Vol. 13(2), pp. 22-29, July-December 2021
DOI: 10.5897/JBR2021.0336
Article Number: 13CC9BA68340
ISSN 2006-9871

http://www.academicjournals.org/JBR

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African Journal of Bacteriology Research

Full Length Research Paper

High prevalence of multidrug resistant enterobacteriaceae isolated from wastewater and soil in Jos Metropolis, Plateau State, Nigeria

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Received 11 August, 2021; Accepted 14 October, 2021

The widespread emergence of antibiotic resistance, particularly multiple antibiotic resistance (MAR) among bacterial species has become one of the most serious challenges in environmental protection. Environmental bacteria are a reservoir of antibiotic resistance genes (ARGs) and a potential source of novel resistance genes in environmental organisms. In the current study, we investigated the high prevalence of multidrug-resistant Enterobacteriaceae isolated from wastewater and soil in Jos metropolis, Plateau State. A total of 150 wastewater and soil samples were obtained from six different locations within Jos metropolis. Serial dilution was carried out for each sample and inoculated using the spread plate method on Eosin Methylene Blue Agar and MacConkey agar respectively. Total viable count for the environmental isolates was carried out and the isolates were identified macroscopically, microscopically, and biochemically. The antibiotic susceptibility profile of the individual isolates was determined using the Kirby-Bauer disk diffusion method and multiple antibiotics resistance index of the isolates determined. The phenotypic and biochemical identification showed that *Escherichia coli* has the highest number of occurrences (70%), seconded by *Klebsiella* spp (20%), and lastly *Proteus* spp. (10%). It was shown that all the isolates were resistant to Ceftazidime (100%), followed by Ampicillin and Augmentin having (95%) each with Cefuroxime (90%) while Gentamicin has the least resistance with (5%), followed by Ciprofloxacin (15%), Ofloxacin (20%) and Nitrofurantoin (25%). Calculations of MAR for individual bacterial species showed that *Klebsiella* spp has the highest MAR index of 0.63, followed by *E. coli* and *Proteus* spp having MAR index of 0.57, and 0.31 respectively. The study suggests proper management for wastes disposal, the prohibition of unregulated use of antibiotics, and regular monitoring for antibiotics resistance in native bacteria of the environment.

Key words: Antibiotics resistance, public health, MAR Index, environmental waste, enterobacteriaceae.

INTRODUCTION

The appearance of antibiotic resistance poses serious health challenges, economic and social problems because infections caused by antibiotic-resistant bacteria often fail to respond to standard treatments, thereby reducing the possibilities of effective treatment and increasing the risk of morbidity and mortality in serious diseases (Carlet et al., 2011). In the past decades, antibiotic resistance has put increasing pressure globally on human healthcare and is estimated to account for 700,000 deaths every year and the environment has repeatedly been identified as a source for resistant genes to pathogens (Bengtsson-Palme and Larsson, 2016).

One of the most serious challenges in clinical therapy is the widespread emergence of antibiotic resistance, particularly multidrug resistance (MDR), among bacterial pathogens (Levy and Marshall, 2004; World Health Organization, 2000). Acquisition of resistance genes through horizontal transfer is ubiquitous in clinical pathogens (Levy and Marshall, 2004). Environmental bacteria are a reservoir of antibiotic resistance genes and a potential source of novel resistance genes in clinical pathogens (Dantas et al., 2008). Horizontal transfer of genes between bacterial strains could be facilitated by mobile genetic elements, such as plasmids, transposons, bacteriophages, integrons, insertion elements (IS), and genomic islands (Li et al., 2010).

Antibiotic residues contained in the environment are alarming because antibiotics might contribute to the appearance of resistant bacteria and could exert selective pressure. The major source of antibiotics in aquatic environments was once considered to be from hospital sewage, followed by municipal, agricultural, and aquacultural wastewater, which has also been shown to be important sources of these compounds and resistant bacteria (Segura et al., 2009). Treated antibiotic-produced-wastewater contains higher concentrations of antibiotic residues than other aquatic environments, thus can serve as an important reservoir of resistant bacteria and genes (Li et al., 2009, 2008a, b; Łukasz et al., 2016).

Enterobacteriaceae belongs to a large family of Gram-negative bacteria which are part of the normal gut flora present in the human intestinal tract. Some species can cause diarrhoea and are the common cause of urinary tract infections (UTIs) (Ngene et al., 2020). These pathogens can cause life-threatening complications when they spread to the bloodstream. They include a number of pathogens such as *Citrobacter*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Escherichia coli*, *Shigella*, *Proteus*, *Serratia* and other species causing healthcare-associated infections (HAIs). Like all bacteria, enterobacteriaceae can develop resistance to antibiotics which includes the carbapenem group of antibiotics [carbapenem-resistant Enterobacteriaceae (CRE) and carbapenemase-producing Enterobacteriaceae (CPE)] (Yuan et al., 2021).

Among the pathogens disseminated in the environment, enteric pathogens such as enterotoxigenic *E. coli*, *Shigella* spp., *Salmonella* spp., and so forth are the ones most frequently encountered that are responsible for a variety of diseases like diarrhea, dysentery, and enteric fever (Poonia et al., 2014). To further compound this problem, enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics (Chitanand et al., 2010). The environment is the source of bacteria with the highest level of resistance and surface water is the main reservoir of antibiotics and antibiotic-resistant bacteria in the environment. In the past two decades, the rise in antibiotic resistance has been reported and remains a global problem (Sharma and Rai, 2012; Verma et al., 2011). In the current study, we investigated the high prevalence of multidrug-resistant Enterobacteriaceae isolated from wastewater and soil in Jos Metropolis, Plateau State.

MATERIALS AND METHODS

Collection of samples

A total of 150 wastewater and soil samples (25 samples for each location) were obtained from 6 different locations (Student Village Hostels 1 and 2, Old Jos University Teaching Hospital, JUTH 1 and 2, and Angwa Rukuba 1 and 2), within Jos North Metropolis, Plateau State, Nigeria. Latitude and Longitudes of their various locations were noted. A 50-ml sterile vial with cover tops were used for this purpose. The containers were immediately disinfected with 70% ethanol at the point of collection, labeled, and kept in a super cool flask for transportation to Africa Center of Excellence in Phytomedicine Research and Development, ACEPRD, University of Jos, Microbiology Laboratory for analysis.

Laboratory Isolation

According to the modified method cited by Ibrahim and Hameed (2015), a total of 10 ml of each sample (after mixing the wastewater and sand and allowed to decant in a conical flask) was diluted in 90-ml of sterile 0.9% NaCl normal saline and homogenized. Thereafter, 100 µl of the fourth and fifth diluent of the samples were inoculated on Eosin Methylene Blue Agar (EMB) agar plates for the isolation of enteric bacteria and MacConkey agar plates are used for both lactose and non-lactose fermenters bacterial isolates using the spread plate method. All the bacteria plates were incubated at 37˚C for 24 h.

Total viable count for environmental isolates

The total viable count was determined using the spread plate

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technique on nutrient agar and counting the colonies developed after incubation at 37˚C for 24 h (Harley and Prescott, 1996).

Identification of isolates

Gram-negative bacteria were isolated on their respective selective and differential media and were identified based on culture characteristics, including Gram stain, motility, and biochemical tests, MacConkey agar, EMB, IMViC, urea, and triple sugar iron (TSI) test (Forbes et al., 2016).

Preservation of isolates

The isolates were subcultured on nutrient agar, incubated at 37°C for 24 h. A single colony was inoculated into a sterile nutrient broth, incubated in a shaker incubator (ZHP-100) at 180 rpm for 24 h at 37°C. The isolates were also incubated on a nutrient agar slant at 37°C for 24 h. They were all stored at 4°C in a refrigerator.

Antibiotics susceptibility profile

The antibiotic susceptibility profile of the Gram-negative isolates was determined using the standard Kirby-Bauer disk diffusion method (Bauer, 1966). These antibiotics with their respective disk concentrations are as follows: Ceftazidime (10 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (10 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg), Ofloxacin (10 µg), and Augmentin (30 µg) (Bhattacharya et al., 2012). Bacterial culture suspension equivalents of 0.5 tube McFarland turbidity standards were spread on Muller-Hinton agar plates using sterile swabs and incubated aerobically at 37˚C for 24 h; then, the diameters of the zone of inhibition surrounding the antibiotic disks was measured. The results are expressed as susceptible or resistant according to the criteria recommended by (CLSI, 2012).

Multiple antibiotics resistance (MAR) index

This MAR index was suggested by Krumperman (1983), according to the following formula in Equation 1 and 2.

$$MAR = a/b \quad (1)$$

Where; a = the number of antibiotics to which the isolate was resistant; b = the number of antibiotics to which the isolate was exposed.

$$MAR = a/(b \times c) \quad (2)$$

Where; a = the aggregate antibiotic resistance score of all isolates from the sample; b = the number of antibiotics; c = the number of isolates from the sample. Also, values of MAR greater than 0.25 pose a high-risk source for contamination.

Statistical analysis

All the experiments were repeated three times and the mean values of the three replicates obtained. The statistical analysis was carried out using SPSS software version 21. Data were analyzed to determine the analysis of variance (ANOVA) using Duncan's multiple range test (JMP v.12 software; SAS Inst., Cary, NC, USA). Significant differences between results were estimated at a P-value less than 0.05 ($P < 0.05$).

RESULTS

In the present study, the samples were collected in six different locations within the Jos metropolis. Angwa Rukuba_1 having the highest mean value of total viable bacteria count ($4.9x10^7$ CFU/ml), followed by Old JUTH_1 ($4.4x10^7$ CFU/ml), Student Village Hostel_2 ($4.35x10^7$ CFU/ml), Old JUTH_2 ($4.25x10^7$ CFU/ml), Angwa Rukuba_2 ($3.7x10^7$ CFU/ml) and Student Village Hostel_1 having the least viable count (3.3x107 CFU/ml) as shown in Figure 1. As illustrated in Figure 2, the phenotypic and biochemical identification showed that *E. coli* has the highest number of occurrences (70%), seconded by *Klebsiella* spp (20%) and lastly *Proteus* spp. (10%). Table 1 showed that Old JUTH 1 has the highest number of positive Enterobacteriaceae (28%), followed by Student Village Hostel_2 (22%). Student Village Hostel_1 and Old JUTH 2 have the same number of Enterobacteriaceae (20%) each while Angwa Rukuba 1 and 2 had the least 7 and 3% respectively. Figure 3 showed that Old JUTH_1 (33%) has the highest distribution of *E. coli* to sample location, followed by Student Village Hostel_2 (29%), Student Village Hostel_1 (24%), and Old JUTH_2 having the least (14%) while Angwa Rukuba 1 and 2 recorded none. For *Klebsiella* spp., Old JUTH_2 had the highest distribution number of (50%), followed by Old JUTH_1 (25%), Student Village Hostel_1 (17%), and the least Student Village Hostel_2 with (8%) and was absent in Angwa Rukuba 1 and 2. Angwa Rukuba_1 has the highest distribution number of *Proteus* spp. (50%) followed by Old JUTH_2 (33%) and the least Angwa Rukuba_2 (17%). As demonstrated in Figure 4**,** it was shown that all the isolates were resistant to Ceftazidime (100%), followed by Ampicillin and Augmentin having (95%) each with Cefuroxime having (90%). Gentamicin had the least resistance with (5%), followed by Ciprofloxacin, Ofloxacin, and Nitrofurantoin having 15, 20, and 25% respectively. Susceptibility of bacteria to different antibiotics (8 items) showed multiple antibiotics resistance (MAR) for the majority of isolates. As indicated in Table 2 and illustrated by Figure 5**,** calculations of MAR for individual bacterial species showed that *Klebsiella* spp has the highest MAR index of 0.63, followed by *E. coli* and *Proteus* spp having MAR index of 0.57, and 0.31 respectively.

DISCUSSION

There is a need for periodic surveillance of laboratory activities to monitor antibiotic resistance and its spread in our environment. This will help in gathering information needed in making policies that matter on antimicrobial resistance (World Health Organization, 2013). It is worth mentioning that, all the study samples exceeded the international standard limits (5000 CFU 100 ml^{-1}) (Collivignarelli et al., 2017; Tebbutt, 1998) and could be a

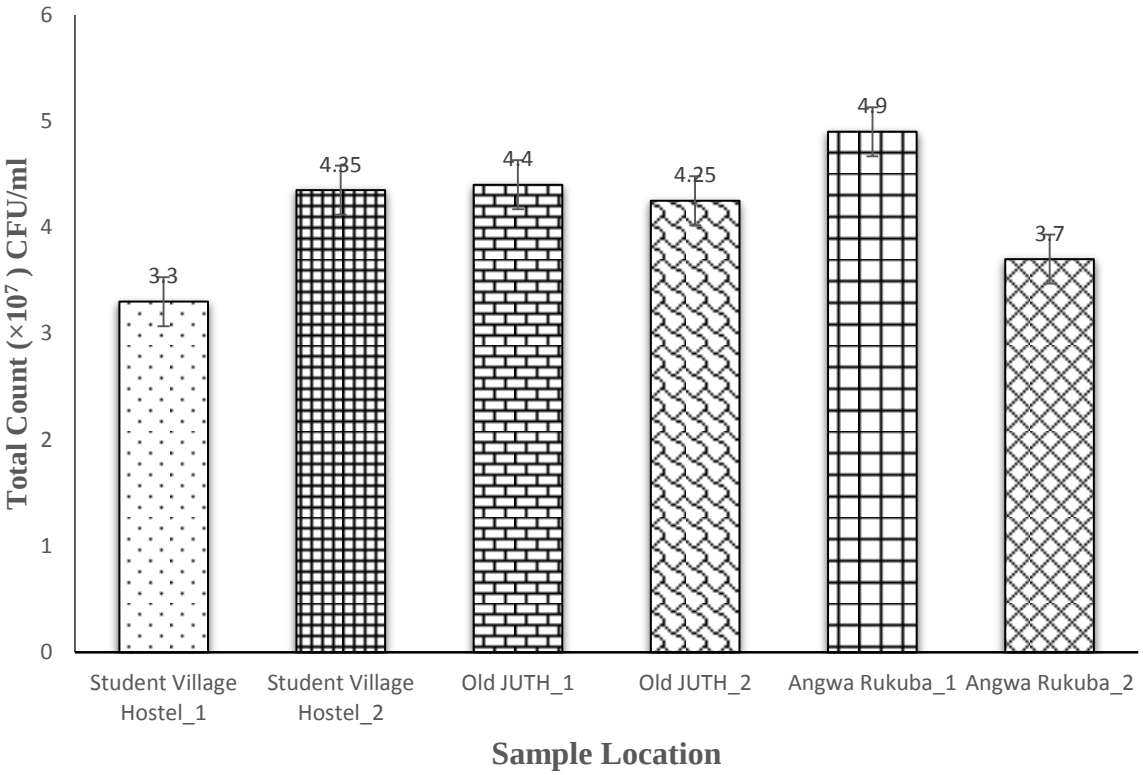

Figure 1. Total viable count of the isolates with respect to sample locations.
CFU = Colony Forming Unit.

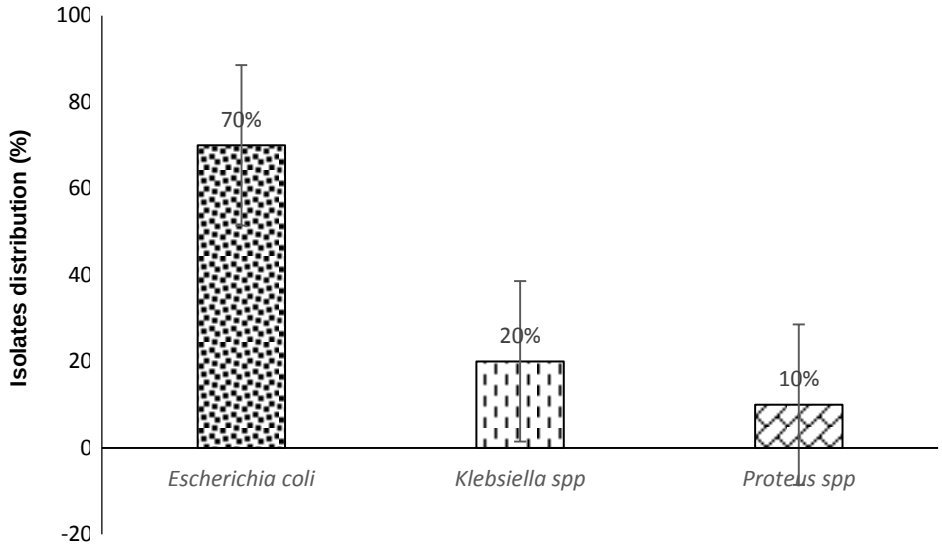

Figure 2. Percentage distribution of the isolates.

result of fecal contamination as reported by Azzam et al. (2017). Some restricted limits have been reported by Efstratiou et al. (2009) and Cabelli (1978), a maximum total coliforms count of 1000 CFU 100 ml^{-1}, particularly in surface water that would be used as a drinking water supply. Bacteria generally identified in this study were reported to be potential human pathogens of a public health concern as described by Sneath (1986),

Table 1. Distribution of samples with positive Enterobacteriaceae.

Sample location	Latitude	Longitude	Enterobacteriaceae Positive Isolates	%
Student Village Hostel_1	9.96565	8.87116	12	20
Student Village Hostel_2	9.96571	8.87128	13	22
Old JUTH_1	9.9187	8.890219	17	28
Old JUTH_2	9.91832	8.890219	12	20
Angwa Rukuba_1	9.93922	8.909185	4	7
Angwa Rukuba_2	9.934	8.908757	2	3

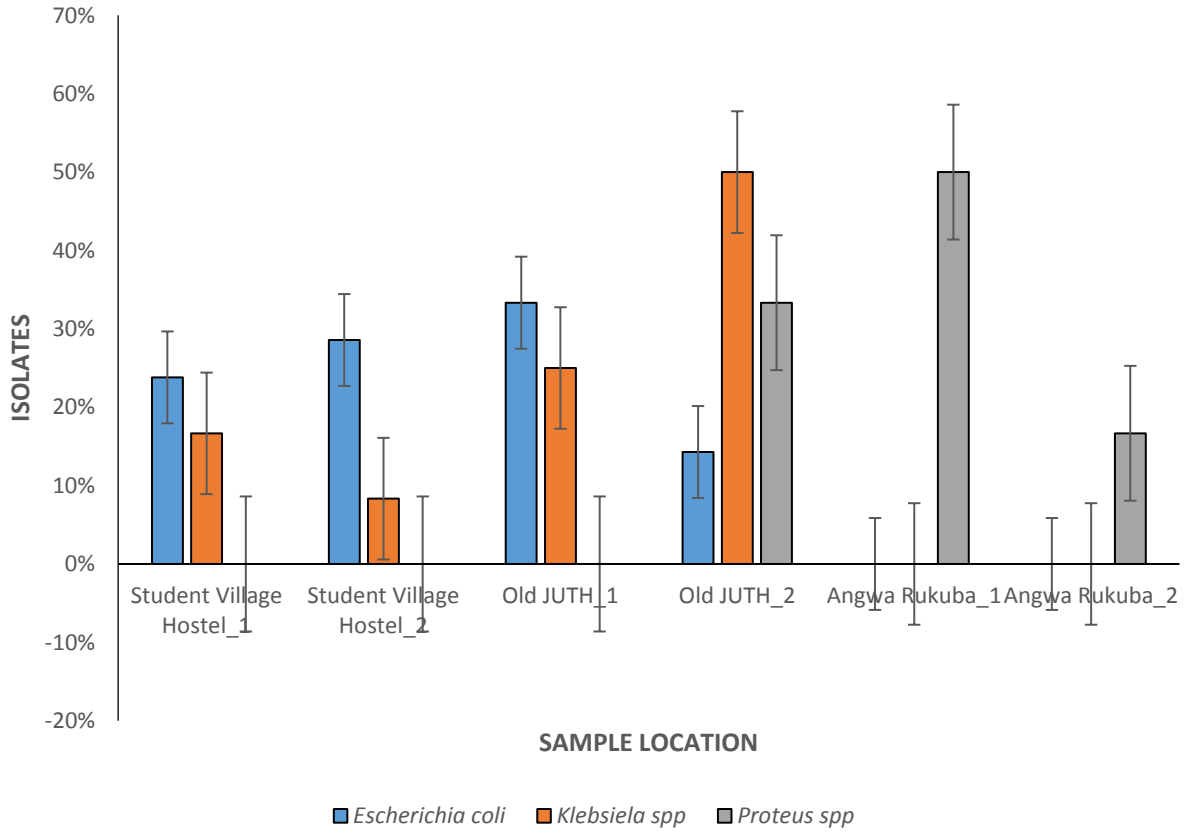

Figure 3. Distribution of enterobacteriaceae in relation to samples.

Cheesbrough (2006), and World Health Organization (2011). The most widespread bacteria obtained was *E. coli*, followed by *Klebsiella* spp and *Proteus* spp which indicates that the samples were subjected mainly to sewage pollution as reported by Ibrahim and Hameed (2015) which recorded *E. coli* to be the most common lactose-fermenting bacterial isolates from the environmental specimens, comprising 54.6% of the total samples, followed by *Klebsiella pneumonia* with 32.8% of samples. The high incidence of *E. coli* correlated with fecal coliforms supports such findings (Edberg et al., 2000; Azzam et al., 2017). The environmental isolated Enterobacteriaceae showed a high level of resistance to Ceftazidime, Cefuroxime, Ampicillin, and Augmentin while susceptible to Gentamicin, Ciprofloxacin, Ofloxacin, and Augmentin which supports the research findings of Ibrahim and Hameed (2015) and Azzam et al. (2017). The high susceptibility profile of the bacterial isolates to the named antibiotics could be related to the less frequent use of these drugs for therapeutic purposes, therefore reducing the chance for resistance as reported by Ibrahim and Hameed (2015). The genetic background of resistance mechanisms is diverse because they are present on chromosomes, plasmids, integrons, and transposons (Brooks et al., 2010). High levels of genetic flux between Gram-negative Enterobacteriaceae have

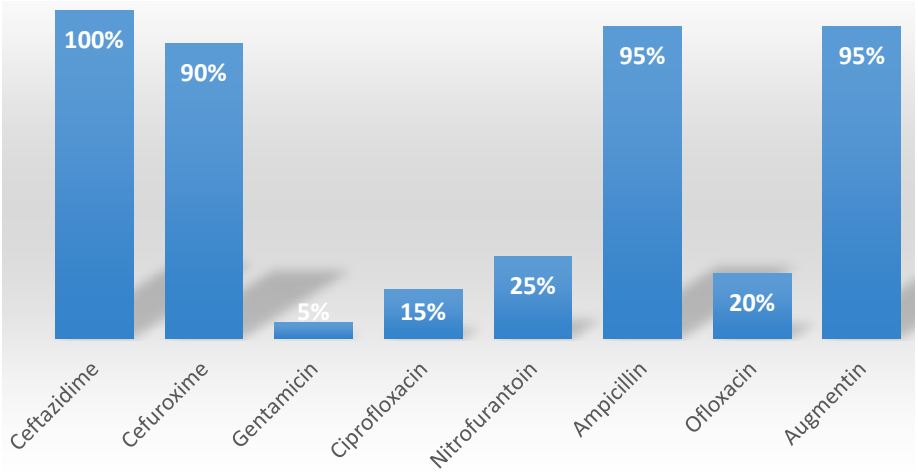

Figure 4. Percentage of antimicrobial resistance of the isolates.

Table 2. Multiple antibiotic resistance (MAR) index of the individual isolates.

S/N	Isolate	MAR Range
1	*Escherichia coli*	0.38 - 0.75
2	*Klebsiella* spp	0.5 - 0.75
3	*Proteus* spp	0.13 - 0.5

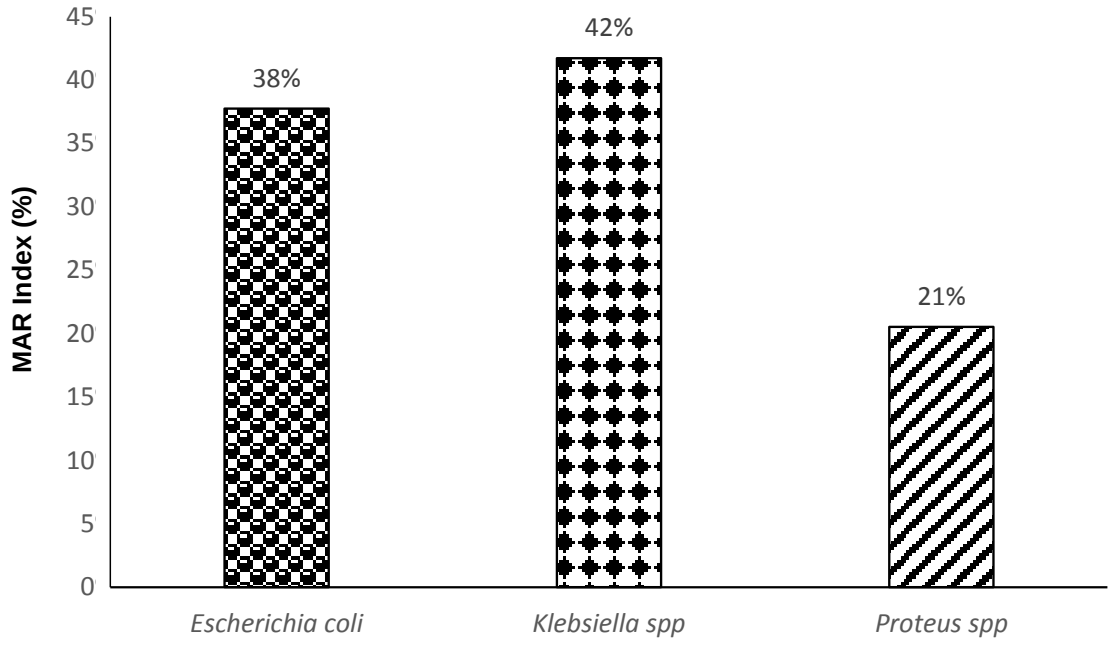

Figure 5. Multiple antibiotic resistance (MAR) index of the total individual isolates.

been suggested by many studies (Stecher et al., 2012) that may favor exchange of plasmid between Enterobacteriaceae members. Study area with MAR index values above 0.25 as calculated by Krumperman

(1983) and Hinton et al. (1985) was classified as potential health risk environments. In both drainage and river water, Munir et al. (2011) reported in Michigan (USA) the incidence of MAR bacteria and was also reported in the work of Azzam et al. (2017) in Egypt. This shows that the issue of multiple antibiotics resistant bacteria in the environment is of global concern since it is of international, rather than national problem (Knapp et al., 2012; Lupan et al., 2017; Okeke and Edelman, 2001).

Conclusion

The study shows that the Enterobacteriaceae isolated were *E. coli*, *Klebsiella* spp, and *Proteus* spp, which demonstrated multidrug resistance for Ceftazidime, Cefuroxime, Ampicillin, and Augmentin. Factors that may be associated with the transmission of resistant strains in the environment include poor hygiene and antibiotic abuse. More bacterial isolates from different sources in conjunction with genetic analysis are to be collected for future studies.

This situation suggests regular monitoring for antibiotics resistance in native bacteria of the environment, the prohibition of unregulated use of antibiotics, and proper management for wastes disposal.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Director, Africa Center of Excellence in Phytomedicine Research and Development University of Jos, Plateau State, Nigeria for funding this research. World Bank also sponsored and is appreciated.

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