Journal of Advances in Microbiology

19(4): 1-9, 2019; Article no.JAMB.53875
ISSN: 2456-7116

The Effect of *Moringa oleifera* and *Ocimum gratissimum* Essential Oils and Extracts on Antimicrobial Resistant Enterobacteriaceae from Environmental Sources

C. I. Chikwendu[1*], C. E. Okere[1], R. K. Obi[1] and N. U. Nwogwugwu[1]

[1]Department of Microbiology, School of Biological Sciences, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author CIC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CEO and NUN managed the analyses of the study. Author RKO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2019/v19i430202
Editor(s):
(1) Dr. Simone Aquino, Instituto de Pesquisas Energéticas e Nucleares (IPEN), Brazil.
Reviewers:
(1) D. Shital Bonde, Sant Gadge Baba Amravati University, India.
(2) Neeta Bhagat AIB, Amity University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53875

Original Research Article

Received 10 November 2019
Accepted 16 January 2020
Published 21 January 2020

ABSTRACT

The continuous increase in resistance to antimicrobials amongst the Enterobacteriaceae constitutes a growing public health threat and thus has necessitated the need to continuously search for alternative antimicrobial chemotherapy. This study was aimed at evaluating the effects *Moringa oleifera* seed and *Ocimum gratissimum* plant extracts and essential oils on antimicrobial resistant Enterobacteriaceae isolated from aquatic sources. Two hundred isolates of *Klebsiella pneumoniae* and *Escherichia coli* were recovered from two different environmental sources. The susceptibility of the isolates to ten (10) different antimicrobials was examined by the Kirby-Bauer technique. The isolates were also tested for extended β-lactamase production (ESβL) by the modified double disc synergy test and the susceptibility of the isolates to essential oils and extracts from *Moringa oleifera* seeds and *Ocimum gratissimum* leaves was analysed using the agar - well diffusion assay. In addition, the phytochemical analysis of the extracts was carried out to determine their constituents.

**Corresponding author: E-mail: chinwe.chikwendu@futo.edu.ng, chinwechikwendu@gmail.com;*

The river water isolates recorded high resistance rates for the following antibiotics: Ampicillin (99%), cefotaxime (83%), imipenem (77%) and low rates for levofloxacine (19%), while the aquaculture isolates expressed high resistance rates to ampicillin (95%) ceftazedime (86%), ertapenem (65%), and low rates to aztreonam (8%). Thirteen (37%) isolates out of 35 tested were positive for ESβL production, four isolates from river water and nine from aquaculture. Antimicrobial analysis of the essential oils against the ESBL producers showed no inhibitory activity while the plant extracts produced zones of inhibition and minimum inhibitory concentrations of between 1.32 and 2.70 mg/ml for the two plant extracts tested. Phytochemical analysis showed the presence of alkaloids, saponins, tannins, flavonoids and glycosides in different quantities. This study has shown that crude extracts of *Moringa oleifera* seeds and *Ocimum gratissimum* leaf could become a potential alternative in the treatment of infections due to antimicrobial-resistant Enterobacteriaceae.

Keywords: Antimicrobials; enterobacteriaceae; Escherichia coli; Klebsiella pneumoniae; Moringa oleifera; Ocimum gratissimum.

1. INTRODUCTION

Enterobacteriaceae are rod-shaped, gram-negative bacteria that are normal inhabitants of the intestinal flora of warm-blooded animals and among the most common human pathogens causing infections that range from cystitis to pyelonephritis, wound sepsis, pneumonia, bacteremia, and meningitis [1]. Entero-bacteriaceae spread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons [2,3,4].

The increasing development of antimicrobial resistance in microorganisms has reached a critical point, threatening the gains over the years on the treatment of infectious diseases, since the discovery of penicillin in the early 1900s. The rate of resistance has become so astronomical that bacteria are developing defenses against antibiotics even as new ones are developed [5]. Unfortunately, this problem is not limited to clinical isolates alone but has extended to native isolates of aquatic environments [6].

The general belief is that an increase in antimicrobial resistance is as a result of extensive use and abuse of antibiotics in medication, veterinary agriculture and aquaculture. Several studies worldwide have reported the presence of antimicrobials in all types of water including mineral water, drinking water, lakes, rivers, ground and wastewater [7-10]. Consequently, antimicrobial-resistant bacteria (AMR) are commonly found in environmental waters including oceans, rivers and lakes [11-14]. In addition, water bodies also contain other substances like biocides and heavy metals that place a selection of pressure amongst the waterborne microbial gene pools [15,16,17].

The continuous increase in resistance to antimicrobials among the Enterobacteriacea constitutes a growing public health threat and so has necessitated the need to continuously search for alternative antimicrobial chemotherapy. Essential oils and plant extracts have great potential in the field of bio-medicine to open new pathways for anti-biotherapy as they effectively destroy several bacterial, fungal and viral pathogens. Medicinal plants have been recognized as potential sources of new antimicrobial molecules, creating a renewed interest in the antimicrobial activities of phytochemicals [18]. Plants produce a wide variety of secondary metabolites including alkaloid, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinolones and coumarines [19]. According to Srivastava, et al. [20] antimicrobial properties of plants are as a result of the presence of these biomolecules. Two examples of such medicinal plants common in the Nigerian environment are *Ocimum gratissimum* and *Moringa oleifera.* According to Sofowora [21] Fakae, et al. [22] and Chitwood [23] *O. gratissimum* leaves are used as vegetables for food preparation while their extracts are an antidote for cough, rheumatism, high fever and gonorrhea. *M. oleifera* on the other hand is a small tree with little foliage and commonly called Horse-radish in English. Every part of the plant is reported to possess medicinal properties and used in the treatment of various ailments [24].

The aim of this work was to evaluate the effect of extracts and essential oils of *Moringa oleifera* seeds and *Ocimum gratissimum* leaves on antimicrobial resistant Enterobacteriaceae isolated from environmental sources.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample types and collection

Bacterial isolates were recovered from river and fish pond water samples. *Moringa oleifera* seeds and *Ocimum gratissimum* leaves were purchased from Relief market Owerri in Owerri Municipal Council Area, Imo State Nigeria. Both plant samples were identified by a taxonomist at the Crop Science Department of the Federal University of Technology, Owerri (FUTO), Imo State Nigeria. The river and fish pond water samples were collected from Otamiri river and fish ponds in FUTO respectively, using sterile plastic containers. Ten antimicrobials were tested for activity against the isolates and they included gentamycin (10 μg), ampicillin (10 μg), ceftazidime (30 μg), aztreonam (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), meropenem (10 μg), imipenem (10 μg) and ertapenem (10 μg). The breakpoints in millimeter (mm) for resistant isolates according to Clinical and Laboratory Standards Institutes (CLSI) [25] for the tested antimicrobials are gentamycin ≤12, ertapenem ≤19, imipenem ≤19, meropenem ≤19, ciprofloxacin ≤15, levofloxacin ≤13, ceftazidime ≤17, aztreonam ≤17, cefotaxime ≤22, ampicillin ≤13.

2.2 Methods

2.2.1 Isolation and characterization of *E. coli* and *Klebsiella pneumoniae* isolates

MacConkey and eosin methylene blue agar were used for primary isolation of *Klebsiella pneumoniae* and *E. coli* after enrichment in nutrient broth for 24 hours at 30ºC. Subsequently, 2 or 3 distinct colonies from these primary plates with characteristic colonies were selected and purified on nutrient agar plates to obtain pure colonies. The isolates were confirmed using IMViC tests for enterobactariaceaea classification [26].

2.2.2 Antimicrobial resistant test

Antimicrobial resistant test was carried out by the Kirby-Bauer technique, according to Cheesbrough [26]. Briefly, isolates standardized to 0.5 McFarland standards were spread on the surface of already prepared and dried Mueller agar plates using swab sticks. The antimicrobial discs were placed on the agar surface using sterile forceps, the bacterial films on the agar left to dry briefly and plates subsequently incubated at 35ºC for 24 hours. After incubation, the zones of inhibition were measured using a meter rule and designated as resistant or susceptible according to already established breakpoints.

2.2.3 Extraction of plant samples

2.2.3.1 Essential oil extraction

The technique adopted by Adepoju, et al. [27] was used to extract the essential oils of the plant and seed samples. A 250 ml soxhlet extractor apparatus and petroleum ether as a solvent were used with 50 g fresh plant materials for the extraction. The quantity of the oil yield was determined gravimetrically as the ratio of the weight of the extracted oil to the weight of the plant powder samples used. The obtained oil was kept in a refrigerator before use.

2.2.3.2 Crude plant extraction

Crude ethanol extraction of the plant sample was carried out by soaking the powdered leaves and seeds in ethanol for 4 days with constant shaking. The mixture was filtered using Whatman Number 1 filter paper, centrifuged at 1500xg for 20 minutes. The extracts were capped and stored at 4ºC as plant stock. The crude extract was evaporated to dryness by placing in a water bath at 40ºC.

2.2.3.3 Phenotypic testing for extended spectrum βeta-lactamase (ESβL) production

All the isolates that showed resistance to ceftazidime and cefotaxime were selected for phenotypic testing for ESβL production. The test was conducted by the modified Double Disk Synergy Test (MDDST) according to Kaur, et al. [28] using amoxicillin – clavulanate (20/10 μg) disc, along with four cephalosporins: Cefotaxime, ceftriazone, cofpodoxime, (3GC) and cefepime (4GC). The organisms were spread on Meuller-Hinton agar plates according to Clinical and Laboratory Standards Institute (CLSI), (2009) [25] and discs of amoxicillin-clavulanate (30 μg) placed in the center of the plates while the discs of the other antibiotics were placed 15 mm and 20 mm apart respectively, center to center to that of the amoxicillin-clavulanate disc [28]. A distortion or increase in the zones of inhibition towards the disc of amoxicillin-clavulanate was considered as positive for ESβL production.

2.2.4 Antimicrobial activity of the essential oils of *M. oleifera* and *O. gratissimum* against ESβL isolates

2.2.4.1 Disc preparation for plant essential oils

The antimicrobial activity of the essential oils was conducted according to Serban, et al. [29]. Paper discs were prepared from Whatman No. 1 filter paper using a puncher and sterilized in separate bijou bottles at 170ºC for 180 minutes. Thereafter, different concentrations of the *Moringa oleifera* seed and *Ocimum gratissimum* leaf essential oils, prepared by dissolving different volumes of the oil in appropriate volumes of dimethyl sulfoxide (DMSO) were dropped on the discs for subsequent analysis. The different quantities used in the assay were 10μl (1 ml of essential oil), 7.5μl (0.75 m1 of essential oil and 0.25 ml of DMSO), 5.0μl (0.5 ml of essential oil and 0.5 ml of DMSO) and 2.5μ1 (0.25 ml of essential oil and 0.75 ml of DMSO) with each disc capable of adsorbing 0.01ml of the solution.

2.2.4.2 Well-diffusion assay of essential oils

Standardized inocula of the *E. coli* and *K. pneumonia* isolates were swabbed on the surface of prepared and solidified Mueller Hinton agar plates in duplicates. 0.1 ml of the active cultures was spread over the plate using a sterile glass spreader in order to get a uniform microbial growth for all the plates [30]. A well was dug using a 6mm diameter cork borer on the agar plate. The wells were filled with 10, 20 and 30 μl of the essential oils. All plates were sealed by paraffin with sterile laboratory conditions to avoid evaporation of the agar plates. The plates were left for 30 minutes at room temperature to allow the diffusion of the oil and then incubated at 37ºC for 24 hours.

2.2.4.3 Antimicrobial activity of the crude plants extracts against the ESβL isolates

The activity of the two crude plant extracts against ESβL isolates was screened by using the agar-in-well diffusion method [31]. An inoculum suspension was swabbed uniformly to solidified 20 ml Mueller Hinton Agar (MHA) plates and the inoculum was allowed to dry for 5 minutes. Holes of 6 mm in diameter were made in the seeded agar using a sterile cork borer. Aliquots of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml from each plant extract was added into each well on the seeded medium and allowed to stand on the bench for one hour for proper diffusion and thereafter incubated at 37ºC for 24 hours. After incubation, the diameters of the growth inhibition zones were measured in millimeters (mm).Three replicates were carried out for each extract against each of the test organisms that produced ESβLs.

3. RESULTS

A total of two hundred isolates of *E. coli* and *Klebsiella pneumoniae* were obtained from two different environmental sources - River and aqua culture water. The isolates from river water expressed high resistance rates to ampicillin, cefotaxime, imipenem, azetreonam, meropenem, ertapenem (99%, 83%, 77%, 75%, 74%, 70%) respectively, while expressing moderate resistances to ceftazidime and ciproflaxacin (53% and 40%) and low resistances to Gentamycin and Lavafloxacin (34% and 19%) respectively (Table 1). Isolates from aquaculture water on the other hand, expressed high rates of resistance to ampicillin, ceftazidime and ertapenem (95%, 86% and 65%) respectively, moderate resistances to imipenem, cefotaxime, ciprofloxacine and meropenem (56%, 44%, 43% and 41%) respectively, while low resistance rates were recorded for levofloxacine, gentamycin and aztreonam (33%, 18% and 8%) respectively (Table 2). On the average, isolates from river water samples generally expressed higher rates of resistance to the antibiotics tested compared to the aquaculture water isolates. The least resistance rate of 8% being recorded for aztreonam amongst the aquaculture isolates (Table 3).

Out of 35 isolates tested phenotypically for ESβL production, 13 (17.5%) isolates were found to be ESβL producers with nine (9) being *E. coli* and four (4), *K. Pneumoniae isolates*. All these Isolates showed a clear extension of the edge of inhibition which was produced by the antibiotic towards the amoxicillin clavulanate disk. Four (11.4%) of the extended spectrum βeta-lactamase (ESβL) producers were from river water, while 9 (25.7%) were from aquaculture samples (Table 4).

The isolates exhibited high levels of variability expressing varying numbers of resistant patterns amongst them as shown on Table 5. *E. coli* from river water and aquaculture exhibited 26 and 36 resistant patterns respectively while *K. pneumonia* exhibited 36 and 43 patterns respectively (Table 5). Multiple antibiotic resistance index (MAR) of between 0.3 and 1

were also identified with 96-98% of the isolates being resistant to between 3 and 10 antibiotics.

The essential oils extracted from the *Moringa oleifera* seeds and *Ocimus gratissimum* leaves were not active against the ESβL producing isolates as there were no zones of inhibition on the Mueller-Hinton agar plates.

The MICs as observed for the two medicinal plants extracts analyzed showed there was inhibition of the Isolates by the extracts at 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, in both aqueous and ethanol extracts and the minimum inhibitory concentrations of between 1.32 mg/ml and 2.70 mg/ml for the two plants extracts were recorded as seen on Table 6.

Table 1. Antimicrobial resistant profile of *E. coli* and *Klebsiella pnuemoniae* isolated from river water

Antibiotics	No(%) of resistant isolates		
	***E. coli* (n=50)**	***Klebsiella pneumoniae* (n=50)**	**Total resistant (n=100)**
Ampicillin	50 (100)	49 (98)	99 (99)
Cefotaxime	39 (78)	44 (88)	83 (83)
Imipenem	43 (86)	34 (68)	77 (77)
Aztreonam	36 (72)	39 (78)	75 (75)
Meropenem	36 (72)	38 (76)	74 (74)
Ertapenem	37 (74)	33 (66)	70 (70)
Ceftazidime	20 (40)	33 (66)	53 (53)
Ciprofloxacine	22 (44)	18 (36)	40 (40)
Gentamycin	16 (32)	18 (36)	34 (34)
Levofloxacin	8 (16)	11 (22)	19 (19)

Table 2. Antimicrobial resistant profile of *E. coli* and *Klebsiella pnuemoniae* isolated from aqua culture

Antibiotics	No(%) of resistant isolates		
	***E. coli* (n=50)**	***Klebsiella pneumoniae (*n=50)**	**Total resistant (n=100)**
Ampicillin	45(90)	50(100)	95(95)
Ceftazidime	43(86)	43(86)	86(86)
Ertapenem	27(54)	38(76)	65(65)
Imipenem	31(62)	25(50)	56(56)
Cefotaxime	23(46)	21(42)	44(44)
Ciprofloxacin	17(34)	26(52)	43(43)
Meropenem	4(8)	37(74)	41(41)
Levofloxacin	17(34)	16(32)	33(33)
Gentamycin	8(16)	10(20)	18(18)
Aztreonam	4(8)	4(8)	8(8)

Table 3. Total resistant rates (%) of antimicrobial resistant enterobacteriaceae isolated from both sources

Antibiotics	No(%) of resistant isolates		
	River water (n=100)	**Aquaculture water (n=100)**	**Total resistant rates (n=200)**
Ampicillin	99 (99)	95 (95)	194 (97)
Cefotaxime	83 (83)	44 (44)	127 (63.5)
Imipenem	77 (75)	56 (56)	133 (66.5)
Aztreonam	75 (75)	8 (8)	83 (41.5)
Meropenem	74 (74)	41 (41)	115 (57.5)
Ertapenem	70 (70)	65 (65)	135 (67.5)
Ceftazidime	53 (53)	86 (86)	139 (69.5)
Ciproflaxacin	40 (40)	43 (43)	83 (41.5)
Gentamicin	34 (34)	18 (18)	52 (26)
Levofloxacin	19 (19)	33 (33)	52 (26)

Table 4. Number (%) ESβL producing isolates among the river and aquaculture water isolates

	Aquaculture (n=17)	River (n=18)	Total (n=35)
E. coli	6 (35.2)	3 (16.7)	9 (25.7)
K. pneumoniae	3 (17.6)	1(5.5)	4 (11.4)

Table 5. Antimicrobial resistant patterns of the *E. coli* and *Kleb. pnuemoniae* isolates from river and aquaculture water sources

Organism	Source	No of patterns expressed	No of organisms expressing pattern	Predominant pattern
E. coli	RV	26	8	CTX+ETP+IPM+MEM+AMP+ATM
E. coli	AQ	36	3	CAZ+ATM+AMP+IPM
			3	CAZ+CTX+ETP+ATM+AMP
			3	CAZ+CTX+ETP+AMP+IMP
Kleb. pneumoniae	RV	36	6	CTX+CAZ+ETP+IPM+MEM+LEV +CN+CIP+APM+ATM
	AQ	43	6	CAZ+ETP+AMP+IPM+CIP+MEM

KEY: RV: River water; AQ: Aquaculture; CN (gentamycin), IMP (imipenem), AMP (ampicillin), LEV (levofloxacin), CAZ (ceftazidime), ATM (aztreonam), CTX (cefotaxime), CIP (ciprofloxacin), MEM (meropenem), ETP (ertapenem)

Table 6. Minimum Inhibitory Concentration (MIC) against *E. coli and Kleb. pneumoniae* isolates

			MIC (mg/ml)/plant	
S/No	Test organism(s)	Sources	*M. oleifeira*	*O. gratissimum*
	Klebsiella pneumoniae	River water	1.59	1.59
		Aqua Culture	1.32	1.32
	E. coli	River water	Indeterminate	2.70
		Aqua Culture	1.32	1.32

Table 7. Phytochemical constituents of water and extracts of the test plants

	O. gratissimum		*M. oleifeira*	
	OE	OW	OE	OW
Alkaloids	+	-	+++	++
Saponins	+	+	++	++
Tannins	+	+	++	+
Flavonoids	+	+	++	+
Steroids	-	-		
Glycosides	+	+	+	++
Terpenoids			+	++

KEY: OE; ethanol extract; OW: water extract; +; slightly present; ++; moderate: +++; abundant

4. DISCUSSION

Members of the Enterobacteriaceae such as *E. coli* and *Klebsiella pneumoniae* are among the most important causative agents of hospital and community acquired infections of human beings [32]. In this study, the susceptibility data of microbial Enterobacteriaceae environmental isolates demonstrated remarkable resistance to commonly used antibiotics with significant resistance to the β-lactams. High resistance rates were identified amongst the aquatic environmental isolates with up to 98% of the isolates being resistant to at least three antibiotics. In a similar work by Sivakumarasmany, et al. [33] 68.4% of their environmental Gram-negative isolates were resistant to at least one antibiotic with 43.4% being multi-resistant. And 31.6% were susceptible to all the twelve (12) antibiotics tested. This is in contrast to the present study where no isolate was susceptible to all the tested antibiotics. Similarly all the isolates in Sivakumarasmany, et al. [33] expressed maximum resistance to ampicillin as in the present study.

In the study by Obayiuwa, et al. [34] 85.8% of their bacterial isolates including *K. pneumonia* were multidrug resistant, similar to the present study where very high rates of multidrug resistance of up to 96–98% were identified. Similarly, resistance was low for gentamicin and 100% for ampicillin similar to the current study were resistances were 26% and 97% for gentamicin and ampicillin respectively. In Onuoha [35] very high proportions of the bacterial isolates were resistant to cefotaxime and cefuroxime, both β-lactam antibiotics. Similar high trends were equally reported for the same group of antibiotics in the present study, with cefotaxime and ceftazidime showing rates of 63.5% and 69.5% resistance rates respectively. Most effective antibiotic in their study was azithromycin at 100% susceptibility rates followed by imipenem at 77% contrary to the present study were rates of resistance to imipenem were 66.5%.

Multiple antibiotic resistance (MAR) index values for the study were high, ranging from 0.3–1, with at least 8 isolates resistant to all 10 antibiotics under study, making the environmental sources tested potential health risk environments. According to Krumperman [36] and Hinton, et al. [37] MAR index above 0.25 classifies an area of study as potentially health risk environment. Many other similar studies in different geographical zones have also led to the classification of several other aquatic environments in different countries as health risk contaminated areas due to high and increasing MAR index values above 0.25. Some of these diverse areas include Egypt [5], Romania [38] USA [39], China [40] and now Nigeria. This classification of widespread areas across the globe as potentially health risk environment supports the assertion that the phenomenon of MAR bacteria in aquatic environments is a worldwide burden being that it is an international rather than a national problem [41,42,43] and measures for amelioration ought to put this fact into consideration.

Extended spectrum beta lactamases were phenotypically identified in some of the environmental isolates. Their presence particularly on plasmids makes it possible for them to be easily transferred from one organism to another. The genes for the production of extended spectrum βeta-lactamases usually co-exist with the resistance genes for several other antibiotics they are encoded by plasmids which also carry resistant genes for other antibiotics. [44].

Phytochemical analysis of the plant extracts showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides and terpenoids in varying quantities. Their antimicrobial activities against these environmental isolates confirmed by the MIC results could be attributed to the above mentioned phytochemicals. *Moringa oleifera* seed and *Ocimum gratissimum* leaf extracts could therefore be used as effective candidates for the treatment of antimicrobial resistant Enterobacteriaceae in these environments.

5. CONCLUSION

Phytochemical analysis showed the presence of alkaloids, saponins, tannins, flavonoids and glycosides in different quantities. *Moringa oleifera* seed and *Ocimum gratissimum* leaf extracts could be used as effective candidates for the treatment of antimicrobial resistant Enterobacteriaceae. The current challenge in Medicare is the race between the creation of effective novel molecules and the spread of multi-drug resistant Enterobacteriaceae. The use of medicinal plants as source of alternative antibacterial agents is very important and should be encouraged at a “certain or well-known dose”. There was wide spread and diverse resistance of the Enterobacteriaceae in the aquatic environments, which may be controlled by the use of *Moringa oleifera* seed and *Ocimum gratissimum* leaf extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mitchell J, Yehuda C. Carbapenem-resistant Enterobacteriaceae. A potential threat. JAMA. 2008;300(24):2911-2913.
2. Partridge SR. Analysis of antibiotic resistance regions in gram-negative bacteria. FEMS Microbiol. Rev. 2011;35: 820–855.
3. Stokes HW, Gillings MR. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into gram-negative pathogens. FEMS Microbiol. Rev. 2011;35:790–819.

4. Toleman MA, Walsh TR. Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. FEMS Microbiol Rev. 2011;35:912-935.
5. Azzam MI, Ezzat SM, Othman BA, El-Dougdoug KA. Antibiotic resistance phenomenon and virulence ability in bacteria from water environment. Water Science. 2017;31:109-121.
6. Kummerer K. Antibiotics in the aquatic environment. Chemosphere. 2009;75(4): 435-441.
7. Messi P, Guerrieri E, Bondi M. Antibiotic resistance and antibacterial activity in heterotrophic bacteria of mineral water origin. Sci. Total Environ. 2005;346:213-219.
8. Oyetayo VO, Akharauji FC, Oghumah M. Antibiotic sensitivity pattern of *Escherichia coli* isolated from water obtained from wells in Akure Metropolis. Res. J. Microbiol. 2007;2:190-193.
9. Emmanuel I, Joseph N, Kingsley E, Egbebor E, Lawrence E. Antibiotic susceptibility profiles of enteric bacteria isolates from dump site, uitsols and water sources in a rural community in cross River State, Southern Nigeria. Nat. Sci. 2011; 9:46-50.
10. Mulamattathil S, Bezuidenhont C, Mbiwe M, Aleta C. Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa and characterization using their antibiotic resistance profiles. J. Pathog. 2014;214:1-11.
11. Marti E, Jofre J, Balcazar JL. Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. PLoS One. 2013;8(10):78906.
12. Hatosy SM, Martiny AC. The ocean as a global reservoir of antibiotic resistance genes. Appl. Environ. Microbiol. 2015; 81(21):7593–7599.
13. Pang YC, Xi JY, Li GQ, Shi XJ, Hu HY. Prevalence of antibiotic-resistant bacteria in a lake for the storage of reclaimed water before and after usage as cooling water. Environ. Sci. Processes Impacts. 2015; 17(6):1182–1189.
14. Rosas I, Salinas E, Martınez L, Cruz-Córdova A, González-Pedrajo B, Espinosa N, Amábile-Cuevas CF. Characterization of *Escherichia coli* isolates from an urban lake receiving water from a wastewater treatment plant in Mexico City: Fecal pollution and antibiotic resistance. Curr. Microbiol. 2015;71(4):490–495.
15. Baquero F, Martınez JL, Cantón R. Antibiotics and antibiotic resistance in water environments Curr. Opin. Biotechnol. 2008;19(3):260–265.
16. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, et al. Tackling antibiotic resistance: The environmental framework. Nat. Rev. Microbiol. 2015;13(5):310–317.
17. Haberecht HB, Nealon NJ, Gulliland JR, Holder AV, Runyan C, Oppal RC, Ibrahim HM, Muller L, et al. Antimicrobial-resistant *Escherichia coli* from environmental waters in Northern Colorado. J. Environ. Pub. Health. 2019;3862949.
18. Anyanwu MU, Okoye RC, Antimicrobial activities of Nigerian Medicinal plants. J. Intercultural Ethnopharmacology. 2017;6 (2):240-259.
19. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. J. Med. Plants Res. 2010;4:104–11.
20. Srivastava J, Chandra H, Nautiyal AR, Kalra SJS. Antimicrobial Resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. Biotech. 2013;4:451–60.
21. Sofowora A. Medical Plants and Traditional Medicine in West Africa, Spectrum Books Ltd., Ibadan 1982;204-208.
22. Fakae BB, Campbell AM, Barrett J, Scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM. Inhibition of Glutathione-S-Transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother. Res. 2000;1148:630-634.
23. Chitwood DJ. Phytochemical based strategies for nematode control. Annu. Rev. Phytopathol. 2003;40:221-249.
24. Fahey J. *M. oleifera*: An overview of the medicinal evidence for its nutritional, therapeutic and prophylactic properties. Tree Life J. 2005;1:5.
25. Cheesbrough M. District laboratory practice in Tropical Countries. Part 2. Cambridge University Press, U.K. 2004; 62-115:135-143.
26. Adepoju TE, Olawale O, Okunola AA, Olatunji EM. Solvent extraction of oil from soursop oil seeds and its quality characterization. International Journal

Sustainable Energy and Environmental Research. 2014;3(2):82.
27. Kaur J, Chopra S, Sheevan, Mahajan, G. Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli and Klebsiella pneumoniae*. J. Clin. Diagnos. Res. 2013; 7(2):229-233.
28. Clinical and Laboratory Standards Institute, Wayne, PA. Clinical and Laboratory Standards Institute Performance standards for, antimicrobial susceptibility testing; nineteenth informational supplement M100-S19; 2009.
29. Şerban ES, Ionescu M, Matinca D, Maier CS, Bojita MT. Screening of the antibacterial and antifungal activity of eight volatile essential oils Farmacia. 2011;59: 440-446.
30. Donaldson JR, Warner SL, Cates RG, Young DG. Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods Pharmaceut. Biol. 2005;43(8):687-695.
31. Perez C, Paul M, Bazerque P. An antibiotic assay by agar well diffusion method. Acta Biol. Med. Exp. 1990;15:111-115.
32. Paterson DL. Resistance in gram-negative bacteria. Enterobacteriacae Am. J. Infect. Control. 2006;34:20-28.
33. Sivakumarasmany SK, Deekshit VK, Vittal R, Akhila DS, Mundanda DM, Raj JRM, Chakraborty A, Karunasagar I. Phenotypic and genotypic study of antimicrobial profile of bacteria isolates from environmental samples Indian J. Med. Res. 2019;149: 232-239.
34. Obayiuwa A, Ogunjobi A, Yang M, Ibekwe M. Characterization of bacterial communities and their antibiotic resistance profiles in waste waters obtained from pharmaceutical facilities in Lagos and Ogun States, Nigeria. Int. J. Environ. Res. Public Health. 2018;15(7):1365.
35. Onuoha C. The prevalence of antibiotic resistant diarrhogenic bacterial species in surface waters, South Eastern Nigeria. Ethiop. J. Health Sci. 2017;27(4):319-330.
36. Krumperman PH. Multiple antibiotic resistance indexing of *E. coli* to identify high risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 1983;46: 165–170.
37. Hinton M, Hedges AJ, Linton AH. The ecology of *E. coli* in market calves fed a milk substitute diet. J. Appl. Bacteriol. 1985;58:27–35.
38. Florea AB. Antimicrobial susceptibility of *E. coli* isolated from Aries River (Romania). Tom. XVIII. 2011;1:34–38.
39. Munir M, Wong K, Xagoraraki I. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res. 2011;45:681–693.
40. Suzuki S, Hoa PT. Distribution of quinolones, sulfonamides, tetracyclines in aquatic environment and antibiotic resistance in Indochina. Front. Microbiol. 2012;3:67.
41. Okeke I, Edelman R. Dissemination of antibiotic resistant bacteria across geographic borders. Clin. Infect. Dis. 2001; 33:364–369.
42. Knapp CW, Lima L, Olivares-Rieumont S, Bowen E, Werner D, Graham DW. Seasonal variations in antibiotic resistance genetransport in the Almendares River, Havana, Cuba. Front. Microbiol. 2012;3: 396.
43. Lupan I, Carpa R, Oltean A, Kelemen BS, Popescu O. Release of antibiotic resistant bacteria by a waste treatment plant from Romania. Microbes Environ. 2017;32(3): 219–225.
44. Jacoby GA, Sutton L. Properties of plasmids responsible for production of extended spectrum β-lactamases Antimicrob. Agents Chem. 1991;35:164-169.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53875