World Applied Sciences Journal 35 (11): 2286-2293, 2017
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2017.2286.2293

Antibiotic and Heavy Metal Susceptibility of Bacteria Isolated from Heavy Metals Contaminated Soil

[1,2]Iyadunni A. Osibote, [2]Adeniyi A. Ogunjobi and [3]Boluwatife A. Osibote

[1]Department of Microbiology, College of Sciences,
Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria
[2]Environmental Microbiology and Biotechnology Unit,
Department of Microbiology, Faculty of Science, University of Ibadan, Nigeria
[3]Department of Environmental Health Science,
Faculty of Public Health, University of Ibadan, Oyo State, Nigeria

Abstract: The presence of heavy metals as contaminants in the soil often creates selective pressure in bacteria found in such environment conferring on them resistance to both antibiotics and heavy metals. The aim of this study was to isolate and identify bacterial species associated with heavy metals contaminated soil and determine the susceptibility of the isolates to antibiotics & heavy metals. Bacteria were isolated from the soil using pour plate technique and identified using biochemical and molecular techniques. The isolates were subjected to antimicrobial susceptibility testing using various antibiotic discs and the isolates were also screened for their susceptibility to heavy metals. Thirty five bacterial isolates were obtained from soil contaminated with heavy metals. The various biochemical identification tests carried out on the isolates revealed that the isolates belonged to *Pseudomonas* sp (19), *Proteus mirabilis* (5), *Alcaligenes faecalis* (5), *Enterobacter* sp (3), *Providencia* sp (2) and *Bacillus* sp (2). Majority of the isolates obtained in this study showed resistance to heavy metals at different concentrations and also showed resistance to multiple antibiotics. This study has shown that most bacterial isolates present in heavy metals contaminated sites are usually resistant to multiple antibiotics and heavy metal salts. The ability of the isolates to tolerate different concentrations of heavy metal salts can however be explored and channelled towards alleviating the burden of heavy metal contamination in the environment through bioremediation.

Key words: Antimicrobial Susceptibility · Bioremediation · Contaminant · Heavy Metal · Molecular

INTRODUCTION

Environmental pollution caused by the release of a wide range of compounds resulting from industrialization has become a major concern worldwide. Thousands of hazardous waste sites have been generated globally as a result of accumulation of xenobiotics in soil and water over the years [1]. Heavy metals contamination of soil is a major culprit in environmental pollution. According to Suruchi and Khanna [2] heavy metals refer to metals such as arsenic, cadmium, chromium, copper, lead, nickel, molybdenum, vanadium and zinc. Also of interest are metals such as aluminium, cobalt, strontium and other rare metals.

Metal contaminants can be produced through industrial processes such as mining, refining, electroplating, metal finishing, leather tanning, chrome preparation, production of batteries, phosphate fertilizers, pigments, stabilizers and alloys [3]. The discharge of wastewaters containing high levels of heavy metals from a wide variety of industries had impacted both the aquatic and soil environments negatively [4]. Metals solubility in soils depends mainly on the soil pH, organic carbon, Cation Exchange Capacity (CEC), redox conditions and clay contents [5-8]. These metal contaminants pose adverse health effects to those who live near these polluted sites. Chronic exposure to these contaminants can cause permanent kidney and brain damage [9]. The

Corresponding Author: Osibote, Iyadunni Adesola, Department of Microbiology, College of Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. Postal Address: P.O. Box: 1056, Ado-Ekiti, Ekiti State, Nigeria.
Tel: +234-8034336722.

heavy metals can accumulate within the body organs over time and constitute serious disruption to normal body function [10-12].

Most bacteria species possessing resistance to heavy metals are also observed to be resistant to most antibiotics [13]. Bacterial resistance to antibiotics and heavy metals is an increasing problem nowadays. Resistance to antibiotics is acquired by a change in the genetic makeup of an organism, this can be as a result of gene mutation or by transfer of antibiotic resistance genes between bacteria in the environment [14]. The increasingly use of antibiotics in health care, in agriculture and animal husbandry is in turn contributing to the growing problem of antibiotic resistant bacteria [15]. Heavy metals used in industry and in household products together with antibiotics are creating a selective pressure in the environment that leads to mutation in microorganism thus aiding them to survive and multiply in such environments [16]. Heavy metals tolerance and antibiotic resistance in bacteria are closely related this is due to the likelihood that resistance genes to both antibiotics and heavy metals may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment [14].

This study was therefore designed to isolate and identify bacterial species associated with heavy metals contaminated sites and to determine the susceptibility of the isolates to antibiotics and salts of heavy metals.

MATERIALS AND METHODS

Sample Collection: Soil contaminated with heavy metals as a result of the activities of a steel rolling company was used for this study. Composite top soil sample was collected from different points of the study site using soil auger and this was conveyed to the laboratory for microbiological evaluation.

Isolation of Microorganisms: The agar medium and the diluents used were sterilized at 121°C for 15 minutes. Serial dilution was carried out and one millilitre of appropriate dilutions was inoculated into sterile Petri dishes and already prepared and cooled nutrient agar (Lab M, United Kingdom) was added to it using the pour plate technique as described by Olutiola *et al*. [17]. Inoculated plates were incubated at 37°C for 24 hours after which distinct bacteria colonies were counted. Morphologically distinct bacteria colonies were subcultured by streaking on fresh nutrient agar plates until pure bacteria colonies were obtained. Pure cultures of each bacteria strain were stored on nutrient agar slants at 4°C for further studies. Pure bacterial isolates were subjected to various biochemical tests to aid their identification.

Molecular Characterisation of Bacterial Isolates

16S rRNA Based Identification: Isolation of 16S rRNA gene of the bacterial isolates was carried out using QIAamp DNA Mini Kit (250) cat no 51306 after which the sequence were amplified using Applied Biosystems Thermocycler, model 9800. Sequencing of the 16S rRNA was carried out using a 16-well Applied Biosystems sequencing plate following the manufacturer's instructions. The obtained sequences of bacterial 16S rRNA were analysed using Sequence Scanner (Applied Biosystems) software and the 16S rRNA sequence contigs were generated using Chromas Pro. The online program BLASTn was used to find out the related sequences with known taxonomic information in the databank at NCBI website (http://www.ncbi.nlm.nih.gov/BLAST) to accurately identify the bacterial strain.

Determination of the Susceptibility of Bacterial Isolates to Heavy Metal Salts: The susceptibility of the bacterial isolates to increasing concentration of heavy metals of chromium, cadmium, lead, copper, cobalt, nickel and zinc was determined quantitatively using the agar diffusion method following the method described by Narasimhulu *et al.* [13]. Concentration of heavy metals in nutrient agar medium was gradually increased from 100-500 µg/mL. The screening was done by streaking a 24 hours old culture of the test organism on nutrient agar plate supplemented with 100 µg/mL of the salt of the heavy metals of interest and was incubated for five days. Isolates that grew at this concentration were sub-cultured to nutrient agar plates supplemented with higher concentration of the heavy metals until 500 µg/mL concentration of heavy metal at increasing level of 50 µg/mL.

Antibiotic Susceptibility of Bacterial Isolates: The isolates obtained from the samples were subjected to antibiotic susceptibility test following the Clinical and Laboratory Standards Institute (CLSI) method using Kirby-Bauer disk diffusion test on Muller-Hinton agar (Oxoid CM0337 Basingstoke, England) [18]. Each isolate was inoculated into nutrient broth separately and incubated for 24 hours at 37°C. The broth was streaked using sterile cotton swabs on Mueller-Hinton Agar plates.

This was followed by aseptic placement of the antibiotic discs using sterile forceps. The plates were incubated aerobically at 37°C for 24 hours, after which the zones of inhibition were measured and interpreted according to CLSI [18]. Antibiotics used for Gram negative isolates were Ceftazidime (CAZ) (30µg), Cefuroxime (CRX) (30µg), Gentamicin (GEN) (10µg), Ceftriaxone (CTR) (30µg), Erythromycin (ERY) (5µg), Cloxacillin (CXC) (5µg), Ofloxacin (OFL) (5µg) and Amoxycillin/ Clavulanate (AUG) (30µg) while those used for Gram positive isolates were Ceftazidime (CAZ) (30µg), Cefuroxime (CRX) (30µg), Gentamicin (GEN) (10µg), Ciprofloxacin (CPR) (5 µg), Ofloxacin (OFL) (5µg), Amoxycillin/ Clavulanate (AUG) (30µg), Nitrofurantoin (NIT) (200µg) and Ampicillin (AMP) (20 µg).

RESULTS

Thirty five bacterial isolates were obtained from soil contaminated with heavy metals. Two of the isolates were Gram positive while the remaining thirty three were Gram negative. The various biochemical identification tests carried out on the isolates revealed that the isolates were distributed in this manner, *Pseudomonas* spp (11), *Proteus mirabilis* (5), *Alcaligenes faecalis* (5), *Pseudomonas putida* (3), *Pseudomonas fluorescens* (3), *Enterobacter* spp (3), *Pseudomonas azotoformans* (2), *Providencia* spp (2), *Bacillus mycoides* (1) and *Bacillus subtilis* (1). Figure 1 shows the distribution of the isolated bacteria into various genera based on the results obtained from the biochemical tests. The result of molecular characterization carried out using the 16S rRNA sequences showed that the bacterial isolates belong to different phylogenetic groups. Twenty-seven (77.14%) of the isolates belong to the group Gamma (?) proteobacteria and in the genera *Proteus, Azotobacter, Pseudomonas, Providencia, Shewanella, Citrobacter and Pantoea* while five (14.29%) of the isolates belong to the Beta (β) proteobacteria and in the genera *Alcaligenes, Paenalcaligenes, Castellaniella.* Two (5.71%) of the bacteria isolates belong to the group of Firmicutes in the genera *Bacillus,* only one (2.86%) isolate was found to belong to the group Alpha (α) proteobacteria in the genera *Brucella* as shown in Figure 2. Based on the data base information available on National Centre for Biotechnology Information (NCBI) site using the Basic Local Alignment Search Tool (BLAST) (blastn), the isolates were classified and identified using the highest percentage similarity with organism of the nearest homology as shown in Table 1.

Table 2 shows the result of the various heavy metal salts on the isolates. It was observed that most of the isolates could tolerate the tested heavy metal salts. Most of the bacteria isolates were observed to grow between 150 and 350 µg/mL of the different heavy metal salts used with the exception of *Alcaligenes aquatilis, Pseudomonas mucidolens* and *Bacillus mycoides* which were able to tolerate some of the heavy metal salts to 450 µg/mL. Some isolates however could not tolerate the varying concentrations of the heavy metals salts used and as such they failed to grow at all the concentrations used for the determination of the minimum inhibitory concentration of heavy metal salts on bacterial isolates.

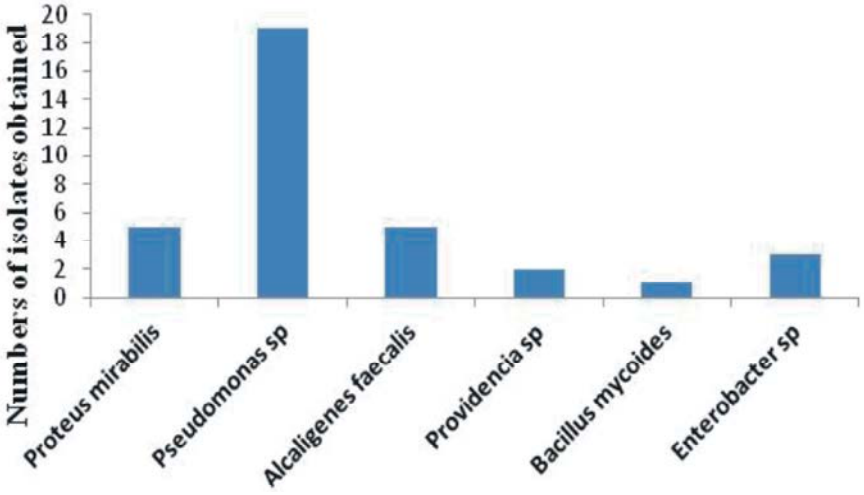

Fig. 1: Distribution of isolated bacteria

Table 1: Phylogenetic Identities of the bacterial isolates using blastn

S/N	Probable organism identified with biochemical test	Length of the nucleotide sequences	% similarity	Accession number of nearest Homology	Name of the organism from NCBI using blastn	Phylogenetic group
1	*Alcaligenes faecalis*	802	99	NR 025357.1	*Alcaligenes faecalis subsp. parafaecalis*	β- proteobacteria
2	*Proteus mirabilis*	973	91	NR 074898.1	*Proteus mirabilis*	?- proteobacteria
3	*Proteus mirabilis*	963	100	NR 074898.1	*Proteus mirabilis*	?- proteobacteria
4	*Proteus mirabilis*	973	70	NR 074898.1	*Proteus mirabilis*	?- proteobacteria
5	*Proteus mirabilis*	878	99	NR 113344.1	*Proteus mirabilis*	?- proteobacteria
6	*Proteus mirabilis*	1008	57	NR 074898.1	*Proteus mirabilis*	?- proteobacteria
7	*Pseudomonas* sp.	826	70	NR 029063.1	*Pseudomonas rhizosphaerae*	?- proteobacteria
8	*Pseudomonas* sp.	1037	99	NR 074597.1	*Pseudomonas syringae*	?- proteobacteria
9	*Alcaligenes faecalis*	973	77	NR 114959.1	*Alcaligenes aquatilis*	β- proteobacteria
10	*Alcaligenes faecalis*	956	70	NR 116967.1	*Paenalcaligenes hominis*	β- proteobacteria
11	*Pseudomonas putida*	927	99	NR 074596.1	*Pseudomonas putida*	?- proteobacteria
12	*Pseudomonas* sp.	966	100	NR 025588.1	*Pseudomonas proteolytica*	?- proteobacteria
13	*Pseudomonas* sp.	923	98	NR 074798.1	*Shewanella oneidensis*	?- proteobacteria
14	*Pseudomonas* sp.	964	100	NR 026395.1	*Pseudomonas graminis*	?- proteobacteria
15	*Pseudomonas azotoformans*	902	76	NR 043422.1	*Pseudomonas mucidolens*	?- proteobacteria
16	*Pseudomonas* sp.	936	95	NR 112075.1	*Pseudomonas veronii*	?- proteobacteria
17	*Providencia* sp.	981	95	NR 102978.1	*Providencia stuartii*	?- proteobacteria
18	*Providencia* sp.	943	90	NR 042412.1	*Providencia heimbachae*	?- proteobacteria
19	*Pseudomonas* sp.	977	53	NR 041296.1	*Shewanella hafniensis*	?- proteobacteria
20	*Pseudomonas* sp.	994	35	NR 119141.1	*Shewanella putrefaciens*	?- proteobacteria
21	*Pseudomonas* sp.	769	100	NR 114233.1	*Shewanella decolorationis*	?- proteobacteria
22	*Bacillus mycoides*	946	97	NR 114582.1	*Bacillus cereus*	Firmicutes
23	*Alcaligenes faecalis*	803	97	NR 044802.1	*Castellaniella denitrificans*	β- proteobacteria
24	*Enterobacter* sp.	971	96	NR 102823.1	*Citrobacter koseri*	?- proteobacteria
25	*Enterobacter* sp.	1004	95	NR 126319.1	*Cedecea lapagei*	?- proteobacteria
26	*Pseudomonas putida*	972	99	NR 040992.1	*Pseudomonas japonica*	?- proteobacteria
27	*Enterobacter* sp.	936	97	NR 111998.1	*Pantoea agglomerans*	?- proteobacteria
28	*Pseudomonas* sp.	975	93	NR 116732.1	*Shewanella xiamenensis*	?- proteobacteria
29	*Alcaligenes faecalis*	988	98	NR 025357.1	*Alcaligenes faecalis subsp. parafaecalis*	β- proteobacteria
30	*Pseudomonas fluorescens*	718	99	NR 028706.1	*Pseudomonas veronii*	?- proteobacteria
31	*Bacillus subtilis*	915	77	NR 113945.1	*Bacillus safensis*	Firmicutes
32	*Pseudomonas fluorescens*	669	100	NR 028986.1	*Pseudomonas poae*	?- proteobacteria
33	*Pseudomonas* sp.	952	56	NR 103935.1	*Brucella suis*	á- proteobacteria
34	*Pseudomonas azotoformans*	973	99	NR 102514.1	*Pseudomonas poae*	?- proteobacteria
35	*Pseudomonas putida*	978	86	NR 074739.1	*Pseudomonas putida*	?- proteobacteria

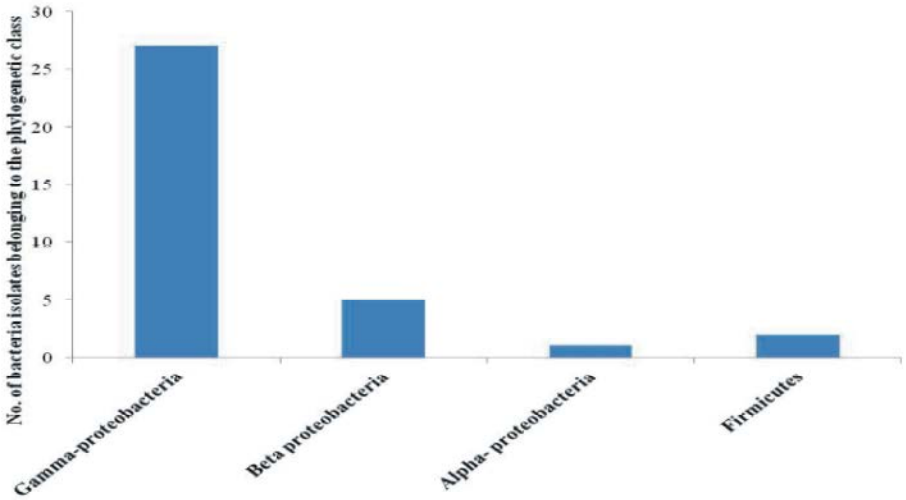

Fig. 2: Distribution of the isolaed bacteria into phylogentic class

Table 2: Susceptibility of isolates to heavy metal salts

S/N	Name of organism based on molecular studies	Concentration of heavy metals (µg/ml)						
		Cadmium	Copper	Chromium	Nickel	Lead	Cobalt	Zinc
1	*Alcaligenes faecalis subsp. parafaecalis*	200	300	-	-	-	200	250
2	*Proteus mirabilis*	-	-	-	-	-	-	-
3	*Proteus mirabilis*	-	300	350	300	350	200	350
4	*Proteus mirabilis*	250	-	-	-	-	-	-
5	*Proteus mirabilis*	-	300	350	300	350	150	350
6	*Proteus mirabilis*	-	-	350	300	300	150	300
7	*Pseudomonas rhizosphaerae*	250	-	350	300	300	300	300
8	*Pseudomonas syringae*	350	300	-	300	350	-	-
9	*Alcaligenes aquatilis*	400	400	400	400	400	-	-
10	*Paenalcaligenes hominis*	-	250	-	-	-	-	300
11	*Pseudomonas putida*	-	300	300	300	350	300	350
12	*Pseudomonas proteolytica*	-	-	200	200	300	-	300
13	*Shewanella oneidensis*	-	300	300	300	300	250	300
14	*Pseudomonas graminis*	-	300	300	-	200	250	300
15	*Pseudomonas mucidolens*	200	150	400	350	400	350	400
16	*Pseudomonas veronii*	-	-	-	-	-	-	-
17	*Providencia stuartii*	-	300	200	300	300	300	300
18	*Providencia heimbachae*	200	-	200	250	-	250	-
19	*Shewanella hafniensis*	-	200	300	300	300	300	300
20	*Shewanella putrefaciens*	-	-	200	300	300	250	300
21	*Shewanella decolorationis*	250	300	200	300	300	200	300
22	*Bacillus cereus*	450	450	250	400	450	250	400
23	*Castellaniella denitrificans*	300	-	-	300	-	-	300
24	*Citrobacter koseri*	-	300	300	300	300	150	300
25	*Cedecea lapagei*	-	-	-	-	-	-	-
26	*Pseudomonas japonica*	250	300	-	300	300	300	300
27	*Pantoea agglomerans*	300	-	-	300	150	150	300
28	*Shewanella xiamenensis*	-	-	-	300	150	-	-
29	*Alcaligenes faecalis subsp. parafaecalis*	200	-	-	300	-	-	259
30	*Pseudomonas veronii*	-	300	-	-	-	-	300
31	*Bacillus safensis*	-	300	-	-	-	-	300
32	*Pseudomonas poae*	-	300	-	-	-	-	300
33	*Brucella suis*	-	300	300	200	300	200	300
34	*Pseudomonas poae*	-	-	-	-	-	-	-
35	*Pseudomonas putida*	300	-	-	-	-	-	300

Table 3: Antibiotic susceptibility of Gram positive isolates

ORGANISM	CAZ (30 µg)	CRX (30 µg)	GEN (10 µg)	CPR (5 µg)	OFL (5 µg)	AUG (30 µg)	NIT (300 µg)	AMP (20 µg)
Bacillus cereus	R	R	S	S	S	R	R	R
Bacillus safensis	R	R	S	I	S	R	S	R

Key: R- Resistant, S- Susceptible, I- Intermediate

Table 3 shows the result of the antibiotic susceptibility test of Gram positive isolates. The two Gram positive isolates were susceptible to antibiotics such as Gentamicin, Ciprofloxacin and Ofloxacin while *Bacillus safensis* alone showed susceptibility to Nitrofurantoin. Both *Bacillus cereus* and *Bacillus safensis* were resistant to Ceftazidime, Cefuroxime, Amoxycillin/Clavulanate and Ampicillin whereas only *Bacillus cereus* was resistant to Nitrofurantoin.

Table 4 shows the result of the antibiotic susceptibility test carried out on the Gram negative isolates. The results showed that 90.91% of the isolates were resistant to cefuroxime, this was followed by the resistance of the bacteria to erythromycin (72.73%), however 66.67% of the isolates were observed to be susceptible to ceftazidime.

Table 4: Antibiotic susceptibility of Gram negative isolates

S/N	Name of organism based on molecular studies	GEN (10ug)	CTR (30ug)	ERY (5ug)	CXC (5ug)	OFL (5ug)	AUG (30ug)	CAZ (30ug)	CRX (30ug)
1	*Alcaligenes faecalis subsp. parafaecalis*	S	S	R	R	S	R	R	R
2	*Proteus mirabilis*	S	S	R	R	S	R	R	R
3	*Proteus mirabilis*	S	R	R	R	S	R	R	R
4	*Proteus mirabilis*	S	S	R	R	S	R	R	R
5	*Proteus mirabilis*	R	S	R	R	S	R	R	R
6	*Proteus mirabilis*	S	S	R	R	S	R	S	R
7	*Pseudomonas rhizosphaerae*	S	S	R	R	S	R	S	S
8	*Pseudomonas syringae*	S	R	R	R	S	R	R	R
9	*Alcaligenes aquatilis*	R	R	R	R	R	S	S	R
10	*Paenalcaligenes hominis*	R	R	R	I	R	R	S	R
11	*Pseudomonas putida*	R	R	R	S	R	R	S	R
12	*Pseudomonas proteolytica*	R	R	R	S	R	R	S	R
13	*Shewanella oneidensis*	R	R	R	R	R	R	S	R
14	*Pseudomonas graminis*	R	R	R	S	I	R	S	R
15	*Pseudomonas mucidolens*	R	R	R	I	R	R	S	R
16	*Pseudomonas veronii*	R	R	R	S	R	R	S	R
17	*Providencia stuartii*	R	R	R	I	R	R	S	R
18	*Providencia heimbachae*	S	R	I	S	S	S	S	R
19	*Shewanella hafniensis*	R	R	R	R	R	R	S	R
20	*Shewanella putrefaciens*	R	R	R	S	S	R	S	R
21	*Shewanella decolorationis*	S	R	R	R	R	R	R	S
22	*Castellaniella denitrificans*	R	R	R	R	R	R	R	R
23	*Citrobacter koseri*	R	R	R	R	R	R	R	R
24	*Cedecea lapagei*	R	R	R	R	R	R	R	R
25	*Pseudomonas japonica*	S	R	R	R	R	R	R	R
26	*Pantoea agglomerans*	S	R	S	R	R	I	S	R
27	*Shewanella xiamenensis*	S	R	S	S	S	S	S	R
28	*Alcaligenes faecalis subsp. parafaecalis*	R	R	S	S	I	S	S	R
29	*Pseudomonas veronii*	S	R	I	I	S	S	S	R
30	*Pseudomonas poae*	R	R	S	S	S	S	S	R
31	*Brucella suis*	R	R	I	S	S	S	S	R
32	*Pseudomonas poae*	R	R	S	R	S	S	S	I
33	*Pseudomonas putida*	R	R	S	S	S	S	S	R
% Susceptibility		39.39	18.18	27.27	45.45	54.55	30.30	66.67	9.09
% Resistance		60.61	81.82	72.73	54.55	45.45	69.70	33.33	90.91

Key: R- Resistant, S- Susceptible, I- Intermediate

DISCUSSION

The isolates obtained in this study were *Pseudomonas* sp. (11), *Proteus mirabilis* (5), *Alcaligenes faecalis* (5), *Pseudomonas putida* (3), *Pseudomonas fluorescens* (3), *Enterobacter* sp. (3), *Pseudomonas azotoformans* (2), *Providencia* sp. (2), *Bacillus mycoides* (1) and *Bacillus subtilis* (1). All these bacteria have been implicated as having the ability to withstand the toxicity of heavy metals in heavy metals contaminated site [19, 20]. The diversity of the microorganisms isolated in this study belonged to four divisions. Twenty-seven (77.14%) of the isolates belong to the group Gamma (□) proteobacteria which were in the genera *Proteus, Azotobacter, Pseudomonas, Providencia, Shewanella, Citrobacter* and *Pantoea*, while five (14.29%) of the isolates belonged to the Beta (â) proteobacteria which are in the genera *Alcaligenes, Paenalcaligenes* and *Castellaniella*. Two (5.71%) of the bacteria isolates belonged to the group of Firmicutes in the genera *Bacillus,* only one (2.86%) isolate was found to belong to the group Alpha (α) proteobacteria in the genera *Brucella.* Majority of these isolates especially *Pseudomonas, Providencia, Shewanella, Alcaligenes* and *Bacillus* species have been cited in earlier studies as having ability to tolerate heavy metals in the environment [21-23].

Majority of the isolates obtained in this study showed resistance to both heavy metals and various antibiotics tested against it. Bacterial resistance to toxic

heavy metals is a widespread phenomenon and reported to enhance the antibiotic resistance ability of microorganisms [24]. Previous studies by Kawane [14] revealed that there is a correlation between tolerance to heavy metals and resistance to antibiotics in bacterial isolates, this is due to the likelihood that resistance genes to both heavy metals and antibiotics may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment.

The two *Bacillus* sp. isolated in this study were observed to be susceptible to Gentamicin, Ciprofloxacin and Ofloxacin while they showed resistance to Ceftazidime, Cefuroxime, Amoxycillin/Clavulanate and Ampicillin. The *Bacillus cereus* obtained in this study showed high tolerance to multiple heavy metal salts and also was multi drug resistant. This is in agreement with the work of Samata and co-workers [25] in which they observed that some *Bacillus* species were multi-drug resistant and could tolerate wide range of heavy metals at the same time.

Among the Gram negative isolates obtained in this study, 90.91% of the isolates showed resistance to Cefuroxime while 33.33% of the isolates were susceptible to Ceftazidime. It was also observed that four of the Gram negative isolates namely: *Proteus mirabilis, Pseudomonas veronii, Cedecea lapagei* and *Pseudomonas poae* could not tolerate the heavy metals salt at all the tested concentrations and this resulted in their failure to grow, others were able to tolerate a wide range of heavy metals salts and were also resistant to multiple antibiotics.

CONCLUSIONS

This study has shown that most bacterial isolates present in heavy metals contaminated sites are usually resistant to multiple antibiotics as well as heavy metal salts. This is a threat both to the flora and fauna of the environment as the resistant genes can be transferred to microorganisms of public health importance in the environment. However, a positive twist to it is that organisms such as *Alcaligenes aquatilis, Pseudomonas mucidolens* and *Bacillus cereus* isolated in this study which were resistant to multiple antibiotics, showed high tolerance to heavy metals salts, this ability to tolerate different concentrations of heavy metal salts can be explored and channelled towards alleviating the burden of heavy metal contamination in the environment through bioremediation.

REFERENCES

1. Boricha, H. and M.H. Fulekar, 2009. Pseudomonas plecoglossicida as a novel organism for the bioremediation of cypermethrin. Biology and Medicine, 1: 1-10.
2. Suruchi and P. Khanna, 2011. Assessment of Heavy Metal Contamination in Different Vegetables Grown in and Around Urban Areas. Research Journal of Environmental Toxicology, 5: 162-179.
3. Dixit, R., M.D. Wasiullah, K. Pandiyan, U.B. Singh, A. Sahu, R. Shukla, B.P. Singh, J.P. Rai, P.K. Sharma, H. Lade and D. Paul, 2015. Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes. Sustainability, 7: 2189-2212.
4. Stephens, W.E. and A. Calder, 2005. Source and health implications of high toxic metal concentrations in illicit tobacco products. Environmental Science and Technology, 39: 479-488.
5. Hough, R.L., S.D. Young and N.M.G. Crout, 2003. Modeling of Cd, Cu, Ni, Pb and Zn uptake by winter wheat and forage maize, from a sewage disposal form. Soil Use and Management, 19: 19-27.
6. Walker, D.J., R. Clemente, A. Roig and M.P. Bernal, 2003. The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. Environmental Pollution, 122: 303-312.
7. Kashem, M.A. and B.R. Singh, 2004. Transformation in solid phase species of metals as affected by flooding and organic matter additions in contaminated soils. Communications in Soil Science and Plant Analysis, 35: 1435-1456.
8. Rieuwerts, J.S., M.R. Ashnore, M.E. Farago and I. Thornton, 2006. The influence of soil characteristics on the extractability of Cd, Pb and Zn in upland and moorland soils. Science of the Total Environment, 366: 864-875.
9. United State Environmental Protection Agency (USEPA) 2004. Cleaning Up the Nation's Waste Sites: Markets and Technology Trends. EPA 542-R-04-015.
10. Jarup, L., 2003. Hazards of heavy metals contamination. British Medical Bulletin, 68: 167-182.
11. Sathawara, N.G., D.J. Parikish and Y.K. Agrwal, 2004. Essentials heavy metals in environmental samples from western Indian. Bulletin of Environmental Contamination and Toxicology, 73: 756-761.

12. Curtis, L.R. and B.W. Smith, 2008. Heavy metals in fertilizers: Considerations for setting regulations in Oregon. Department of Environmental and molecular Toxicology: Oregon State university Corvallis Oregon, http: www Oregon.gov/ODA/TEST/docs/pdf/ferthes yy met.pdf
13. Narasimhulu, K., P.S. Rao and A.V. Vinod, 2010. Isolation and identification of bacterial strains and study of their resistance to heavy metals and antibiotics. Journal of Bacterial and Biochemical Technology, 2: 074-076.
14. Kawane, R.S., 2012. Studies on antibiotics and heavy metal resistance profiling of Escherichia coli from drinking water and clinical specimens. Bioscience Discovery, 3: 292-295.
15. Osibote, I.A., P.A. Okiki, E.A. Ekundayo and A.C. Adekunle, 2014. Prevalence of Multidrug Resistant Bacterial Isolates from Meat Processing Equipment and Abattoir Environment in Ado Ekiti Advances in Biological Research, 8: 207-211.
16. Calomiris, J., L. Armstrong and J. Seidler, 1994. Association of Metal Tolerance with multiple antibiotic resistances of bacteria isolated from drinking water. Applied Environmental Microbiology, 47: 1238-1242.
17. Olutiola, P.O., O. Famurewa and H.G. Sonntag, 2000. An introduction to general microbiology: A practical approach, Hygiene Institute Der Universitat Heldelberg, pp: 157-180.
18. Clinical and Laboratory Standards Institute (CLSI), 2013. M100-S23 Documents: Performance standards for antimicrobial susceptibility testing; twenty third informational supplement.
19. Guha, H., K. Jayachandranand and F. Maurrasse, 2001. Kinetics of chromium (VI) reduction by a type strain Shewanella alga under different growth conditions. Environmental Pollution, 115: 209-218.
20. Camargo, F.A., B.C. Okeke, F.M. Bento and W.T. Frankenberger, 2003. In vitro reduction of hexavalent chromium by a cell-free extract of Bacillus sp. ES 29 stimulated by Cu^{2+}. Applied Microbiology and Biotechnology, 62: 569-573.
21. Rajendran, P., J. Muthukrishman and P. Gunasekaran, 2003. Microbes in heavy metal remediation. Indian Journal of Experimental Biology, 41: 935-944.
22. De, J., N. Ramaiah and I. Vardanyan, 2008. Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Marine Biotechnology, 10: 471-477.
23. Pandey, B. and M.H. Fulekar, 2012. Bioremediation technology: A new horizon for environmental clean-up. Biology and Medicine, 4: 51-59.
24. Edlund C., E.J. Bjorkmanl, G. Sadbargh–England and C.E. Nord, 1996. Resistance of the normal human micro flora to mercury and antimicrobial after exposure to mercury from dental amalgam fillings. Clinical and Infectious Disease., 22: 944-950.
25. Samata, A., P. Bera, M. Khatun, C. Sinha, P. Pal, A. Lalee and A. Mandal, 2012. An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain Bacillus sp. isolated from municipal waste. Journal of Microbiology and Biotechnology Research, 2: 178-189.