INVESTIGATION OF MULTIDRUG RESISTANT *ESCHERICHIA COLI* ON MEDICAL EQUIPMENT AND SURFACES FROM SELECTED HOSPITALS IN MINNA, NIGERIA

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ABSTRACT

Nosocomial infections are often caused by multidrug-resistant (MDR) bacteria contaminating hospital environments which can cause outbreaks as well as sporadic transmission. This study was carried out to investigate the presence of multidrug resistant Escherichia coli on medical equipment and surfaces in selected hospitals in Minna, Nigeria. A total of 130 samples were collected by swabbing medical equipment (28) and surfaces (102), the samples were screened for Escherichia coli isolates by culturing on MacConkey agar and Eosin methylene blue agar (EMB). A total 22 (17.9%) Escherichia coli were isolated, with the highest level of contamination observed in beds (31.8%) and chair surfaces (13.6%). Antibiotic susceptibility results revealed that 19(86.4%) of the E. coli isolates were multidrug-resistant showing high level of resistance to trimethoprim-sulfamethoxazole 19(86.4%), ampicillin 18(81.8%), cefixime 17(77.3%), nalidixic acid 17(77.3%), ciprofloxacin 15(68.2%) and cefpodoxime 14(63.6%). Escherichia coli isolated exhibited high level of resistance to commonly prescribed antibiotics in the treatment of E. coli infections. This is quiet alarming, as such existing prevention strategies and infection control programs should be intensified in the control of nosocomial infections.

Keywords: *Escherichia coli,* Gram negative bacteria, Hospital equipment, Inanimate surfaces, Multidrug resistance

INTRODUCTION

For more than a century, nosocomial infections have been acknowledged as a serious issue impacting the standard of care in healthcare and as a significant contributor to unfavourable patient outcomes. A new strain on hospital medical care has been brought about by the rise of multidrug-resistant bacteria (Aly et al., 2008; Cornejo-Juárez et al., 2015). The effects of nosocomial infections and antibiotic resistance on people who are impacted are immeasurable. According to the World Health Organization

(WHO), each year, 52.3% of patients in intensive care units and more than 24% of patients with nosocomial sepsis die. Antibiotic-resistant infections increase the risk of death by a factor of two to three in patients (AMvomo et al., 2023). The microbes present in the hospital setting are frequently multi-resistant to antibiotics and are the primary conduit for the spread of highly pathogenic strains. One of the top goals of healthcare organizations is the battle against nosocomial diseases, especially those

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connected to microorganisms in the hospital setting.

According to studies, contaminated or even visually clean inanimate surfaces and medical equipment are strongly linked to the transmission of nosocomial infections (Jaboska-Trypu et al., 2022). Microorganisms possess traits that allow them to endure poor environments for a short period of time up to several months. One of the most significant pathogens that spreads most readily from inanimate surfaces to skin is *Escherichia coli* (Wißmann et al., 2021).

Multidrug resistance in the *Enterobacteriaceae* is starting to pose a threat to world health. *E. coli* that is resistant to antibiotics is also spreading and is becoming a serious hazard to human health worldwide. Over the past few decades, multidrug-resistant *E. coli* has emerged in a number of nations. For the treatment of *E. coli* infections, the rising cephalosporin resistance, in particular the corresponding rise in the incidence of multidrug-resistant *E. coli*, is of growing concern. Recent estimates show that antibiotic-resistant illnesses contributed to around 5 million fatalities worldwide in 2019 alone (Murray et al., 2022). One of the top three threats to public health in the twenty-first century is antibiotic resistance (WHO 2014). Infections brought on by resilient microorganisms now happen often. Although it is a natural occurrence, the egregious overuse of antimicrobial drugs expedited its growth considerably (Michael et al. 2014).

It is crucial to pay special attention to inanimate surfaces and medical equipment's propensity to retain potentially hazardous microorganisms given that many of them come into direct contact with healthcare professionals, patients, technicians, cleaners, and occasionally care providers in hospitals. Therefore, this study was aimed at investigating the prevalence and distribution of multidrug resistant *Escherichia coli* on medical equipment and surfaces from selected hospitals within Minna, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in two major hospitals (Ibrahim Babangida Specialist Hospital and general Hospital Minna) attended by the populace in Minna, the capital of Niger state in North-Central Nigeria.

Inclusion and Exclusion Criteria

All medical equipment and surfaces used by patients or the ones patients have contact with, in all the units within the selected hospital were included in this study while units/places that have no record of contact with patients were excluded.

Ethical Approval

Ethical approval was obtained from the Research and Ethics Committee of the selected hospitals.

Sample Size

Sample size was determined using the Fisher's formula (equation 1) and a prevalence of 9.09% reported by Mohammed et al. (2016). The calculated sample size was 127 and a total of 130 samples were collected from each hospital.

$$n = \frac{z^2 pq}{d^2} \qquad (1)$$

Sample Collection

Samples were collected by swabbing surfaces of medical equipment and inanimate surfaces in selected hospitals. A total of 130 non-clinical samples were obtained from predefined surfaces such as ultra sound machines (2), X-ray machine (1), Air conditioners (3), infant radiant warmer (1), beds (32), chairs (15), tables (21), drip stand (17), Reception Counters (3), baby swings (2), sinks (15), scale (3), emesis basin (7), hospital trolley (2) and electrical appliances switch (6) using swab sticks moistened with normal saline. The samples were transported in ice pack to the Centre for Genetic Engineering and Biotechnology (CGEB),

Federal University of Technology Minna for analysis.

Bacteria Isolation

Samples were inoculated onto nutrient broth (Oxoid) and incubated at 37 ^{0}C for 24 hours. Subsequently, growth was noted by the turbidity of the medium, broth was cultured on MacConkey agar (Oxoid) and incubated for 37 ^{0}C for 24 hours. Characteristically distinct colonies obtained after incubation were sub-cultured onto Eosin methylene blue agar (EMB) (Oxoid) repeatedly to obtain pure cultures which were stored on agar slants for further identification and analysis (Kibet et al. 2017).

Identification of Isolates

Gram-negative bacterial isolates stored on agar slants were identified with the aid of colony morphology and conventional biochemical tests including Gram staining, oxidase, voges proskauer, indole, methyl red, citrate, Triple Sugar Iron, catalase, urease and motility tests using Bergey's manual of bacteriology (Dawodu and Akanbi, 2021).

Molecular Identification of isolates

DNA extraction: Following the manufacturer's instructions, genomic DNA was extracted using a column-based JENA Bioscience Bacteria DNA Preparation Kit. Bacteria cells were collected using a micro centrifuge at 10,000g for 1 minute from a 500 μL aliquot of bacteria broth culture. The leftover pellet was resuspended in 300 μL of resuspension buffer and 2 μL of lysozyme solution. The mixture was inverted several times to homogenize before incubation at 37 °C for 1 hour. Centrifugation was used to recover resuspended cells, which were then lysed with 300 μL of lysis buffer, 2 μL RNase A, and 8 μL proteinase-K-solution, and then incubated at 60 °C for 10 mins and the tube was allowed to cool on ice for 5 min. About 300 μL of binding buffer was then added and vortexed briefly before cooling on ice again for 5 mins and centrifuging at 10,000g for 5 minutes. To trap the DNA, the supernatant was directly placed into the spin column and centrifuged at 10,000g for 1 minute. The trapped DNA was rinsed twice with washing buffer before being eluted in a clean eppendorf tube with 50 μL elution buffer (Nwokeh et al. 2022).

Polymerase chain reaction (PCR)

Each PCR reaction mixture consisted of 12.5 μL mastermix (2x JENA Ruby hot start mastermix), 1μl (10 pmol) each of 27FAGA GTTTGATCMTGG CTC AG and 1492R-TACGGYTACCTTGTTACG ACT T, 1 μL DNA template and 9.5μl sterile nuclease free water to make up a total reaction of 25 μL. PCR amplification was carried out in an Applied Biosystem2720 Thermalcycler. The mixture was subjected to denaturation at 94 °C for 3mins; followed by 35 cycles of 94 °C for 45s, 55°C for 60 seconds and 72°C for 60 seconds; and a final extension at 72°C for 10mins (Lane et al. 1991).

Gel Electrophoresis

The PCR product was visualized on a 2% agarose gel containing ethidium-bromide in 0.5x Tris-borate buffer (pH 8.0).

Antibiotic Susceptibility Testing

Antibiotic susceptibility test was carried using the Kirby Bauer disk diffusion method on Mueller Hinton Agar. Single disc antimicrobial disc of Imipenem (10μg), Meropenem (10μg), Colistin (10μg), Trimethoprim-sulfamethoxazole (25μg), Amoxicillin-clavulanic acid (30μg), Fosfomycin (50μg), Gentamicin (30μg) Ciprofloxacin (10μg), Nalidixic acid (30μg), Cefixime (5μg), Cefpodoxime (10μg), Ampicillin (30μg) and Chloramphenicol (30μg) were applied aseptically on the surface of the inoculated plates. The plates were incubated at 37°C for about 18-24 hours. The diameter of the zone of inhibition around the discs was measured and interpreted in accordance to the standard criteria recommended by the Clinical Laboratory Standards Institute (CLSI 2018).

Multiple antibiotic resistance index (MARI)

According to Krumperman (1983), the multiple antibiotic resistance index is determined as the ratio of the number of antibiotics to which the isolates are resistant to the number of antibiotics against which they were tested.

$$MARI = \frac{Number\ of\ antibiotics\ isolate\ is\ resistant\ to}{Total\ number\ of\ antbiotics\ tested}$$

RESULTS

Molecular Identification of *Escherichia coli* isolates

Identification of four isolate was carried out by the 16rRNA sequence analysis. The result of pulsed field gel electrophoresis of selected isolates is shown in Figure 1. The sequences obtained using basic local alignment search tool in GenBank of NCBI showed that the test organisms had 96%-98% similarity with *Escherichia coli*. Two isolates (BW-1 and EP-1) were identified as *Escherichia coli strain* NBRC 102203 with accession number NR_114042.1. The other isolates (F-6 and FM-7) were identified as *Escherichia coli strain* U 5/41 with accession number NR_024570.1 (Table 3).
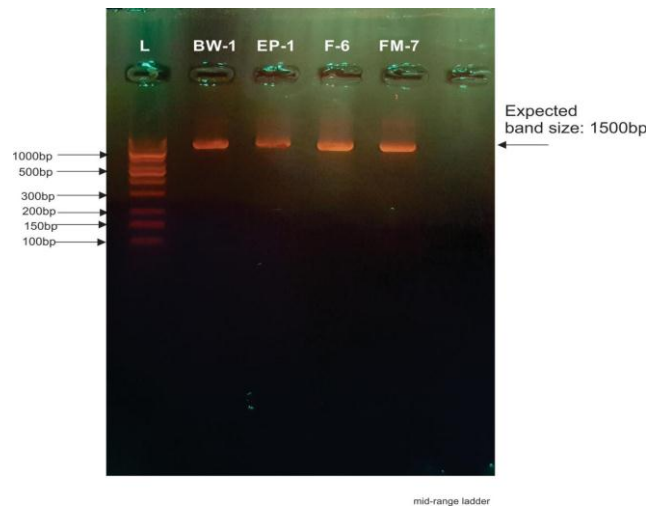

Figure 1. Gel electrophoresis of the PCR product

Table 3. Sequencing Result Alignment for the Selected Isolates

Sample code	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
BW-1	*Escherichia coli*	1937	1934	96%	0.0	96.06%	1467	NR_114042.1
F-6	*Escherichia coli*	2087	2087	95%	0.0	97.90%	1450	NR_024570.1
Ep-1	*Escherichia coli*	2025	2025	96%	0.0	97.32%	1467	NR_114042.1
FM-7	*Escherichia coli*	2006	2006	94%	0.0	96.11%	1450	NR_024570.1

Distribution and Prevalence of *Escherichia coli* on hospital surfaces and equipment

Out of 130 samples collected 22 *Escherichia coli* isolates were obtained giving a prevalence of 17.9%. The most contaminated hospital surfaces and equipment were beds (32%), chair surfaces (14%), Bedside cabinet (9%), Bed rails (9%), Weighing scale (9%), Wheel chair (5%), Sink (5%), Kidney Tray (4%), Door knob (4%), baby radiant warmer (4%) and ultra sound machines (4%) as presented in Figure I.

Antimicrobial susceptibility profile of *Escherichia coli* Isolates

Escherichia coli isolates showed high level of resistance to Trimetoprim- sulfamethoxazole 19(86.4%), Ampicillin 18(81.8%), Cefixime 17(77.3%), Nalidixic acid 17(77.3%), Ciprofloxacin 15(68.2%) and Cefpodoxime 14(63.6%). The isolates were highly susceptible 22(100.0%) to Carbapenems (Imipenem and Meropenem) as indicated in Table 1.

Multiple antibiotic resistance index (MARI) of *Escherichia coli* Isolates

About 86.4% of the *Escherichia coli* isolates were multi-drug resistant and showed MARI of ≥0.2 while 13.6% had a MARI of <0.2 as presented in Table 2.
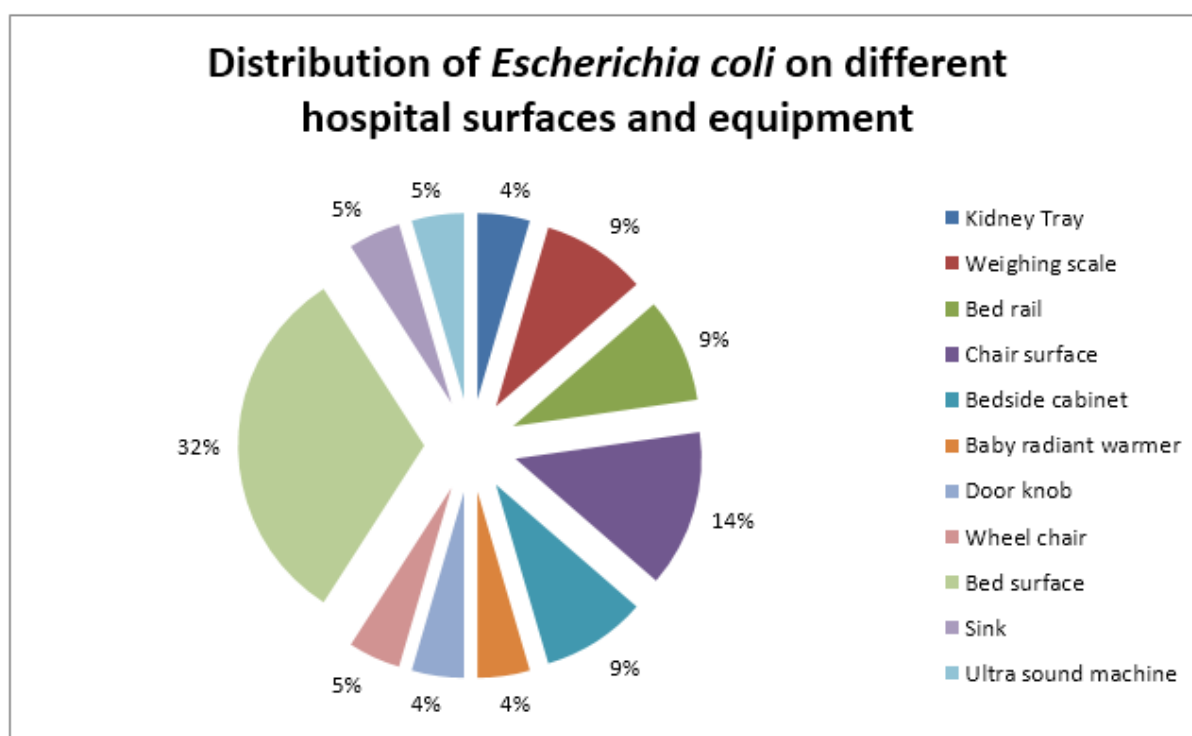

Figure I. Distribution and prevalence of *Escherichia coli* on different hospital surfaces and equipment

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Table 1. Antimicrobial susceptibility profile of *Escherichia coli*

Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Meropenem	22(100.0)	0(0.0)	0(0.0)
Imipenem	22(100.0)	0(0.0)	0(0.0)
Ciprofloxacin	6(27.3)	1(4.6)	15(68.2)
Trimetoprim-sulfametoxazole	3(13.6)	0(0.0)	19(86.4)
Fosfomycin	22(100.0)	0(0.0)	0(0.0)
Colistin	15(68.2)	7(31.8)	0(0.0)
Amoxicillin-clavulanic acid	4(18.2)	11(50.0)	7(31.8)
Gentamicin	17(77.3)	2(9.1)	3(13.6)

Cefpodoxime	5(22.7)	3(13.6)	14(63.6)
Cefixime	5(22.7)	0(0.0)	17(77.3)
Nalidixic acid	4(18.2)	1(4.6)	17(77.3)
Chloramphenicol	17(77.3)	3(13.6)	2(9.1)
Ampicillin	1(4.6)	3(13.6)	18(81.8)

Table 2. Resistance pattern of *Escherichia coli* isolates

Isolate code	Antibiotic resistant pattern	MARI	No of Antibiotics classes Resistant to	No of Antibiotics Resistant to	Resistance Category
EP-8	CPX, SXT, CPD, CFM, NA, PN	0.4	3	6	MDR
EP-1	CPX, SXT, AMC, CPD, CFM, NA, PN	0.5	3	7	MDR
E-11	SXT, AMC, CPD, CFM, NA, PN	0.4	3	6	MDR
EP-7	CPX, SXT, CPD, CFM, NA, PN	0.4	3	6	MDR
E-9	CPX, SXT, AMC, CPD, CFM, NA, PN	0.5	3	7	MDR
NT-8	CPX, SXT, CFM, CPD, NA, PN	0.4	3	6	MDR
US-4	CPX, SXT, CPD, CFM, NA, PN	0.4	3	6	MDR
E-6	CPX, SXT, CPD, CFM, PN	0.3	3	5	MDR
BW-1	CPX, SXT, AMC, CPD, CFM, CH, PN	0.5	4	7	MDR
E-5	CPX, SXT, CPD, CFM, NA, PN	0.4	3	6	MDR
BW-9	CPX, SXT, CPD, CFM, NA, PN	0.4	3	6	MDR
NT-1	CPX, SXT, CPD, CFM, NA, PN	0.4	3	6	MDR
EP-5	CPX, SXT, CFM, CPD, NA, PN	0.4	3	6	MDR
FS-11	CPX, CN, SXT, AMC, CFM, NA	0.4	4	6	MDR
FM-5	SXT, NA, PN	0.2	3	3	MDR
FM-7	CPX, SXT, CN, CFM, CPD, NA, PN	0.5	4	7	MDR
FM-6	--	0	0	0	Susceptible
S-4	AMC, CFM, NA, CH, PN	0.3	3	5	MDR
S-5	SXT, NA, PN	0.2	3	3	MDR
S-6	--	0	0	0	Susceptible
F-6	CPX, CN, SXT, AMC, CPD, CFM, NA, PN	0.6	4	8	MDR
W-4	SXT	0.1	1	1	Non multi drug resistant

MEM: Meropenem; IPM: Imipenem; CPX: Ciprofloxacin; NA: Nalidixic acid; CH: Chloramphenicol; PN: Ampicillin; AMC: Amoxicillin-clavulanic acid; SXT: Trimetoprim-sulfametoxazole; FOS: Fosfomycin; CT: Colistin; CPD: Cefpodoxime, CFM: Cefixime; CN: Gentamicin; MDR: Multi-drug resistant; --: No resistance

DISCUSSION

Microorganisms of human and environmental origin are ubiquitous and naturally contaminating hospital environments (Ebongue et al. 2018). MDR pathogens can live on inert hospital surfaces and medical equipment. *Enterobacteriaceae* are considered to be one of most common cause of nosocomial infections and outbreaks (Anibijuwon et al. 2018). In this study, 108(83.1%) out of 130 samples collected, were found to be positive for bacterial growth. The high prevalence is clearly an indication of improper cleaning and sterilization of hospital surfaces and equipment. This is consistent with the findings of Yusha'u et al. (2012) who reported a prevalence of 76% in hospital equipment in Kano, Nigeria but lower than the prevalence of 96% reported by El Ouali et al. (2016) in a hospital in Fez city, Morocco. On the other hand, it is higher than the prevalence of 59% reported by Otokunefor et al. (2018). This variation could be attributed to the frequency of decontamination of equipment and surfaces, types of disinfectant used, nature of medical equipment and surfaces (Abrar-Nasser et al. 2016).

The predominant Gram-negative bacteria isolated were *K. pneumoniae, E. coli*, *P. aeruginosa*, *Salmonella*, *Enterobacter* and *Citrobacter* species. This is consistent with

the findings of other studies which reported members of the family *Enterobacteriaceae* as the most predominant Gram-negative bacteria isolated from hospital environments (Otokunefor wt al. 2018, Zubair et al. 2018, Gruszecka et al. 2019). The presence of *Enterobacteriaceae* in hospital environments is a strong indicator of faecal contamination and poor hand hygiene among healthcare staffs and patients (Sserwadda et al. 2018).

The prevalence rate of *Escherichia coli* (17.9%) from this study is higher than the prevalence of 7.46% reported by Zubair et al. (2018) but lower than 28.8% reported by Otokunefor et al. (2018). Discrepancies in results could possibly be due to differences in patient colonization load, sample size, study area and hospital's cleaning/disinfection protocols (Freeman et al. 2014).

Almost all equipment and surfaces had high prevalence of Gram-negative bacteria. Similar finding was also observed in Egypt by Abdallah et al. (2018) where they reported that various hospital surfaces and equipment were heavily contaminated with Gram-negative bacteria. According to this study, surfaces of beds, chair, bedside drawers, door knobs and bed rails had the highest prevalence of microorganism. This is similar to the study of Oumokhtar et al. (2017) that reported bedrails, bedsides tables, door knobs and electricity buttons as the most contaminated surfaces. These surfaces are high contact surfaces, the pathogens found may have been shed by infected/colonized patients, hospital employees, or visitors, such surfaces may pose a risk of infection to other patients, caregivers and visitors. The high level of contamination could be attributed to the inadequate cleaning, improper hygiene and lack of strict infection control measures in the hospital.

In this study, all the *E. coli* isolates were susceptible (100.0%) to Carbapenems (Imipenem and Meropenem), the result being consistent with similar studies by Al-Salamy (2012) and Daoud et al. (2020) that reported 100% susceptibility rate to Carbapenems.

However, it is contrary to the report of Malik et al. (2021) that reported susceptibility rate of 75.3% and 62.4% to Imipenem and Meropenem respectively. The high susceptibility rates to carbapenems observed in this study may be due to limited use of the antibiotics in the study area. Carbapenems are not readily available as they are reserved for life threatening Gram-negative bacterial infections as such they are not abused. Also, they are intravenously administered and are not frequently prescribed.

Significant susceptibility rate to Fosfomycin (100%) and Colistin (68.2%) was also observed. Similarly, Malik et al. (2021) recorded significant susceptibility rate of 100% and 96.2% against Fosfomycin and Colistin respectively, Seo et al. (2014) reported 100% susceptibility rate to Fosfomycin while Oladipo et al. (2018) recorded high susceptibility rate of 96% against Colistin. Fosfomycin and Colistin are old antimicrobial agents that have re-surfaced as a drug of choice against MDR Gram-negative bacilli (Delgado-Valverde et al. 2013, Giske 2015, Seok et al. 2020). Fosfomycin structure is unrelated to any other antimicrobial agent, therefore chances of cross-resistance are low (Sastry and Doi 2016). While the high susceptibility to Colistin could be attributed to its low usage as they are rarely prescribed by clinicians or subjected to abuse due to its nephrotoxicity and other side effects.

Antibiotic susceptibility pattern of *E. coli* isolates to commonly prescribed antibiotics in the study area revealed high rates of resistance to Nalidixic acid, Trimethoprim-sulfamethoxazole, Cefixime, Cefpodoxime, Ciprofloxacin and Ampicillin. These antibiotics are traditional first-line therapeutic drug options for treating Gram-negative bacterial infections (Delgado-Valverde et al. 2013). Several studies have reported high rates of resistance to these drugs worldwide (Sastry and Doi 2016, Liu et al. 2017, Otokunefor et al. 2018). This high level of resistance to first line drugs in nonclinical isolates poses a major public health concern.

The isolates also showed significant resistance rate to Fluroquinolones (Ciprofloxacin (77.3%) and Nalidixic acid (77.3%)). The resistance to Ciprofloxacin is comparable to the study of Malik et al. (2021) that reported resistance rate of 73.6% to Ciprofloxacin. However lower resistance rate of 24.5% and 17.8% to Ciprofloxacin was reported by Ya'aba et al. (2020) and Najim et al. (2019). Resistance to Nalidixic acid is comparable to results of Sharma et al. (2013) and Wagle et al. (2018) who reported resistance rates of 78.9% and 71.5% respectively. The high level of resistance possibly suggests the injudicious use of the drugs, due to their cheap costs and availability or frequent prescription by clinicians against bacterial infections in the study area (2020).

Escherichia coli isolates had a moderate resistance rate of 31.8% to Amoxicillin/clavulanic acid. This is in contrast to various other studies where higher resistance rate was recorded. Engda et al. (2018) and Najim et al. (2019) reported that *E. coli* isolates were totally resistant (100%) to amoxicillin-clavulanic acid while Ya'aba et al. (2020) and Malik et al. (2021) reported resistance level of 61.22% and 81.1% to amoxicillin-clavulanic acid respectively. Lower resistance rate to Amoxicillin/clavulanic acid observed in this study could be due to the effectiveness of the drug combinations. The clavulanic acid targets β-lactamase enzymes that are responsible for resistance against β-lactams and other antibiotics, aiding the drug overcome resistance (Odongo et al. 2020).

From this study the *E. coli* isolates were highly susceptible to Chloramphenicol (77.3%) and Gentamycin (77.3%). However, moderate resistance to Chloramphenicol (36.5%) and Gentamycin (26%) was reported by Najim et al. (2019). Susceptibility to Gentamicin could be attributed its nature of administration, as Gentamicin are administered intravenously as such they are not easily abused.

Multiple antibiotic resistance index reveals the spread of bacterial resistance in a given population (Krumperman 1983, Joseph et al. 2017). MARI value greater than or equal to 0.2 indicate high-risk source of contamination where antibiotics are often used. About 86.4% of the *E. coli* isolates obtained in this study were multi-drug resistant having MARI above 0.2 which is an indication that a large proportion of the isolates have been previously exposed to several antibiotics. This could be explained by high and uncontrolled use of antibiotics in the study area, these antibiotics are easily procured over the counter, without a proper investigation and prescription (Joseph et al. 2017). Hence, antimicrobial susceptibility testing is imperative in selecting therapeutic options. Similarly, several studies have reported the occurrence of MDR *E. coli* on inanimate hospital surfaces, Ibrahim et al. (2012) and Shams et al. (2014) reported the prevalence of 92.2% and 83% MDR *E. coli* respectively.

CONCLUSION

From this study it is worth noting that carbapenems remain an effective drug for the treatment of multidrug resistant *Escherichia coli* as no carbapenem resistance was recorded. However, the high prevalence (86.4%) of multidrug-resistance observed among *Escherichia coli* isolates from nonclinical sources is quiet alarming; as such the role of infection prevention and control and antibiotic stewardship programs cannot be overemphasized.

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