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Full Length Research Paper

Antimicrobial susceptibility of *Pseudomonas aeruginosa* strains in Bamako, Mali

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***Pseudomonas aeruginosa* is generally susceptible to antibiotics of the families of Beta-lactam, aminoglycosides and quinolones. The aim of this study was to evaluate the antimicrobial susceptibility of *P. aeruginosa* strains in Bamako, Mali. *P. aeruginosa* strains were isolated on Drigalski agar. Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar. Among 317 non repetitive strains recovered from 2010 to 2019, there were 246 (77.6%) hospital strains and 71 (22.4%) extra-hospital strains. Colistin (100%), imipenem (98.4%), ceftazidime (89.3%), amikacin (85.2%) and piperacillin (72.3%) were the most active antibiotics against our *P. aeruginosa* strains. Of the strains 11 (3.5%) were multi-drug resistant (MDR) and 5 (1.6%) were extensively drug-resistant (XDR). The extra-hospital *P. aeruginosa* strains were more susceptible to aztreonam (91.5% vs 60.6%; P = 0.0000018), piperacillin (84.5% vs 68.7%; P = 0.013), gentamycin (84.5% vs 62.2%; P = 0.00071), netilmicin (56% vs 32.5%; P = 0.0045) and ciprofloxacin (79% vs 65.4%; P = 0.0455) than the hospital strains. Colistin, imipenem, ceftazidim, amikacin and piperacillin have a high-level activity against *P. aeruginosa* in Bamako.**

Key words: *Pseudomonas aeruginosa*, antimicrobial susceptibility, Bamako, Mali.

INTRODUCTION

Pseudomonas aeruginosa is a strictly non-fermenting aerobic Gram-negative bacillus that belongs to the Pseudomonadaceae family. *P. aeruginosa* is involved in various infections: urinary tract infections, abscesses, bacteremia, pulmonary infections, bone and joint infections, eye infections, infections of the otolaryngological sphere, meningeal infections, skin infections, enteritis, and endocarditis Avril et al., 2000; Wu et al., 2015). *P. aeruginosa* which is catalase and oxidase-positive, generally produces two pigments: pyocyanin (blue-green) and pyoverdine (yellow-green and fluorescent), Avril et al., (2000). *P. aeroginosa* is susceptible to carboxypenicillins, ureidopenicillins (mezlocillin, piperacillin), some 3rd generation cephalosporins (cefsulodine, cefoperazone, ceftazidime, cefepime, and cefpirome), carbapenems (imipenem),

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monobactams (aztreonam), certain aminoglycosides (gentamicin, netilmicin, and amikacin), fluoroquinolones, and colistin (Avril et al., 2000). *P. aeruginosa* has a natural resistance to aminopenicillins, 1st and 2nd generation cephalosporins, cefotaxime, cotrimoxazole, tetracyclines, chloramphenicol and nalidixic acid (Avril et al., 2000).

In addition to this natural resistance, there are acquired resistances which constitute the whole problem with this bacterium, particularly in hospitals and increasingly in community settings. These acquired resistances could be found in β-lactam (cephalosporin), aminoglycosides, fluoroquinolones (Avril et al., 2000; Kouamé et al., 2016; Weldaghen et al., 2003).

In Mali, there are very limited data on the susceptibility of *P. aeruginosa* to antibiotics, and given the increasing antibiotic resistance worldwide, the aim of this study was to evaluate the susceptibility of *P. aeruginosa* to antibiotics in Bamako.

MATERIALS AND METHODS

Study site and setting

This was a retrospective study carried out in the Medical Biology and Hospital Hygiene Laboratory of the University Teaching Hospital of the Point G, Bamako, Mali from January 1st, 2010 to December 31st, 2019. The University Teaching Hospital of the Point G is the third-pyramidal reference in Mali, and has 522 beds divided between the surgical, intensive care and medical departments.

Bacterial strains

While patients admitted to the different departments of the University Teaching Hospital of the Point G, were hospitalized patients (in-patients), those who were coming to the hospital, for medical consultation, laboratory tests and/or X-Ray were not hospitalized and were called out-patients.

The hospital strains of *P. aeruginosa* were isolated from samples from hospitalized patients at the Point G University Teaching Hospital.

The extra-hospital strains of *P. aeruginosa* (community strains) were isolated from samples from out-patients.

The 317 non-repetitive strains isolated from samples collected from in-and out-patients visiting the University Teaching Hospital of the Point G. The strain isolated was done on Drigalski agar (Bio-Rad, France) at 37°C.

The identification of the strains was made either on the production of pyocyanin and pyoverdine on respectively King A and King B (Bio-Rad, France) media, on oxidase (Bio-Rad, France) and catalase (bioMérieux, France) positive reactions, and by the API 20 NE systems (bioMérieux, France).

Susceptibility to antibiotics test

The antimicrobial susceptibility testing was carried out on Mueller-Hinton agar (Bio-Rad, France) by the disc method (Agar diffusion method). The strains of *P. aeruginosa* were classified as "susceptible", "intermediate" or "resistant" according to the recommendations of the Antibiotic Committee of the French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing in 2015 (CA-SFM/EUCAST) (Jehl et al., 2015). The strains of *P. aeruginosa* classified as <<intermediate>> to the antibiotics tested were considered resistant.

Laboratory procedure

The antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Bio-Rad, France) poured into a Petri dish. A colony isolated from an 18-24 h culture of *P. aeruginosa* was suspended in 5 ml of sterile saline solution which was calibrated to 0.5 MacFarland. Two drops of this suspension are then added in 10 ml of sterile distilled water. This second suspension is poured over the entire surface of the Mueller-Hinton agar poured into a Petri dish. The excess is poured into bleach. The seeded agar is left to dry for 15 min at 37°C inside the incubator. Please kindly note that the incubator is not used to dry seeded agar plate. Instead, the plates are allowed to stand on the bench for a while, before the antibiotic discs are introduced. After the seeded agar has dried, the blotting paper discs impregnated with the antibiotics to be tested are placed on the surface of the agar using a disc dispenser according to manufacturer's instructions (Bio-Rad, France). After a first diffusion of the antibiotics in 30 min at room temperature, the Petri dish is incubated at 37°C for 18 to 24 h, in the inverted position (cover down). The reading is performed in measuring the diameter of inhibition of each antibiotic disc using a caliper in contact of growth.

Antibacterial agents tested

The antibiotics tested were ticarcillin (75 µg), piperacillin (75 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), gentamicin (15 µg), tobramycin (10 µg), netilmicin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), and colistin (50 µg) (Bio-Rad, France).

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes

The strains of *P. aeruginosa* intermediate or resistant to at least one molecule in three groups of antibiotics active against *P. aeruginosa*: (1) β-lactams except imipenem (ticarcillin, piperacillin, ceftazidime, and aztreonam), (2) imipenem, (3) aminoglycosides, and (4) ciprofloxacin were considered to be MDRs. The strains of *P. aeruginosa* intermediate or resistant to at least one molecule in each group of antibiotics have been considered as XDR (Barbier and Wolff, 2010; Magiorakos et al., 2012; Horcajada et al., 2019).

Ethics statement

The clinical specimens included in this manuscript were collected under public health surveillance of antimicrobial testing, and not as human subject research. Thus, submission to institutional review boards was not applicable. Participants were explained, and they consented to use the results. In addition, permission was received from Hospital Director for this manuscript.

Statistical analysis of data

The samples were collected under public health surveillance of antimicrobial resistance, and thus an estimated sample size was not previously determined. The data were entered and analyzed using Epi Info software 7.1 version. For the comparison of the results, we used the test of χ^2 with a significance level $P \leq 0.05$.

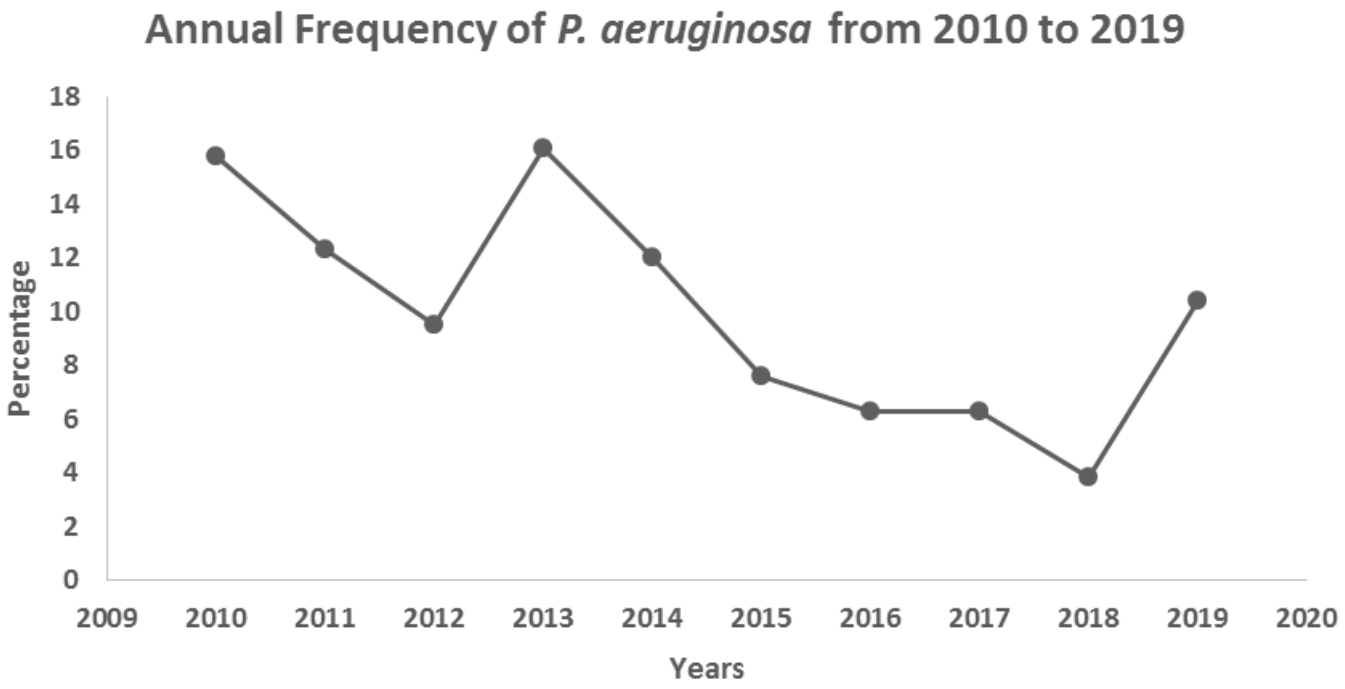

Figure 1. Distribution of the frequency of *P. aeruginosa* in the Point-G University Teaching Hospital between 2010 and 2019.

RESULTS

A total of 317 non-repetitive strains of *P. aeruginosa* were identified from 317 persons between 2010 and 2019. The mean age of patients was 48.77±18.5 years old, and the sex ratio (male/female) was 1:4 ratio.

The annual frequency of strains during the ten-year period is presented in Figure 1. Among these strains, 246 (77.6%) were of hospital and 71 (22.4%) of extra-hospital origin. The hospital strains were isolated in the wards of medicine (n = 185), surgery (n = 49) and intensive care unit (n = 12).

The distribution of *P. aeruginosa* strains according to the samples is shown in Table 1. The strains of *P. aeruginosa* have been isolated primarily from urine 143 (45.1%), pus 81 (25.6%), vaginal swabs 41 (12.9%), and/or sputum 26 (8.2%).

The antibiotic susceptibility of *P. aeruginosa* is reported in Figure 2. Thus, colistin, imipenem, ceftazidime, amikacin and piperacillin were the most active antibiotics against *P. aeruginosa*. Of the 317 strains 11 (3.5%) were MDR and 5 were XDR (1.6%). More specifically, 7 (2.8%) MDR-strains of *P. aeruginosa* were isolated in in-patients, and 4 (5.6%) in the out-patients setting, while 3 (1.2%) XDR-strains were isolated in in-patients, and 2 (2.8%) in the out-patients setting.

The susceptibility of antibiotics to *P. aeruginosa* strains isolated either from in- or out-patients is reported in Table 2. The strains isolated from out-patients were statistically more susceptible to aztreonam ($P < 0.0000$), piperacillin ($P = 0.0130$), gentamicin ($P=0.007$), netilmicin ($P=0.0045$) and ciprofloxacin ($P=0.0455$) than in-patients' strains.

DISCUSSION

This study was carried out to evaluate the antimicrobial susceptibility of different *P. aeruginosa* strains isolated in our laboratory between 2010 and 2019. To the best of our knowledge, this study is the first study of its kind conducted in Bamako, Mali.

The identification of our strains of *P. aeruginosa* was based on their morphological and biochemical characteristics (Avril et al., 2000). The interpretation of the results was done with regard to international recommendations (CA-SFM/EUCAST) (Jehl et al., 2015).

This has public health implication in Mali as it was a surprise to find out that we have resistance to some antibiotics such as imipenem, ceftazidime, and amikacin which are not used in routine care. Thus, both in hospital area and outside hospital, the use of antibiotics should be guided by antimicrobial susceptibility testing results.

In this study, the strains of *P. aeruginosa* were of hospital and non-hospital origin, and the hospital strains were mainly from the medical and surgical departments. In Monastir in Tunisia, *P. aeruginosa* strains were isolated in intensive care, surgery and ear, nose, and throat (ENT) departments, mostly (Ben Abdallah et al., 2008).

This difference in the study site may explain the difference between the two studies.

Table 1. Distribution of 317 *Pseudomonas aeruginosa* strains according to the specimen and the patients' origin.

Specimen	Hospital strains (No. of strains)	Extra-hospital strains (No. of strains)	Total (Rate in %)
Urines	119	24	143 (45.1)
Pus	70	11	81 (25.6)
Vaginal secretions	12	29	41 (12.9)
Sputums	19	7	26 (8.2)
Blood cultures	12	0	12 (3.8)
Pleurisy	4	0	4 (1.3)
Catheters	4	0	4 (1.3)
Cerebro-spinal fluid	1	0	1 (0.3)
Prostatic fluid	1	0	1 (0.3)
Peritoneum fluid	1	0	1 (0.3)
Broncho-alveolar fluid	1	0	1 (0.3)
Articular fluid	1	0	1 (0.3)
Gastric fluid	1	0	1 (0.3)
Total	246	71	317(100)

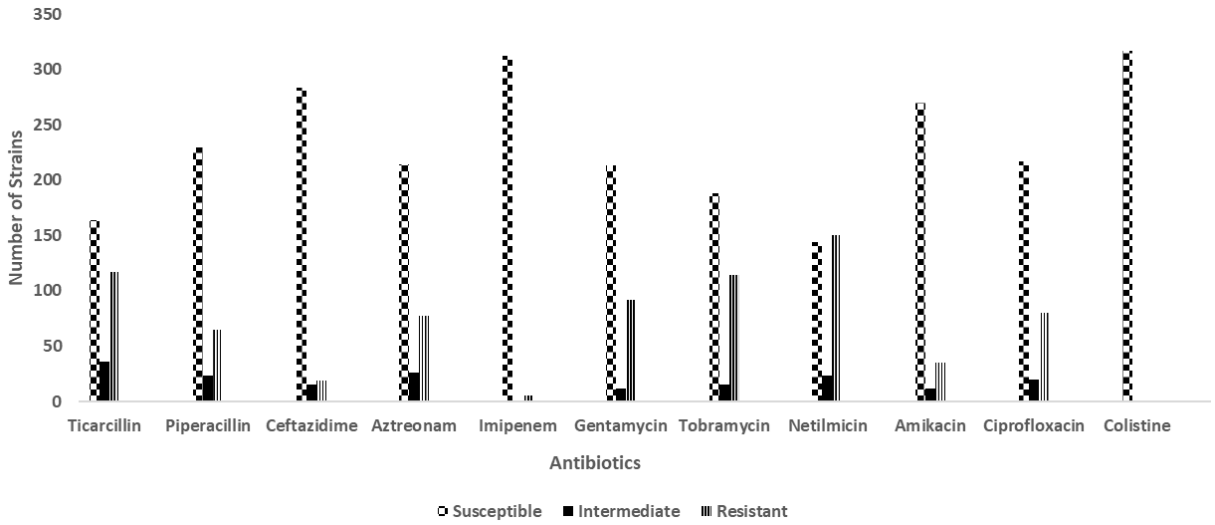

Figure 2. Distribution of 317 *Pseudomonas aeruginosa* strains according to the antimicrobial susceptibility.

In the present study, *P. aeruginosa* strains were isolated from different samples: urine, pus, vaginal samples, and blood cultures (Table 1), while in Monastir in Tunisia the strains of *P. aeruginosa* were isolated from pus (52.9%), respiratory samples (19.5%), urine (10.6%) and blood cultures (5%) (Ben Abdallah et al., 2008). The *P. aeruginosa* strains of Abdou-Souley Lié Moustapha (2002) were isolated in 2002 at the Point G University Teaching Hospital in the same samples as the present study. The sampling sites fit well with the pathogenicity of *P. aeruginosa* which determines various infections (Avril et al., 2000; Wu et al., 2015; Michel-Briand, 1992).

In Europe, 74% of *P. aeruginosa* strains were susceptible to ticarcillin, 80% to ceftazidime, 73% to aztreonam and 82% to imipenem (Rossolini and Mantengoli, 2008). Ticarcillin, ceftazidime and aztreonam were, respectively active in 46.9, 89.6 and 67.5% of the strains of *P. aeruginosa* in this study.

Piperacillin was not active in 27.7% of the strains. The proportion of *P. aeruginosa* strains resistant to piperacillin varies from one country to another: it was 48.5% in Germany, 38.4% in France and 5.4% in the United Kingdom (Nordmann and Naas, 2012). This difference could be explained by the previous exposition to this antibiotic. Generally, this ureidopenicillin is not available in Mali. The susceptibility of the strains to imipenem is

Table 2. Comparative antimicrobial susceptibility of *Pseudomonas aeruginosa* hospital and extra-hospital strains.

Antibiotics	Hospital strains (in-patients)		Extra-hospital strains (out-patients)		P value
	S [n (%)]	I+R [n (%)]	S [n (%)]	I+R [n (%)]	
Ticarcillin	121 (49.2)	125 (50.8)	43 (61)	28 (39)	0.091
Piperacillin	169 (68.7)	77 (31.3)	60 (84.5)	11 (15.5)	0.013
Ceftazidime	218(88.6)	28 (11.4)	65 (91.5)	6 (8.5)	0.482
Aztreonam	149 (60.6)	97 (39.4)	65 (91.5)	6 (8.5)	0.0000018
Imipenem	243 (98.8)	3 (1.2)	69 (97)	2 (3)	0.341
Gentamycin	153 (62.2)	93 (37.8)	60 (84.5)	11 (15.5)	0.00071
Tobramycin	143 (58.1)	103 (41.9)	42 (59)	29 (41)	0.985
Netilmicin	80 (32.5)	166 (67.5)	40 (56)	31 (44)	0.0045
Amikacin	210 (85)	36 (15)	60 (84.5)	11 (15.5)	0.857
Ciprofloxacin	161 (65.4)	85 (34.6)	56 (79)	15 (21)	0,0455

S = Susceptible; I = intermediate; R = resistant; P= probability.

almost the same (Figure 2). The proportion of *P. aeruginosa* strains resistant to ceftazidime is 18.6% in France and 21.8% in Monastir in Tunisia (Ben Abdallah et al., 2008; Nordmann and Naas, 2012). This proportion was 10.4% in the present study (Figure 2).

The proportion of *P. aeruginosa* strains resistant to carbapenems (imipenem or meropenem) is 18.4% in France (Nordmann and Naas, 2012). In Monastir in Tunisia, the resistance rate of *P. aeruginosa* to imipenem was 19.6% (Ben Abdallah et al., 2008), while resistance to imipenem was 1.6% in the present study (Figure 2).

In this study the prevalence of *P. aeruginosa* strains MDR and XDR were low regardless of origin. Usually, the prevalence of MDR strains of *P. aeruginosa* varies from 15 to 30% in many regions (Horcajada et al., 2019). In 2017 in Spain, a multicenter study of *P. aeruginosa* infections found 26% of MDR strains and 17% XDR strains (Ben Abdallah et al., 2008). In the United States, out of 7,868 strains of *P. aeruginosa* isolated in 94 hospitals between 2013 and 2016, 1,562 (19.8%) were MDR and 717 (9.1%) XDR (Sader et al., 2017).

In 1990 at Henri Mondor Hospital in France, the resistance rate of *P. aeruginosa* strains to gentamicin and amikacin was 39.81 and 12.03%, respectively (Caron and Humbert, 1993). This rate is close to the present study with regards to gentamicin (Figure 2 and Table 2). This difference of resistance to specific and/or MDR strains could be explained by the low prevalence of *P. aeruginosa* and imipenem is rarely prescribed in setting of the present study.

The *P. aeruginosa* strains of Ben Abdallah et al., (2008) isolated in Monastir were more resistant to gentamicin (39.3%) as the present study. This strains of *P. aeruginosa* appear to be more resistant to amikacin than those from the Henri Mondor Hospital in France (Figure 2 and Table 2), and the strains of Ben Abdallah et al. (2008) isolated in Monastir, Tunisia, were resistant to amikacin at 19.2%.

In France, the *P. aeruginosa* resistance rate to ciprofloxacin was stable at around 25 to 30% according to Soussy (2012). The resistance rate of our extra-hospital strains to ciprofloxacin was identical to that of Soussy (2012) (Figure 2), and probably due to the baseline prescription of ciprofloxacin to many other bacterial diseases such as typhoid fever which is very common in the setting of the present study.

The strains of *P. aeruginosa* were susceptible to colistin (Avril et al., 2000), and the susceptibility of the strains of the present study to colistin was constant, because there was no resistance to this polymyxin (Figure 2).

This study has some limitations; first the data were retrospectively collected, and also were limited by the number of discs to be purchased for a complete full profile of antimicrobial resistance to the *P. aeruginosa* strains.

Despite these limitations, the study is unique as it collects consecutive strains of the *P. aeruginosa* isolated in both hospital and outside-hospital area in Bamako, Mali.

Conclusion

Colistin, imipenem, ceftazidime, amikacin and piperacillin were the most active antibiotics against *P. aeruginosa*. Ciprofloxacin and ticarcillin were active in every other strain in hospital area. The frequency of multidrug-resistant strains of *P. aeruginosa* is low in Bamako. Out-patients' strains were more susceptible to aztreonam, piperacillin, gentamicin, netilmicin and ciprofloxacin than hospital strains.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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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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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

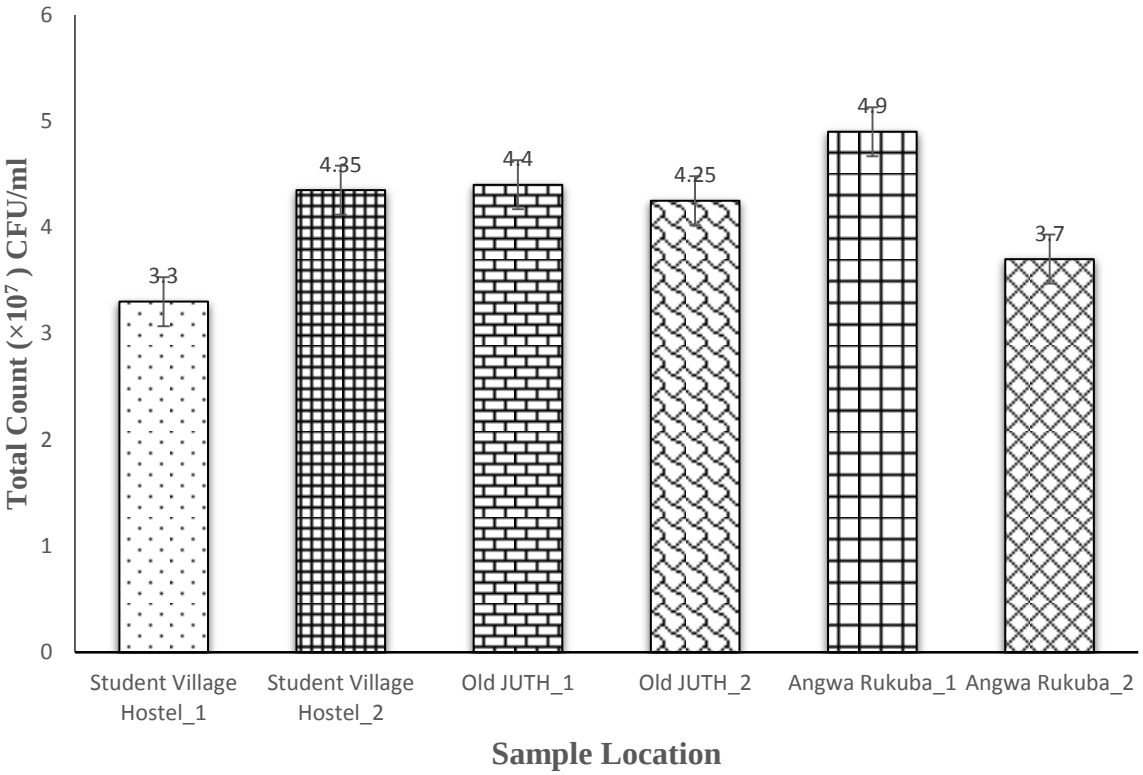

Figure 1. Total viable count of the isolates with respect to sample locations.
CFU = Colony Forming Unit.

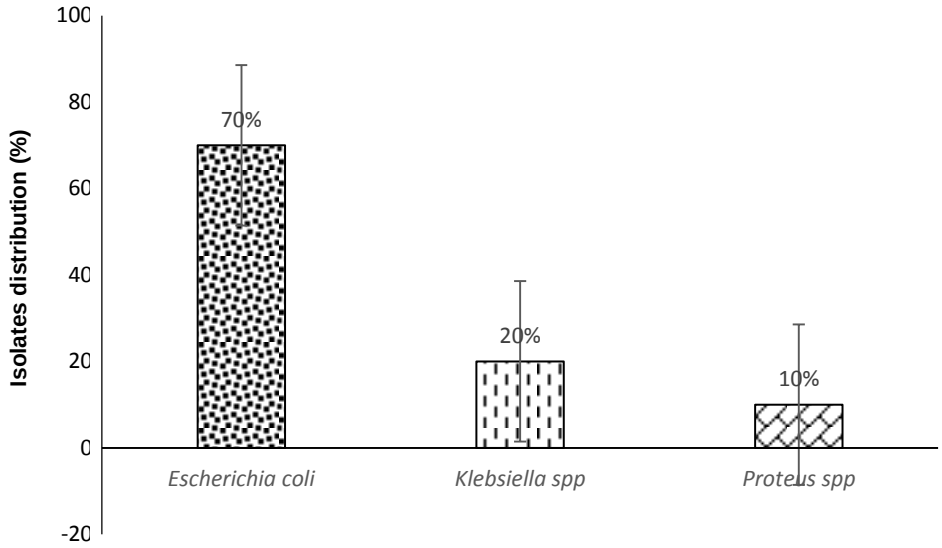

Figure 2. Percentage distribution of the isolates.

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

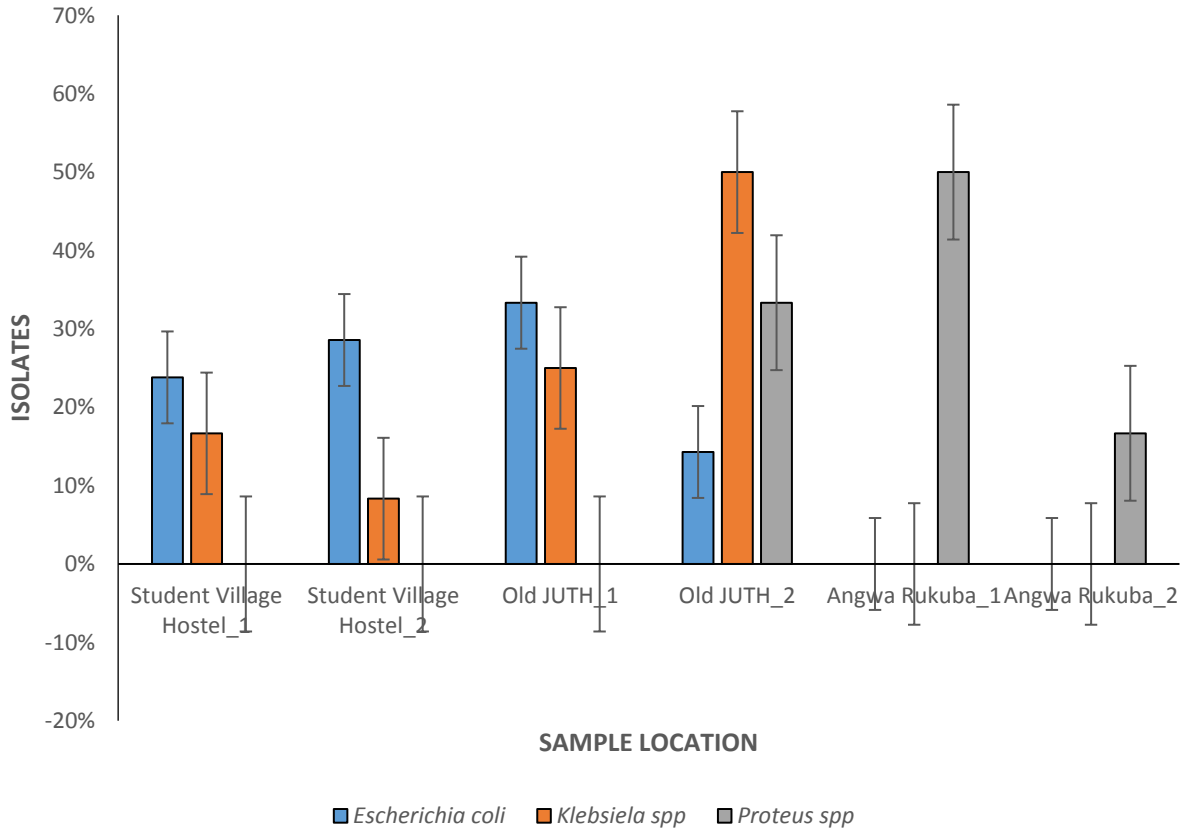

Figure 3. Distribution of enterobacteriaceae in relation to samples.

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

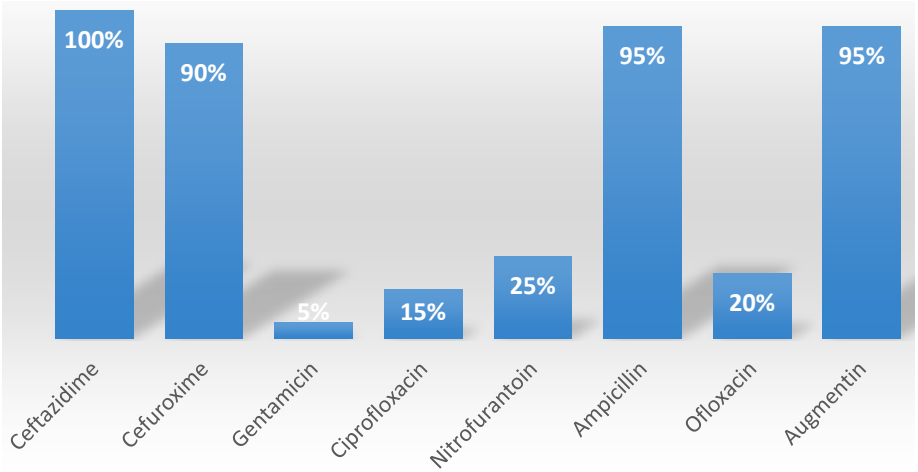

Figure 4. Percentage of antimicrobial resistance of the isolates.

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

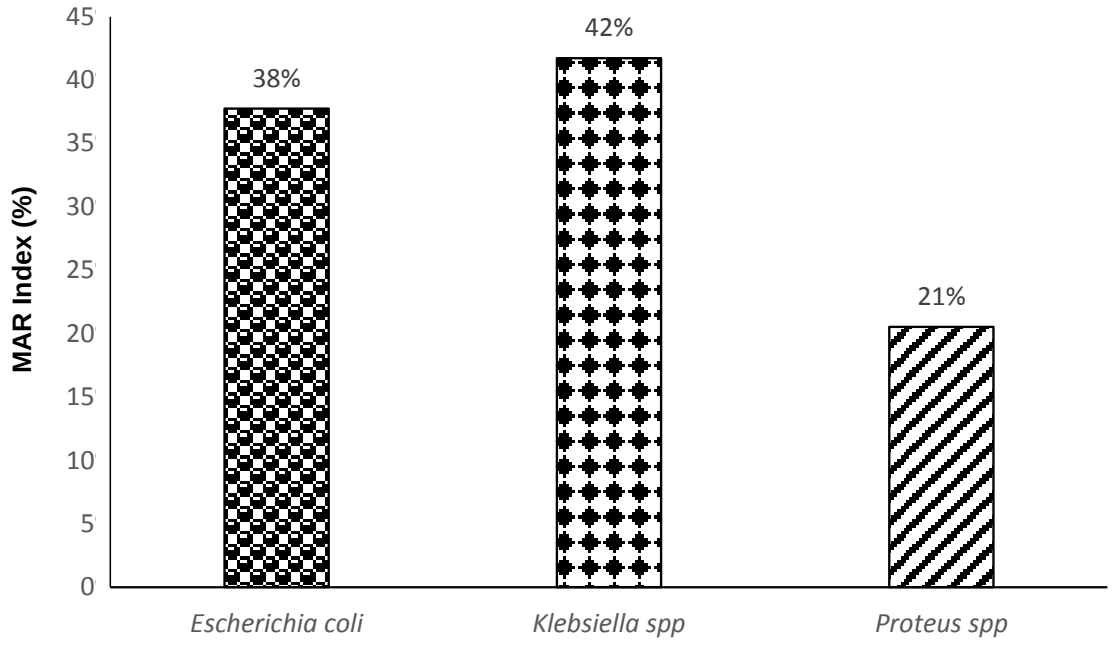

Figure 5. Multiple antibiotic resistance (MAR) index of the total individual isolates.

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.

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