Antibiotic Sensitivity Pattern of *Escherichia coli* Isolates From Urine And Stool of Apparently Healthy Individuals In Port Harcourt Nigeria

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Abstract

Antibiotic-resistant strains of once-controllable pathogens are emerging and spreading beyond the healthcare system. It places an immense economic strain on the healthcare system and raises serious concerns about infection management and prevention. This research aims to identify any trends in antibiotic resistance among *Escherichia coli (E. coli)* strains recovered from urine and stool of outpatients of the University of Port Harcourt Teaching Hospital. *E. coli* was isolated and identified using standard laboratory techniques. Antimicrobial susceptibility testing was performed using the modified Kirby - Bauer disc diffusion method, and results were reported using an interpretive chart developed by the Clinical and Laboratory Standards Institute (CLSI). The proportion of antibiotics to which an isolated strain is resistant was used to establish the Multiple Antibiotic Resistance Index (MAR). A total of 62 *E. coli* isolates were identified, with a large proportion (>70%) resistant to cefuroxime, cefotaxime, cefixime, ceftriaxone, imipenem, ampiclox, and amoxicilin clavulanate, intermediately susceptible to nalidixic acid and gentamycin, and very sensitive to nitrofurantoin, ofloxacin, and levofloxacin in both urine and stool. All isolates were multi-drug resistant (MDR) to at least 3 classes of antibiotics. Hundred percent of the samples with a MAR index > 0.2. A high MAR index in *E. coli* isolates suggests that these bacteria have been exposed to antibiotics in the past and have evolved resistance to routinely used antibiotics; thus, antimicrobial susceptibility testing is crucial in deciding on a course of treatment. Spread among people can be slowed by focusing on proper infection prevention and control measures.

Keywords: *Escherichia coli*, antibiotics, resistance, MAR index and MDR

1.0 Introduction

In both humans and animals, *E. coli* is a common commensal. Most members of this genus of enterobacteria are non-pathogenic, meaning they do not harm their hosts in any way and instead form a mutually beneficial symbiotic relationship. However, *E. coli* is a very complicated species since it has mutated into several pathogenic strains. Strains of *E. coli* are categorized as either zoonotic intestinal pathogenic *E. coli* (IPEC) or extraintestinal pathogenic *E. coli*

(ExPEC) (Lindstedt et al., 2018) depending on the virulence factor type present and the associated symptoms of the host.

When it comes to infectious diseases, the gastrointestinal tract and extraintestinal sites are both vulnerable to pathogenic *E. coli*. UTIs, septicaemia, neonatal meningitis, CNS infections, and respiratory infections are all examples of extraintestinal illnesses (Croxen et al., 2013; Kim et al., 2019). About 0.1% of the microflora in a healthy human digestive tract consists of *Escherichia coli* and similar bacteria (Eckburg et al., 2005). It is important to note that faecal-oral transmission is the primary mode of disease transmission caused by pathogenic strains of the bacterium. Patients with healthy immune systems and those with pre-existing disorders are both susceptible to infections caused by *E. coli* and its close relatives (Pitout 2012). Infectious gastrointestinal pathogens like *E. coli* are a major reason for irregular cases of diarrhoea all around the world, particularly in underdeveloped regions (Alizade et al., 2019).

E. *coli's* pathogenicity is largely within the reach of its adaptable gene pool due to its capacity for material gain and loss (Ulett et al., 2013; Wiles et al., 2008). In the 2000s and in some places, the number of antibiotic-resistant isolates has dramatically grown, particularly those that are fluoroquinolones-resistant and those expressing extended-spectrum ß-lactamases, while many nosocomial and community-acquired *E. coli* are now resistant to numerous essential antimicrobials (Pitout 2012). *E. coli* is successfully inhibited by a wide spectrum of antimicrobial drugs. Treatments for *E. coli* infections in hospitals and the community frequently involve the use of β-lactams, fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole (Eckburg et al., 2005). Limited clinical research on the effects of antimicrobial therapy and the results of patients infected with carbapenemase-producing *E. coli* compared to patients infected with susceptible strains indicate worse clinical outcomes for patients with infections caused by resistant isolates (Schultz et al., 2012). This study was designed to isolate, identify, and determine the susceptibility index of *E. coli* from clinical sources obtained from outpatients with no confirmed infections at the University of Port Harcourt   Teaching   Hospital   (UPTH),   Rivers,   Nigeria.

2.0 Methods

2.1 Sample population

A total of 100 (50 urine and 50 stool) samples were randomly taken from the outpatients' department of the University of Port Harcourt Teaching Hospital (UPTH), Rivers, Nigeria and

transferred to the medical laboratories of the Department of Microbiology, University of Port Harcourt, Rivers, Nigeria for subsequent culturing and biochemical tests. This study excluded outpatients with confirmed infection or under antibiotic treatment at the time of sample collection.

2.2   Bacterial isolation and identification

The isolates were identified preliminary using morphology and traditional biochemical testing for Enterobacteriaceae using normal microbiological procedures. Eosin Methylene Blue (EMB) medium was used to purify the positive samples. Gram staining and biochemical studies, such as Kliger iron agar, indole, citrate, and urease tests were used (Cheesbrough, M. 2006; Neama et al., 2020).

2.3   Antibiotic susceptibility test

On Mueller-Hinton agar plates, an antibiotic susceptibility test was performed using the disc diffusion (Kirby-Bauer) method. For each isolate, bacterial suspension was made in 0.5 McFarland turbidity standard and swabbed onto Mueller-Hinton agar plates that had already been prepared. The impregnated antibiotic discs (Oxoid, UK) were placed within the plates, which were then incubated at 37 8C for 24 hours. 12 antibiotics were utilized in the experiment: Amoxicillin clavulanate (AUG; 30 mg), cefotaxime (CTX; 25 mg), imipenem (IMP; 10mg), gentamicin (GN; 10 mg), nalidixic acid (NA; 30mg), levofloxacin (LBC; 5 mg), ofloxacin (OFX; 5 mg), ampliclox (ACX; 10 mg), nitrofurantoin (NF; 300 mg), cefixime (ZEM; 5mg), cefuroxime (CXM; 30 mg), and ceftriaxone sulbactam (CRO; 45mg). As per the advice of the Clinical and Laboratory Standard Institute, zones of diameter were measured and classified as susceptible, intermediate, and resistant. Clinical and Laboratory Standards Institute (CLSI) (2020).

2.4   Calculating the MAR index

The method stated in Osundiya et al., (2013) was used to calculate the MAR index, which is calculated by dividing the number of antibiotics (a) that an isolate is resistant to by the total number of antibiotics used in study (b). The calculating formula is shown below:

MAR   index   =a/b

2.5 Statistical analysis

Descriptive statistics, frequencies, and bivariate analyses (cross-tabulations) were used for the presence   of   antibiotic   resistance   patterns.

3.0 Results

Of the 100 samples collected 62 (62%) were identified as *E. coli* (31 from urine and 31 from Stool). The antibiotic susceptibility profiles of *E. coli* strains in urine and stool were classified as "Resistant," "Intermediate," and "Susceptible" (Figures 1 and 2, respectively). The Kirby-Bauer disc diffusion method was utilised to test multiple strains against 12 distinct antibiotics. The evaluation and standardisation of this process were conducted in accordance with the National Committee for Clinical Laboratory Standards' methodologies. The comparison focused on the resistance patterns of *E. coli* between urine and stool (figure 3) and the resistance pattern of *E. coli* in both (figure 4). MDR in this study was defined as the resistance to 3 or more classes of antibiotics used. The study derived the percentile distribution for the incidence of resistance among isolates to various antibiotic classes, such as penicillin (ampicillin, amoxicillin-clavulanate), aminoglycosides (gentamycin), fluoroquinolones (ofloxacin, and levofloxacin), nitrofurantoin, extended-spectrum cephalosporins (cefixime, cefuroxime, ceftriaxone, and cefotaxime), and carbapenem (imipinem) (figure 5). Table 1 displays the MAR indices for both entities.
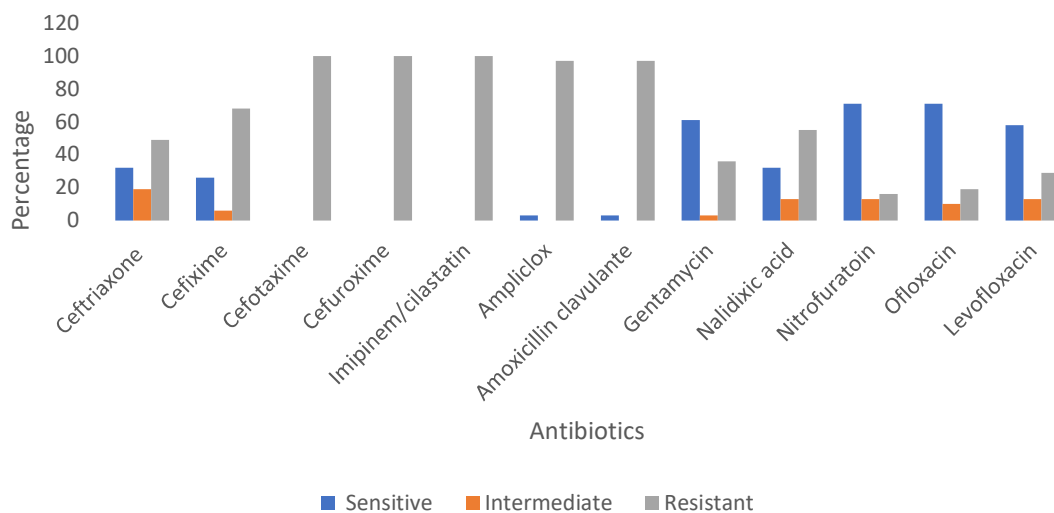
Figure 1: The sensitivity patterns to various antibiotics of Escherichia coli isolated from urine were compared, as illustrated
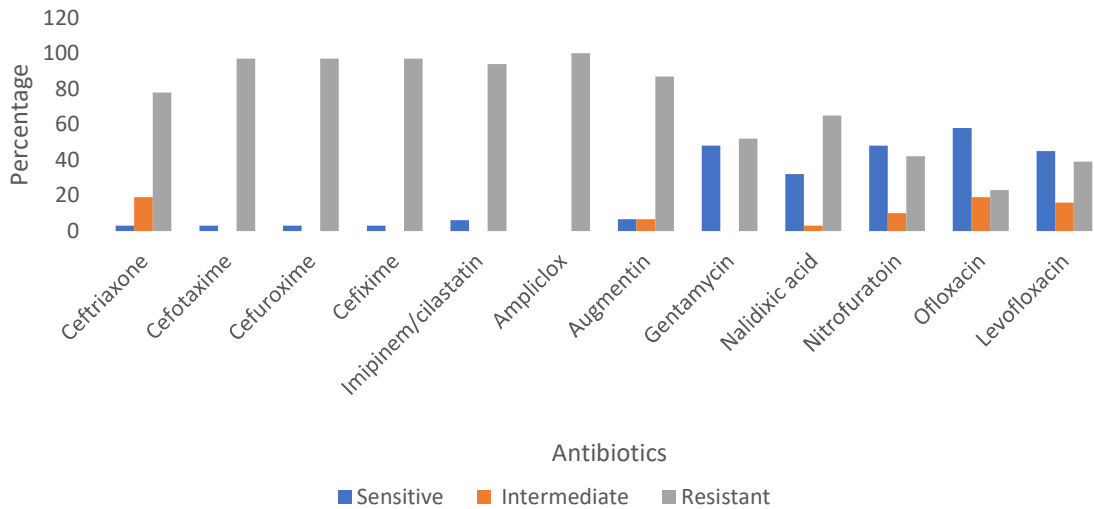

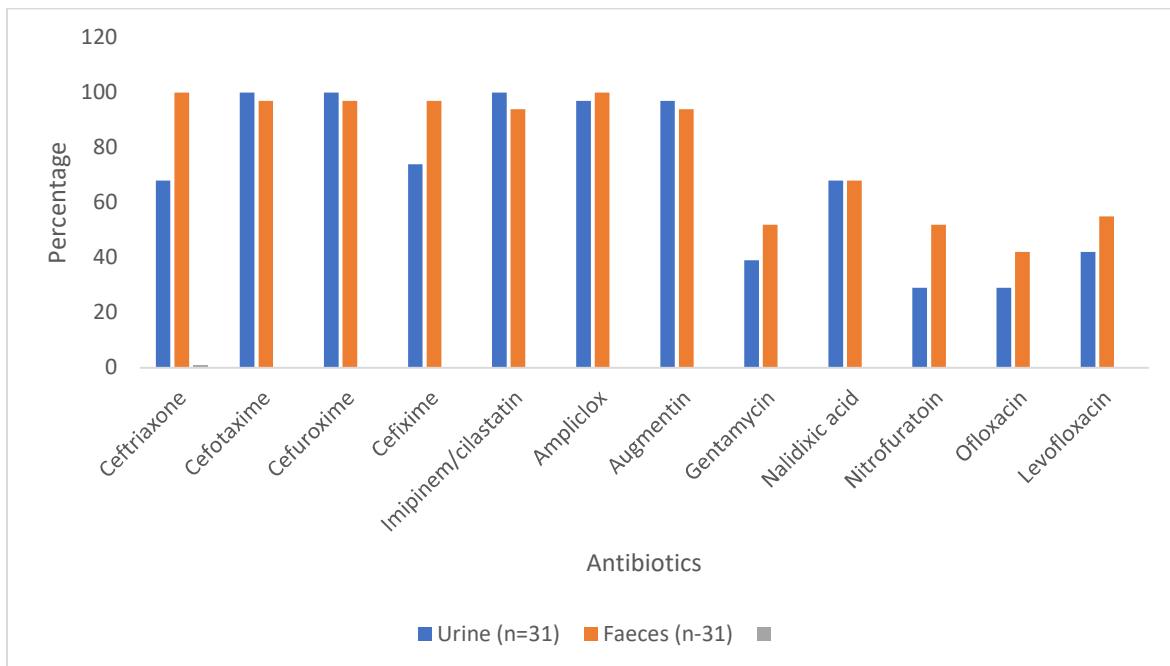Figure 2: Analysing the susceptibility of *E. coli* isolated from faeces to various antibiotics

Figure 3: Patterns of antibiotic resistance in urine and stool isolates of Escherichia coli.
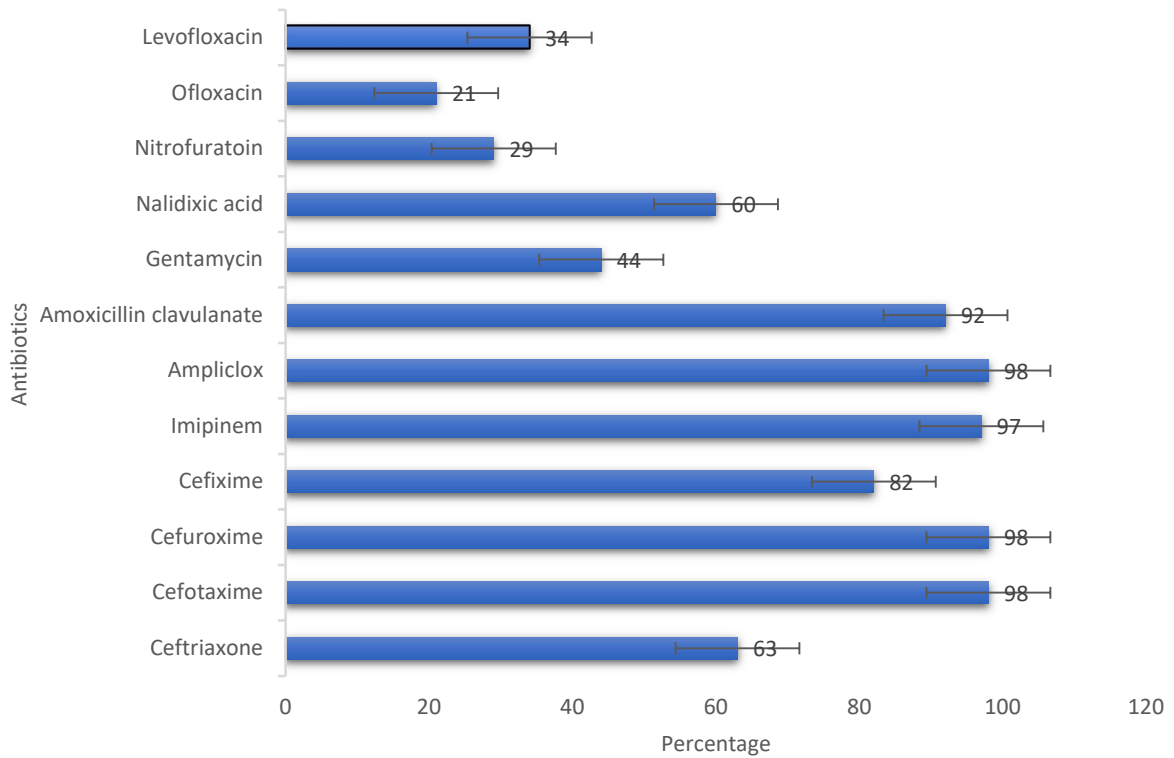
Figure 4: Rate of antibiotic resistance among the strains of *E. coli* tested

Table 1: The MAR index and the prevalence of *E. coli* with resistance.

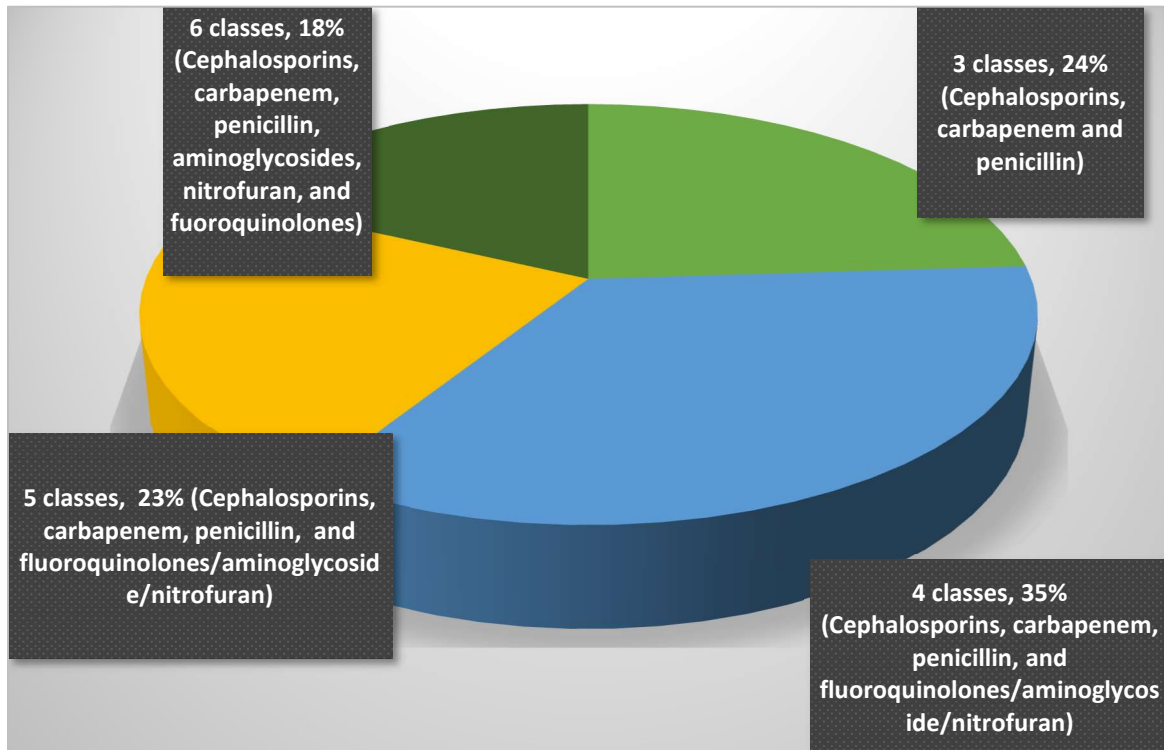| No of antibiotics	MAR index	Urine (no of isolates)	Stool (no of isolates)
1	0.08	0	
2	0.17	0	0
3	0.25	0	0
4	0.33	1	0
5	0.42	3	0
6	0.5	7	3
7	0.58	7	5
8	0.67	6	8
9	0.75	0	6
10	0.83	1	1
11	0.92	4	4
12	1.0	2	4

Figure 5: Multi-drug resistance (MDR) of *E. coli* in this study.

4.0   Discussion

Before now, therapeutic management of *Escherichia coli* infections required the use of tetracycline, β-lactams, fluoroquinolones, aminoglycosides, and cotrimoxazole. However, due to antimicrobial resistance, new lines of antimicrobials such as carbapenems, cephalosporins, and monobactams were introduced. Nonetheless, the phenomenon of *E. coli* exhibiting resistance to antibiotics as observed is increasing dramatically. In the present study, the *E. coli* strains that were obtained from urine specimens were all observed to be resistant (31/31, 100%) to cefotaxime, cefuroxime, and imipenem/cilastatin (belonging to cephalosporin and carbapenem groups of antibiotics). The isolates also showed very high resistance to amoxicillin-clavulanate (30/31, 97%) and ampicillin (30/31, 97%). The lowest resistance (9/31, 29%) exhibited by the isolates from the urine samples was to nitrofurantoin and ofloxacin (belonging to nitrofuran and fluoroquinolones groups of antibiotics). *E. coli* found in stool samples were completely resistant (31/31, 100%) to ceftriaxone, ampicillin, and imipenem, and

generally resistant to all antibiotics tested. The pattern of resistance to gentamycin and levofloxacin was generally moderate.

Nitrofurantoin and ofloxacin antibiotics were the most efficacious against isolates from both clinical sources. Shakya et al., (2017) similarly reported the susceptibility of *E. coli* to nitrofurantoin. Le and Rakonjac (2021), reported that due to antimicrobial resistance, the use of nitrofuran an old drug class has been revived in the treatment of *E. coli*. A report on the regional antibiotic resistance patterns by Tadesse et al., (2020) revealed a low level of resistance *E. coli* to fluoroquinolones in Africa. Abd El-Baky et al., (2020) in a study of the prevalence of *E. coli* in Egypt, observed that meropenem and imipenem were the most effective antibiotics against *E. coli* from clinical samples.

All the isolates showed high resistance to cefotaxime, imipenem, cefuroxime, ampicillin, and cefixime, with resistance >70%. Abd El-Baky et al., (2020) similarly reported >70% resistance to cefotaxime, cefixime, and ceftazidime by *E. coli* from clinical sources. The resistance to imipenem is particularly worrisome because carbapenems are often the treatment option of last resort against infections caused by multidrug resistance (MDR) *E. coli*. Before now, the greatest epidemiological and clinical impact of carbapenamase globally has been due to the high incidence of nosocomial infections caused by *Klebsiella pneumoniae* (Ortega et al., 2016). But evidence has begun to emerge of the prevalence of carbapenems resistance among *E coli* (Govindaswamy et al., 2020; Ortega et al., 2016; Sekar et al., 2016), as supported by the present study. The increased prevalence of carbapenemase-producing *E. coli* could lead to a new epidemiological crisis, owing to the failure of empirical therapy (Sekar *et al*., 2016; Al Tamimi *et al*., 2017).

Multidrug resistance (MDR), defined as the resistance to three or more antimicrobial agents belonging to different classes, was frequently observed among bacterial isolates obtained from urine and stool samples in this study. Abd El-Baky et al., (2020) observed MDR as common only in clinical samples. According to Kayastha et al., (2020) MDR pathogens are more common in hospital settings and are mostly of nosocomial origin.

This study also found that *E. coli* with a MAR score of 1.00 exhibited the highest level of multi-drug resistance and that 6 of 62 isolates (10%) were resistant to all 12 antibiotics. These findings are similar to the results of Ayandele et al., (2020). As indicated in Table 1, the Multiple Antibiotic Resistance (MAR) index is a crucial assessment tool for evaluating antibiotic resistance and health risk factors. The calculation of the MAR index involves the division of

the count of antibiotics to which an organism exhibits resistance by the overall count of antibiotics to which it is subjected. Bacteria with a MAR score of 0.2 originate from a high-risk contamination source that utilizes multiple antibiotics. Organisms with MAR indices greater than 0.2 indicate the existence of multi-drug resistant genes coming from a drug-abusing environmental factors and plasmids harbouring antibiotic-resistance genes that express single-phenotype resistance (Ejiofor et al., 2016; Riaz et al., 2011). Using the MAR index analysis is similarly simple, requiring neither specialized knowledge nor costly equipment (Ejiofor et al., 2016; Sandhu et al., 2016), and it also provides the necessary data.

Resistance to antibiotics among *E. coli* has been reported to be a result of their expression of different virulence factors and antibiotic resistance genes (Alqasim et al., 2020; Escudeiro et al., 2019; Rubini et al., 2020). Both virulence factors and antibiotic-resistance genes can be borne by plasmids (Lee et al., 2016; Turton et al., 2019).

5.0   Conclusion

The majority of the bacterial isolates were resistant to multiple antimicrobials; consequently, it is proposed that adequate antimicrobials be administered to limit the possibility of multidrug-resistant organisms emerging and to prevent antibiotic inefficacy. It is clear from this that empirical treatment is not the best option, and that antibiotic selection should be based on information about the local abundance of bacterial organisms and antibiotic sensitivities. The best antibiotics for treating a potential *E. coli* infection in this research area were nalidixic acid (NA), gentamycin (GN)., levofloxacin (LBC), nitrofurantoin (NF), and ofloxacin (OFX) in that order.

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