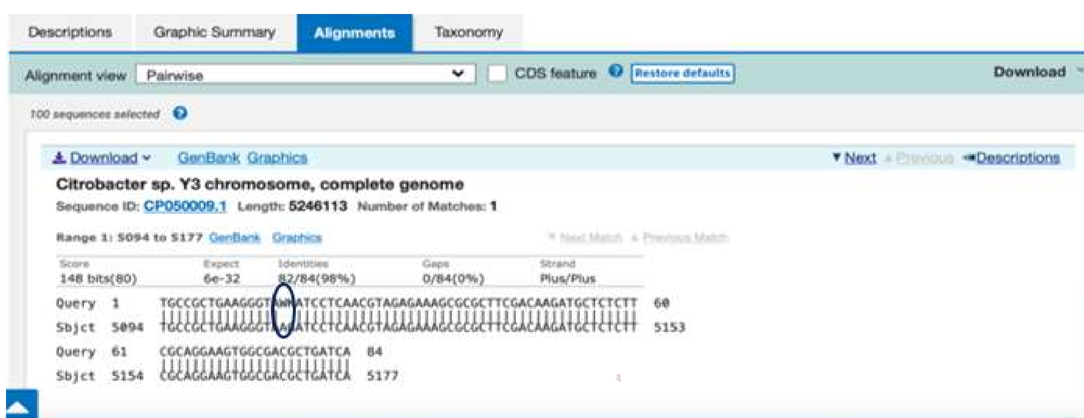

Figure 7: Alteration in gyr B (codon 16&17). Nucleotide sequence of a gyr B region of the Citrobacter Sp. FQs-susceptible, (AG 17,17WK).

examined in clinical isolates of Enterobacteriaceae from patients using medical facilities in the Yola city, Adamawa state. There were four hundred and seventy-two (472) positive samples from the selected health facilities in the study area were collected. Out of the total, 44.9% of the isolates are males subjects while 55% are females. It makes the ratio of male to female 1:1.33. Their ages range between <10 to 80 years. Further analysis revealed that the difference in the distribution of the enteric pathogens statistically in the study area concerning gender and age was not significant ($p>0.05$). The age group with the highest prevalence of infections of Enterobacteriaceae is 21 - 30 years, with 33.6%. This slightly varied with the study conducted by (Islam et al., 2018) and (Guan et al., 2013), who stated that 12 years old are more sensitive and affected by enterobacterial infections and also, studies conducted by (Rojas et al., 2017) and (Yadav et al., 2015) in Pakistan showed that 50 years and above are more prone enterobacterial infections. However, this result agrees with the work carried out by (Van den Bunt et al., 2016), who state that Patients in age groups 21 – 30 years and 31 – 40 years had the highest percentage of occurrence of infections with bacteria. Furthermore, the results of (38) affirmed that children and young adults are more susceptible to enterobacterial infections than any other age group. This is due to risk factors such as feeding and other social activities. Furthermore, the presence of Enterobacteriaceae was confirmed biochemically using motility, indole, methyl-red, vogues Proskauer, lysine, Kliglers Ion test (KIA), lactose, citrate and urease test. A total of 181 samples were confirmed positive biochemically for gram-negative bacteria. Four groups of bacteria have exhibited the same characteristics biochemically. These agreed with the work carried out by (Onwubiko and Chinyeaka, 2015), who stated that 397(54.0%) out of 735 isolates yielded Gram-negative bacteria. The antibiogram structure of the organisms reveals that 93.9% of the isolates were susceptible to Sparfloxacin, 89.5% to chloramphenicol, 85.08% to Pefloxacin, 90.06% to Gentamycin, 92.27% to Augmentin, 95.03% to

ciprofloxacin, 88.95% to Septrin, 82.32% to streptomycin, 94.48% to amoxicillin, and 90.06% to Tarivid respectively. The percentage susceptibility ranges between 82 to 95 per cent. The highest susceptibility was observed in ciprofloxacin and the lowest in streptomycin, as shown in Table 7. The result of this study disagrees with the work of (Abraham et al., 2019) and (Subramani and Vignesh, 2012), who stated that the highest susceptibility was observed in amoxicillin with 86.6% and 99% of Ampicillin while Krumperman, (1983) and Azargun, (2019) agrees with our results which stated that Enterobacteriaceae particularly E. coli is susceptible to Norfloxacin, Ciprofloxacin, Levofloxacin Nalidixic Acid. Similarly, the resistance profile for Enterobacteriaceae to antibiotics from the study area further revealed the percentages of different drugs in Table 7. The results showed the highest resistance observed in streptomycin with 18%, Perfloxacin with 15%, and Septrin with 11% resistance. Furthermore, low resistance is observed in ciprofloxacin, having 5%, and Amoxicillin and Sparfloxacin, with 6% resistance each. Findings from this study on the prevalence of MDR. *Enterobacteriaceae* is much lower than the 93.6% reported in Egypt by (Khalifa et al., 2019) and in Southwest, Nigeria by (Adenipekun et al., 2016) and (Akinbami et al., 2018). The author further stated that MAR is reported to be a good tool for risk assessment as its gives idea of the number of bacteria showing antibiotic resistance in the risk. The values of the MAR index of 0.2 differentiates the low and high risk. If the value is between 0.2 and 0.5, it becomes a very risky phase with equal chances that MAR may fall in the high-risk and low-risk phases (Suzuki et al., 2019). The results in this study disagreed with the work conducted in Egypt by (Temkin et al., 2014), who showed that 93.6% (118/126) of the clinical isolates had a multidrug-resistant phenotype. It is reported by Zaidah et al., (2017) that bacteria having a MAR index of greater than 0.200 originates from an environment where several antibiotics are used. This study's result agrees with the work of Aurilio et al., (2022) who stated that the majority (96.84 %) of Enterobacteriaceae isolates were found to be MDR. Also, (36) observed 64.04 % MDR Enterobacteriaceae and 73.68 % MDR *E. coli* isolates. The very high MAR index in this study is a possible indication that a large proportion of antibiotics has been exposed to several antibiotics. The buoyant access to antibiotics without prescription in the state and the shortage of health professionals necessitates abuse and misuse of antibiotics which could be responsible for the high MAR index. Furthermore, most people in Adamawa State do not attend the hospital for proper diagnosis before embarking on self-medication. They only visit the hospital when such an attempt failed to provide the needed relief. Others resort to the use of herbal medicines while a few combines both. Also, Mirzaii et al. (2018) stated that resistance to β-lactams such as Penicillin, Cephalosporins etc., in Enterobacteriaceae is mainly due to the production of β-lactamases, which may be encoded either chromosomally or on plasmids. The widespread use of antibiotics could be associated with the selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic isolates. Finally, Gram-negative bacteria have been isolated worldwide, including Nigeria and Yola Metropolis of Adamawa state will not be an exception.

To our best knowledge, this is the first study on the prevalence of aac(60)-Ib-cr plasmid-mediated quinolone resistance in the Yola metropolis of Adamawa State, Nigeria and present findings suggest that gyrB and parC genes were prevalent. The result showed that 14 organisms indicate the presence of the gyrB gene. Out of which, 3/16(18.8%) are *Salmonella Species*, 4/16(25%) are *Citrobacter Species*, 2/16(12.5) are *Enterobacter Species*, 1/16(6.25%) and finally 1/16(7.1%). The result also agrees with another research conducted in Azerbaijan and Iran by AbdulAziz et al., (2016) who stated that out of 205 isolates of *E. coli* and *K. Pneumoniae,* one (1) was identified with FQ genes of gyrB and ParE. The work of (Rafalskiy et al., 2020) stated that Forty-five out of fifty clones carried mutations in *gyrA*. The outstanding may have mutations in other CFX target sites in *gyrB, parC, or parE* genes. The result further showed that 11 organisms indicate the presence of the ParC gene. Out of which, 2/11(18%) are Salmonella Species, 6/11(54.5%) are Citrobacter Species, 1/11(9.1%) are Enterobacter Species, 1/11(9.1%) are Klebsiella Pneumoniae. This is in agreement with the work of (Weigel et al., 1998) stated that GyrA and parC genes were detected in both susceptible and resistant isolates of *E. coli* and *K. pneumoniae*.

The result of this study also revealed One-point mutation in the gyrB gene of *Salmonella Spp.* at the nucleotide sequence 31, where Cys replaced Gly. Two (2) deletions and one double mutation were noticed in genes of 3 *Citrobacter Spps*. Where AG replaced WK at positions 16 and 17. One point mutation where Thr-76 replaced Gly-76 and one double mutation where Cys93 and Gly94 replaced Gly93 and Cys94 was noticed in *Aeromonas taiwanensis*, one (1) deletion was noticed where Ala11 was deleted and 2-point mutations were noticed in *Kluyvera genomosp* where Tyr28 replaced Thr28, and Tyr73 replaced Thr73, and finally, 10-point mutations were noticed in the genes of *E. Coli* where Thr11 replaced Ala11, Arg13 replaces Gly13, Cys19 replaced Gly19, Tyr28 replaced Cys28, Tyr34 replaced Cys34, Lys40 replaced Gly40, Tyr43 replaced Thr43, Tyr67 replaced Cys67, Ser82 replaced Gly82, Tyr91 replaced Thr91 and Arg94 replaced Gly94. This is in disagreement with work carried out by Mahapatra *et al*., 2020 who stated that QRDR-positive genes showed point mutations with amino acid changes at codons 83 and 87 in the gyrA gene and 80, 84, and 88 positions in the parC gene.

These mutations may be responsible for the multidrug resistance in the identified. Mutations or altering the drug targets (most common) and plasmids coding for protective proteins can protect the bacteria from the lethal effects of quinolones.

Conclusion and recommendation

There is the presence of a quinolone resistance gene with varying degrees of mutations in organisms identified in Adamawa State. Hence the need to regulate the use of fluoroquinolones and its derivatives without a diagnosis at patent stores, Pharmacies, and Hospitals. Also, this study should have been carried out in all the senatorial zones and on the environment to discover and have an in-depth view of the prevalence of infections caused by MDR. Enterobacteriaceae. There is a need to develop a surveillance strategy against the threat to public health caused by antimicrobial resistance, which causes serious untreatable infections.

Declaration of Interest

The authors declare that they have no conflict of interest regarding the authorship and publication of this article.

REFERENCES

AbdulAziz, Z. A., Ehinmidu, J. O., Adeshina, G. O., Pala, Y. Y., Yusuf, S. S., & Bugaje, M. A. (2016). Plasmid mediated resistance in multidrug resistant bacteria isolated from children with suspected septicaemia in Zaria, Nigeria. Bayero Journal of Pure and Applied Sciences, 9(2), 114-120.

Abraham, S., O'Dea, M., Sahibzada, S., Hewson, K., Pavic, A., Veltman, T., ... & Jordan, D. (2019). Escherichia coli and Salmonella spp. isolated from Australian meat chickens remain susceptible to critically important antimicrobial agents. PLoS One, 14(10), e0224281.

Adenipekun, E. O., Jackson, C. R., Ramadan, H., Iwalokun, B. A., Oyedeji, K. S., Frye, J. G. and Oluwadun, A. (2016). Prevalence and multidrug resistance of Escherichia coli from community-acquired infections in Lagos, Nigeria. The Journal of Infection in Developing Countries, 10(09), 920-931.

Adetoye Adesanya, Oluwafolajimi, and Hilda Amauche Igwe (2020). “Carbapenem-Resistant <em>Enterobacteriaceae</Em> (CRE) and Gram-Negative Bacterial Infections in South-West Nigeria: A Retrospective Epidemiological Surveillance Study.” AIMS Public Health 7(4): 804–15.

Akinbami, O. R., Olofinsae, S. and Ayeni, F. A. (2018). Prevalence of extended spectrum beta lactamase and plasmid mediated quinolone resistant genes in strains of Klebsiella pneumonia, Morganella morganii, Leclercia adecarboxylata and Citrobacter freundii isolated from poultry in South Western Nigeria. PeerJ, 6, e5053.

Allocati, N. M., Alexeyev, M. F., Dillio, C. (2013) “Escherichia coli in Europe: An overview,” International Journal of Environmental Research and Public Health, 10(12), pp. 6235–6254. doi: 10.3390/ijerph10126235.

Amelia, Audrey, Agung Nugroho, and Paul N. Harijanto (2016). “Diagnosis and Management of Infections Caused by Enterobacteriaceae Producing Extended-Spectrum b-Lactamase.” Acta medica Indonesiana 48(2): 156–66.

Aurilio, C., Sansone, P., Barbarisi, M., Pota, V., Giaccari, L. G., Coppolino, F., ... & Pace, M. C. (2022). Mechanisms of action of carbapenem resistance. Antibiotics, 11(3), 421.

Aworh, M. K., Kwaga, J., Okolocha, E., Mba, N., & Thakur, S. (2019). Prevalence and risk factors for multi-drug resistant Escherichia coli among poultry workers in the Federal Capital Territory, Abuja, Nigeria. PloS one, 14(11), e0225379.

Aworh, M. K., Kwaga, J., Okolocha, E., Mba, N., & Thakur, S. (2019). Prevalence and risk factors for multi-drug resistant Escherichia coli among poultry workers in the Federal Capital Territory, Abuja, Nigeria. PloS one, 14(11), e0225379.

http://www.ncbi.nlm.nih.gov/blast.

Azargun, R. (2019) “Frequency of DNA gyrase and topoisomerase IV mutations and plasmid-mediated quinolone resistance genes among Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections in Azerbaijan, Iran,” Journal of Global Antimicrobial Resistance, 17, pp. 39–43. doi: 10.1016/j.jgar.2018.11.003.

Banerjee, Ritu, and Romney Humphries. (2017). “Clinical and Laboratory Considerations for the Rapid Detection of Carbapenem-Resistant Enterobacteriaceae.” Virulence 8(4): 427–39. https://doi.org/10.1080/21505594.2016.1185577.

Chandrasekaran, S., Abbott, A., Campeau, S., Zimmer, B. L., Weinstein, M., Thrupp, L., ... & Humphries, R. M. (2018). Direct-from-blood-culture disk diffusion to determine antimicrobial susceptibility of Gram-negative bacteria: preliminary report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. Journal of clinical microbiology, 56(3), e01678-17.

Chessbrough, M. (2010). District laboratory practice in tropical countries, Part-2, New York, USA, Cambridge University, 184- 186.

CLSI (2012). Performance Standards for Antimicrobial Susceptibility Testing; 21st Informational Supplement. CLSI Document.

Codjoe, F. S., & Donkor, E. S. (2017). Carbapenem resistance: a review. Medical Sciences, 6(1), 1.

De Angelis, G., Del Giacomo, P., Posteraro, B., Sanguinetti, M. and Tumbarello, M. (2020). Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in enterobacteriaceae. International Journal of Molecular Sciences, 21(14), 5090.

De Angelis, G., Del Giacomo, P., Posteraro, B., Sanguinetti, M. and Tumbarello, M. (2020). Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in enterobacteriaceae. International Journal of Molecular Sciences, 21(14), 5090.

Guan, X., Xue, X., Liu, Y., Wang, J., Wang, Y., Wang, J., ... & Pan, L. (2013). Plasmid-mediated quinolone resistance–current knowledge and future perspectives. Journal of International Medical Research, 41(1), 20-30.

Hamzah, H. A., Sirat, R., Mahmud, M. I. A. M. and Baharudin, R. (2021). Mutational Analysis of Quinolone-Resistant Determining Region gyrA and parC Genes in Quinolone-Resistant ESBL-Producing E. Coli. IIUM Medical Journal Malaysia, 20(3).

Islam, S., Selvarangan, R., Kanwar, N., McHenry, R., Chappell, J. D., Halasa, N., Gomez-Duarte, O. G. (2018). Intestinal carriage of third-generation cephalosporin-resistant and extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy US children. Journal of the Pediatric Infectious Diseases Society, 7(3), 234-240.

Khalifa, H. O., Soliman, A. M., Ahmed, A. M., Shimamoto, T., Nariya, H., Matsumoto, T. and Shimamoto, T. (2019). High prevalence of antimicrobial resistance in Gram-negative bacteria isolated from clinical settings in Egypt: recalling for judicious use of conventional antimicrobials in developing nations. Microbial Drug Resistance, 25(3), 371-385.

Khawcharoenporn, T., Vasoo, S. and Singh, K. (2013). Urinary tract infections due to multidrug-resistant Enterobacteriaceae: prevalence and risk factors in a Chicago Emergency Department. Emergency medicine international, 2013.

Khilnani, G. C., Zirpe, K., Hadda, V., Mehta, Y., Madan, K., Kulkarni, A., ... & Bhattacharya, P. (2019). Guidelines for antibiotic prescription in intensive care unit. Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine, 23(Suppl 1), S1.

Krumperman, P. H. (1983) Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods). Applied Environmental Microbiology 46 (1): 165-170.

Mahapatra, A., Patro, A. R. K., Khajuria, A., Dhal, S., & Praharaj, A. K. (2020). Ciprofloxacin-resistant Gram-negative isolates from a tertiary care hospital in Eastern India with novel gyrA and parC gene mutations. Medical Journal Armed Forces India.

Mirzaii, M., Jamshidi, S., Zamanzadeh, M., Marashifard, M., Hosseini, S. A. A. M., Haeili, M., ... & Khoramrooz, S. S. (2018). Determination of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in Escherichia coli and Klebsiella pneumoniae isolated from patients with urinary tract infection in

Iran. Journal of Global Antimicrobial Resistance, 13, 197-200.

Ngbede, E. O. Adekanmbi, F., Poudel, A., Kalalah, A., Kelly, P., Yang, Y., Adamu, A. M., Daniel, S.T., Adikwu, A. A., Akwuobu, C. A., Abba, P. O., Mamfe, L. M., Maurice, N. A., Adah, M. I., Lockyear, O. Butaye, P. and Wang, C. (2021) "Concurrent Resistance to Carbapenem and Colistin Among Enterobacteriaceae Recovered From Human and Animal Sources in Nigeria Is Associated With Multiple Genetic Mechanisms," Frontiers in Microbiology, 12(October). doi: 10.3389/fmicb.2021.740348.

Odeyemi, A. T. (2012). Antibiogram status of bacterial isolates from air around dump site of Ekiti State Destitute Centre at Ilokun, Ado-Ekiti, Nigeria. Journal of Microbiology Research, 2(2), 12-18.

Olowo-Okere, A., Ibrahim, Y. K. E., Nabti, L. Z., & Olayinka, B. O. (2020). High prevalence of multidrug-resistant Gram-negative bacterial infections in Northwest Nigeria. Germs, 10(4), 310.

Onwubiko, N. E., & Chinyeaka, A. H. (2015). Isolation and identification of bacterial contaminants from door handle in a tertiary institution in Umuahia, Abia State, Nigeria. Nigerian Journal of Microbiology, 29, 3139-3147.

Padmini, P., Chung, I. M., Abdul Rahuman, A., Marimuthu, S., Vishnu Kirthi, A., Anbarasan, K., & Rajakumar, G. (2017). Green synthesis of copper nanoparticles using Eclipta prostrate leaves extract and their antioxidant and cytotoxic activities. Experimental and therapeutic medicine, 14(1), 18-24.

Patel, A. K., Singhania, R. R., Pandey, A., Joshi, V. K., Nigam, P. S. and Soccol, C. R. (2014). Enterobacteriaceae, Coliforms and E.Coli. In C. A. Batt, and M. L. Tortorello, (Eds.), Encyclopedia of Food Microbiology: Second Edition (Vol. 1, pp. 659-666). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-384730-0.00096-3.

Rafalskiy, V., Pushkar, D., Yakovlev, S., Epstein, O., Putilovskiy, M., Tarasov, S., ... & Gorelysheva, N. (2020). Distribution and antibiotic resistance profile of key Gram-negative bacteria that cause community-onset urinary tract infections in the Russian Federation: RESOURCE multicentre surveillance 2017 study. Journal of global antimicrobial resistance, 21, 188-194.

Rafalskiy, V., Pushkar, D., Yakovlev, S., Epstein, O., Putilovskiy, M., Tarasov, S.& Gorelysheva, N. (2020). Distribution and antibiotic resistance profile of key Gram-negative bacteria that cause community-onset urinary tract infections in the Russian Federation: RESOURCE multicentre surveillance 2017 study. Journal of global antimicrobial resistance, 21, 188-194.

Ranjbar, R., & Farahani, A. (2019). Shigella: antibiotic-resistance mechanisms and new horizons for treatment. Infection and drug resistance, 12, 3137.

Ranjbar, R., & Farahani, A. (2019). Shigella: antibiotic-resistance mechanisms and new horizons for treatment. Infection and drug resistance, 12, 3137.

Rojas, L. J., Salim, M., Cober, E., Richter, S. S., Perez, F., Salata, R. A., .& Antibacterial Resistance Leadership Group. (2017). Colistin resistance in carbapenem-resistant Klebsiella pneumoniae: laboratory detection and impact on mortality. Clinical Infectious Diseases, 64(6), 711-718.

Subramani, S., & Vignesh, S. (2012). MAR index study and MDR character analysis of a few golden staph isolates. Asian Journal of Pharmacy and Life Science ISSN, 2231, 4423.

Suzuki, Y., Ida, M., Kubota, H., Ariyoshi, T., Murakami, K., Kobayashi, M., & Sadamasu, K. (2019). Multiple β-lactam resistance gene-carrying plasmid harbored by Klebsiella quasipneumoniae isolated from urban sewage in Japan. Msphere, 4(5), e00391-19., T., Vasoo, S. and Singh, K. (2013). Urinary tract infections due to multidrug-resistant Enterobacteriaceae: prevalence and risk factors in a Chicago Emergency Department. Emergency medicine international, 2013.

Tang, S. S., Chee, E., Teo, J. Q., Chlebicki, M. P., & Kwa, A. L. (2021). Incidence of a subsequent carbapenem-resistant Enterobacteriaceae infection after previous colonisation or infection: a prospective cohort study. International Journal of Antimicrobial Agents, 57(6), 106340.

Temkin, E., Adler, A., Lerner, A. and Carmeli, Y. (2014). Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management. Annals of the New York Academy of Sciences, 1323(1), 22-42.

Van den Bunt, G., Liakopoulos, A., Mevius, D. J., Geurts, Y., Fluit, A. C., Bonten, M. J. and van Pelt, W. (2016). ESBL/AmpC-producing Enterobacteriaceae in households with children of preschool age: prevalence, risk factors and co-carriage. Journal of Antimicrobial Chemotherapy, dkw443.

Weigel, L. M., Steward, C. D., & Tenover, F. C. (1998). gyrA mutations associated with fluoroquinolone resistance in eight species of Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*, *42*(10), 2661-2667.

Yadav, K.K., Adhikari, N., Khadka, R. (2015). Multidrug-resistant Enterobacteriaceae and extended-spectrum β-lactamase producing Escherichia coli: a cross-sectional study in National Kidney Center, Nepal. Antimicrob Resist Infect Control 4, 42.

Zaidah, A. R., Mohammad, N. I., Suraiya, S. and Harun, A. (2017). High burden of Carbapenem-resistant Enterobacteriaceae (CRE) faecal carriage at a teaching hospital: cost-effectiveness of screening in a low-resource setting. Antimicrobial Resistance & Infection Control, 6(1), 1-6.