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Characterization of Multidrug Antibiotics Resistant Strains from Soil Samples

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A B S T R A C T

The spread of antibiotic residues increases the burden of antibiotic resistance microorganisms. The purpose of this study was to determine the antibiotic resistance pattern in isolated bacteria from Isfahan soils. The soil samples collected from agricultural lands (19 samples) and non-agricultural (industrial) (16 samples). The resistant bacterial population was determined using five antibiotics including erythromycin, vancomycin, and co-trimoxazole (0.5 mg/L), gentamicin (1 mg/L) and, penicillin G (2mg/L) and identified by standard cultural, morphological and biochemical characteristics. Antibiotic susceptibility of the isolates determined by minimum inhibitory concentration (MIC) by macro broth dilution. Subsequently, minimum bactericidal concentration (MBC) and multiple antibiotic resistance (MAR) profiles were determined. A total of 152 bacteria from different genera such as *Staphylococcus*, *Corynebacterium* and *Bacillus* sp were determined. The incidence of gentamicin and erythromycin-resistant bacteria were highest in agricultural and industrial soils, respectively. The MIC and MBC concentration of penicillin G were significantly higher than other antibiotics. Results revealed that 84% of isolates were MAR which half of them were tetra and penta-resistance to antibiotics. Many factors such as the Zayanderud River and using of livestock is affected by bacterial resistance in soil and water.

1. Introduction

Antibiotic-resistant bacteria are serious public health because of antimicrobials compounds which enter an environment in different ways [1, 2]. A natural environment is a source of antibiotic-resistant bacteria. Farm animals when exposed to a considerable amount of antimicrobial compounds act as a reservoir of antibiotic resistance genes they transmit to humans through the food chain, direct animal contact, and the environment. Using of Livestock manure spread the antibiotic resistance [2]. The antibiotic could easily degrade the environment although, an unmetabolized case is passed into wastewater and then affected by soil and sediment. The high active microbes that exposed to the disposal of antibiotics and resistance gene transmitted into bacteria in contaminated environments [2, 3].The soil is an important niche for many kinds of microorganisms can produce antibiotics. Many of soil-dwelling bacteria develop diverse ways to survive or resist in front of antimicrobial compounds produced by other neighboring in the soil [4] In this paper, the

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antibiotics resistant bacteria studied in soil samples of Isfahan. The study about antibiotic resistance is scanty in this area and could be useful to explorer the contaminant factors and their effects.

2. Materials and Methods

2.1. Antibiotics

The following antibiotics used in this study included: Erythromycin, vancomycin, gentamicin, penicillin G, and co-trimoxazole. The antibiotics prepared from Sigma-Aldrich, diluted according to manufacturer's instructions and kept at -20°C. The used concentration of erythromycin, vancomycin and, co-trimoxazole was 0.5 mg/L, 1 mg/L for gentamicin and, 2mg/L for penicillin G. The used concentration of erythromycin, vancomycin and, co-trimoxazole was 0.5 mg/L, 1 mg/L for gentamicin and, 2mg/L for penicillin G.

2.2. Soil samples analysis

The soil samples collected as random places from 19 agricultural lands, most of them obtained from Lenjan city near to Zayanderud River that is known by rice farms and 16 samples collected from non-agricultural area especially around Isfahan steel company and industrial factory sites. The map shows the sampling locations in Lenjan which the most of agricultural samples collected from this area (Figure 1). The soil samples collected from the surface (0-15cm depth). The collected soil diluted by water at the ratio 1:2 and pH determined using pH-meter and the texture of the soil monitored by hydrometer. The Electrical conductivity (EC) and NaCl concentration for all of the samples determined by the conductivity meter [5]. The serial dilution of soil prepared and the solution was shaken and allowed to settle for 5 minutes.

2.3. Bacteriological Examination

2.3.1. Total bacterial population: The total number of bacteria was determined using standard plate count (SPC) method in nutrient agar. One gram of soil sample dissolved in 10 mL of distilled water and serial dilution was made up to 10^{12} times. The plates incubated for 2-4 days at 30°C and the bacterial population of bacteria determined by colony forming unit (CFU) [3, 6].

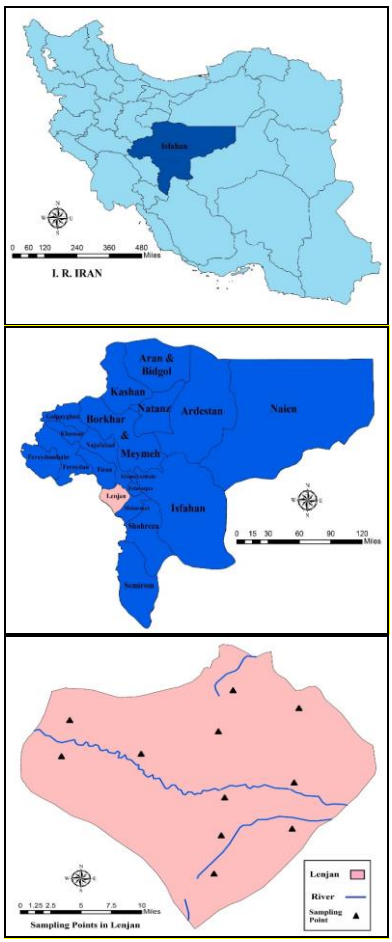

Figure 1. Agricultural sampling location from Lenjan (Isfahan Province) near of Zayanderud River

2.3.2. Determination of antibiotic-resistant bacteria: The population of the resistant bacteria were determined in Muller Hinton agar media containing antibiotics by agar dilution method [7]. The using concentration of erythromycin, vancomycin, and co-trimoxazole was 0.5 mg/L and gentamicin and penicillin G used by 1 mg/L and 2mg/L, respectively. The resistant bacteria evaluated by colony morphology (pigment, surface, margin, and elevation) and the selected isolates were cultured in nutrient agar plates containing antibiotic. The bacteria identified using different staining's such as gram and acid-fast as well as the biochemical test [3, 8].

2.3.3. Determination of MIC and MBC for resistant bacteria: The minimum inhibitory concentration (MIC) of antibiotics defined by macro broth

dilution. The minimum bactericidal concentration (MBC) were determined using culture on Muller Hinton agar medium. The multiple antibiotic resistance (MAR) profiles were appointed for all resistant isolates based on tested antibiotics [9, 10].The multiple antibiotic resistance (MAR) index for each isolates determined by ration a/b that (a): the number of antibiotics to which the isolate was resistant and (b): represents the total number of antibiotics to which the isolate exposed.

3. Results & Discussion

The results of soil sample analysis shown all samples had pH at the range of 7.3-8.1 and the NaCl concentrations were very low. The texture of most of the soil samples from agricultural and industrial lands area was loam and loam-clay, respectively. The proportion of 0.1 to 100% of the total soil bacteria was resistant to at least one antibiotic. The population of antibiotics resistant bacteria determined for both types of soils which their mode show in Table 1.

Table 1. The results of antibiotic susceptibility assay (the mode percentage) of resistant bacteria according to sampling places

Antibiotic	Agricultural soils (%)	Industrial soils (%)
Erythromycin	38.1	34.5
Vancomycin	37	28.7
Gentamicin	41.2	28.7
Penicillin G	7.5	1.5
Co-trimoxazole	13	11

The erythromycin-resistant bacteria isolated by concentration of 0.5 mg/L that the range of resistance population was variable from > 0.1 to 100% of the total population and the mode of erythromycin resistance was 38.1% from the 19 agricultural soils that indicate a high level of resistance among these bacteria. In the second group (non-agricultural soils), the resistance to erythromycin was in the range of 12-100% of total bacteria with the mode of isolates was 34.5% of the total erythromycin-resistant population. The vancomycin-resistant bacteria were in the range of 8-100% and 0.1-100% of the total bacteria for the agricultural and industrial soils, respectively. The mode of them is 37% and 28.7% for the first and second groups at the concentration of 0.5 mg/L of vancomycin. The range of gentamicin-resistant bacteria to 1 mg/L in agricultural samples was varied at the range of 16-88% of the total bacteria population and in industrial soils, the resistance was at range 11-84% which the mode of resistance were 41.2 and 28.7% for the first and second groups, respectively. The results for resistance to penicillin G showed the lower population percentage of resistant bacteria than other antibiotics because of used concentration (2 mg/L) of penicillin G, that was 0.2- 66% of the total isolates in agricultural lands with the mode of 7.5% and 0.1-8.4% from industrial soils by mode of 1.5% were resistant. The last antibiotic was co-trimoxazole at the concentration of 0.5 mg/L which 0.1-90% and 0.1 - 84% of isolates from agricultural soils and industrial soils were resistant, respectively. The resistance modes were 13% and 11% for the first and second groups, respectively. The results showed the population of antibiotics resistant isolates from an agricultural area were higher compared with industrial area. The sample collected from a vegetable garden used animal manure and the soils No. 10 and No. 13 which collected from Lenjan city. They were showing the high percentage of antibiotic-resistance that the rice farms frequently were irrigated by Zayanderud River. Among the collected soils from an industrial area, the sample No. 28 around of Isfahan steel Company, showed the highest population of resistant bacteria for all tested antibiotics. A total of 152 bacteria were determined to belong to different genera mostly gram-positive strains such as *Staphylococcus*, *Corynebacterium* and *Bacillus*. The results of bacterial identification showed in Table 2. The population of resistant bacteria to erythromycin is highest that contained 25.6% of total resistant bacteria and population of *Staphylococcus* was more than others which included 25.6%. The vancomycin-resistant bacteria contained 24 isolates. The high population of resistance belongs to *Bacillus firmus* which was 24.3% of resistant bacteria to vancomycin. The resistant bacteria to gentamicin contained 17.7% of total isolates which the abundance of *Staphylococcus aureus* with 33.3% of gentamicin resistance isolates. In the penicillin G resistance that contained 13.1% of total isolates, the

Table 2. The identified resistant bacteria based on used antibiotics

Antibiotics	Total number of isolated bacteria	Resistant bacteria to antibiotic (%)	Type of resistant Bacteria isolated	Number of genuses resistant to antibiotic	Strain resistant to antibiotic (%)
Erythromycin	39	25.6	*Bacillus set A*[1]	9	23
			Bacillus set B[2]	5	12.8
			Bacillus set C[3]	1	2
			Bacillus set D[4]	1	2
			Bacillus firmus	2	5.1
			Corynebacterium	7	17.9
			Kurthia	2	5.1
			Soprolactobacillus	2	5.1
			Staphylococcus	10	25.6
Vancomycin	37	24.3	*Bacillus set A*	7	18.9
			Bacillus set B	5	13.5
			Bacillus set C	1	2.7
			Bacillus set D	2	5.4
			Bacillus firmus	9	24.3
			Corynebacterium	2	5.4
			Kurthia	4	10.8
			Sporolactobacillus	1	2.7
			Staphylococcus	5	13.5
			Micrococcus	1	2.7
Gentamicin	27	17.7	*Bacillus set A*	2	7.4
			Bacillus set B	3	11.1
			Bacillus set C	1	3.7
			Bacillus firmus	1	3.7
			Corynebacterium	3	11.1
			Kurthia	5	18.5
			Staphylococcus	9	33.3
			Streptomyces	1	3.7
			Listeria	1	3.7
			Micrococcus	1	3.7
Penicillin G	20	13.1	*Bacillus set A*	2	10
			Bacillus set B	3	15
			Bacillus set D	2	10
			Corynebacterium	6	30
			Kurthia	4	20
			Overskovia	3	15
Co-trimoxazole	29	19	*Bacillus set A*	8	27.5
			Bacillus set B	1	3.4
			Bacillus firmus	1	3.4
			Corynebacterium	8	27.5
			Kurthia	5	17.2
			Nocardia	1	3.4
			Staphylococcus	2	6.8
			Rhodococcus	1	3.4
			Overskovia	2	6.8

Bacillus set A included to: *B. cereus*, *B. thuringiensis*, *B. laterosporus*, *B. anthracis*
Bacillus set B: *B. alvei*, *B. circulans*, *B. marcescens*, *B. polymyxa*
Bacillus set C: *B. licheniformis*, *B. coagulans*
Bacillus set D: *B. megaterium*, *B. subtilis*, *B. pumilus*, *B.brevis*

presence of the *Corynebacterium* was highest with 30% of total resistant bacteria to penicillin G.

Finally, the population of resistant bacteria to co-trimoxazole was 19% of total resistant bacteria which among them, *Bacillus* set A and *Corynebacterium* with 27.5% showed the highest percentage of resistant strains to co-trimoxazole.

The presence of *Bacillus* sp was considerably higher than other species because this group is extensive in soils and their structures such as spore can help them for high durability in an unusual condition.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determined for all the resistant bacteria. The range of bacterial MIC for each concentration of antibiotic showed in Table 3.

The most common MIC (MIC mode) for resistant bacteria to erythromycin for the agricultural soils and industrial soils were 16 and 2 mg/L, respectively. The MBC for both groups were in the range of 16-256 mg/L. Both MIC and MBC among isolates from agricultural soils were higher than another group. The MIC mode and MBC of vancomycin was 8 mg/L and 128 mg/L for both groups. Vancomycin is a known antibiotic that uses for many infection treatments. The MIC and MBC range for gentamicin for the first group was at range 1-128 mg/L and 8-256 mg/L, respectively and the mode of MIC show 16 mg/L. In the second group, the MIC was at range 2-64 mg/L and the MBC concentration was 8-128 mg/L with MIC mode 4 mg/L. The MIC and MBC range for penicillin G resistant bacteria were at the range of 1-256 mg/L and 8-512 mg/L, respectively. The MIC for co-trimoxazole in the first group and second group were at the range of 1-128 and 1-16 mg/L, respectively. The MIC mode for co-trimoxazole resistant bacteria in the first group was 4 and in the second group was 2 mg/L. The MBC in the first and second groups were at the range of 8-256 mg/L however, the MBC concentration for co-trimoxazole was higher in the agricultural soils. The isolates bacteria from no18, 10, 13 in the first group and no 28 from the second group of soils shown high MIC concentration than other isolates which their previously determined by the high population of antibiotic-resistant bacteria. In some isolates, multidrug resistance (MDR) determined which the resistance profiles, incidence and phenotype of antibiotic-resistant bacteria showed in Table 4. In the total, 152 bacteria identified which 24 isolates equal by %15.78 of total bacteria were resistant to five antibiotics (Penta-R). The number of isolates with four antibiotics resistance (Tetra-R) was 36 cases that included %23.68 of the total cases. In the total, 152 bacteria identified which 24 isolates equal by %15.78 of total bacteria were resistant to five antibiotics (Penta-R). The number of isolates with four antibiotics resistance (Tetra-R) was 36 cases that included %23.68 of the total cases. The most common type of tetra-resistances determined by the simultaneous resistant to erythromycin, vancomycin, penicillin G and co-trimoxazole that contains %12.5 of the isolates.

Table 3. The MIC for the antibiotic-resistant isolates. The parenthesis number shows the percentage of antibiotic-resistant bacteria for each concentration

Antibiotic	mg/L Antibiotic concentration									Number of strain
	1	2	4	8	16	32	64	128	256	
Erythromycin	4* (10%)	8 (20%)	2 (5%)	2 (5%)	8 (20%)	6 (15%)	5 (13%)	4 (10%)	-	39
Vancomycin	5 (14%)	2 (5%)	10 (27%)	14 (38%)	4 (11%)	2 (5%)	-	-	-	37
Gentamicin	1 (3%)	2 (7%)	11 (42%)	6 (22%)	3 (11%)	3 (11%)	1 (3%)	-	-	27
Penicillin G	1 (5%)	2 (10%)	3 (15%)	1 (5%)	1 (5%)	4 (20%)	2 (10%)	3 (15%)	3 (15%)	20
Co-trimoxazole	6 (20%)	6 (20%)	5 (17%)	4 (14%)	5 (17%)	1 (4%)	1 (4%)	1 (4%)	-	29

* The number present the rate of MIC concentration for the selected antibiotic. The number in parenthesis shows the percentage of antibiotic-resistant isolated bacteria related to the same MIC concentration.

The number of resistant bacteria to three antibiotics (Tri-R) was 40 isolates corresponded by %26.31 of the total resistance bacteria which the phenotype resistance to erythromycin, vancomycin and penicillin G with %5.26 of the total resistant cases was the most common type. In double resistance, 28 bacteria determined equally by %18.42 of the total bacterial isolates. The most common type showed by both resistance to erythromycin and co-trimoxazole which show 3.28% of total cases. Finally, the number of isolated bacteria with single resistance (Mono-R) was 24 bacteria which contains %15.78 of the total resistant bacteria which the highest population determined by erythromycin resistance that was %4.6 of the total isolates.

Table 4. Review on the phenotype of antibiotics resistance in the bacteria isolated from soils

Type of multiple resistance	Isolates		Isolates with different multiple resistant			Resistance pattern				
	No (1)	%(2)	No(3)	%(4)	%R(5)	E	V	G	PG	SXT
Penta – R	24	15.78	24	100	15.78	+	+	+	+	+
Tetra- R	36	23.68	8	22.22	5.26	+	+	+	+	-
			7	19.44	4.6	+	+	+	-	+
			14	38.88	12.5	+	+	-	+	+
			2	5.55	1.31	+	-	+	+	+
			5	13.88	3.28	-	+	+	+	-
Tri-R	40	26.31	5	12.5	3.28	+	+	+	-	-
			8	20	5.26	+	+	-	+	-
			1	2.5	0.65	+	-	+	+	-
			1	2.5	0.65	-	+	+	+	-
			7	17.5	4.6	+	+	-	-	+
			3	7.5	1.97	+	-	+	-	+
			4	10	2.63	-	+	+	-	+
			2	5	1.31	+	-	-	+	+
			5	12.5	3.28	-	+	-	+	+
			4	10	2.63	-	-	+	+	+
Double- R	28	18.42	4	14.28	2.63	+	+	-	-	-
			2	7.14	1.31	+	-	+	-	-
			4	14.28	2.63	+	-	-	+	-
			5	17.85	3.28	+	-	-	-	+
			1	3.57	0.65	-	+	+	-	-
			2	7. 14	1.31	-	+	-	+	-
			3	10.71	1.97	-	+	-	-	+
			2	7.14	1.31	-	-	+	+	-
			2	7.14	1.31	-	-	+	-	+
			3	10.71	1.97	-	-	-	+	+
Mono-R	24	15.78	7	29.1	4.6	+	-	-	-	-
			3	12.5	1.97	-	+	-	-	-
			4	16.6	2.63	-	-	+	-	-
			5	20.8	3.28	-	-	-	+	-
			5	20.8	3.28	-	-	-	-	+

E: erythromycin V: vancomycin G: gentamycin P_G: penicillin G SXT: co trimoxazole

(1) The number of resistant bacteria based on selected resistance phenotype (resistance for five antibiotics)
(2) The percentage of isolated antibiotic-resistant bacteria according to the resistance phenotype
(3) The number of isolated bacteria based on antibiotic resistance pattern
(4) The percentage of isolated bacteria with antibiotic resistance pattern in comparison to the selected phenotype
(5) The percentage of resistant bacteria for each antibiotic to total antibiotic-resistant isolates (n=152)

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4. Conclusions

The high prevalence of indigenous antibiotic-resistant bacteria in natural harboring determined as a serious health risk for human and ecosystem [4, 11].. Each variation in natural environments, such as the release of large amounts of antimicrobials compounds change the population dynamics of microorganisms which the prediction and control of their consequences for the human community are so difficult. Inappropriate use of antibiotics in medical and other activities have been pointed out as one of the reasons which resulted to selection and development of drug-resistant microbes [12].The high prevalence of antibiotic-resistant bacteria in natural harbouring is a health risk for human and ecosystem [4, 11]. Their antimicrobial pollution transfers from water and wastewater into soil that impressed all organisms. The aim of this study was isolation and evaluation of antibiotic-resistant bacteria that isolated from different soils in Isfahan Province. The thirty-five samples collected from different soils in agricultural and industrial sites.

Some soil properties such as solubility, electronegative charge, hydrophobicity, and organic matter affected on microbial population and distributions of antibiotic resistance in the environment [13] which in this study a number of physical properties of soil samples including pH, texture, Electrical conductivity (EC) and NaCl concentration didn't show effects to bacterial resistance. In this study, 30-34% of isolates exposed erythromycin resistance. In another study in India, the erythromycin resistance was between 30-70% of isolates from soil and sediment in the industrial area [7]though, in another study the high resistance (97%) was reported[14].

The range of vancomycin-resistant bacteria was at range 28-37% that was similar to study in India which was the range 30-60% [7]. The high rate of resistance (>90%) to vancomycin reported for isolates from urban and agricultural soils of Switzerland [14]. The incidence of vancomycin-resistant bacteria from dairy farm soil and residential garden that enriched with organic manure were considerably high. The anthropogenic factors enhance the frequency and spread of environmental drug-resistant bacteria[1].

The results for gentamicin-resistant bacteria was varied among 28-41% which were similar by some reports including 10-40% [7], 23% [12] however, the higher rate by 56% of gentamicin resistance from agricultural, urban and pristine soils resistant was reported [14]. In some studies, the range of resistant isolates was considerably less than above results at ranged 5-8% of isolates were resistant to gentamicin [4, 15]. In the present study, the population of resistant isolates to penicillin G was less than 10% probably the high concentration of penicillin G used. But, in many studies, the incidence of penicillin resistance was higher than other antibiotics about 31-38% of dairy farm soils[1]. The high percentage were resistant to penicillin G about 85-97% of isolates from the soil, sediment and municipal dumpsite [12, 14]. The mode of co-trimoxazole resistance was varied at range 10-13% similar with the other studies that 5-20% of isolates were resistant to co-trimoxazole [16, 17]. The antibiotics resistant bacteria were higher population compared with industrial soils because of several reasons; the first, the antibiotic-resistant population increase when the agricultural soils treated by livestock waste or inorganic compound [1]. The second reason, the population of antibiotic-resistant bacteria increased when the farms irrigated by Zayanderud River. The microbial community also affected by type, concentration and half-life of antibiotic [13]. In the present study, the MIC mode for erythromycin was at range 2-16 mg/L similar result determined by MIC for *Staphylococcus* isolates were at the range of 1-8 µg/mL [18] and 0.1 to >256 µg/mL in the agricultural soils [19, 20]. The MIC mode for vancomycin was 8 mg/L that was similar by the report from Beverley Beck River in England that were 4-8 mg/L [20, 21] and the MIC range for *Staphylococcus* was 8-16 µg/mL [18].

The MIC mode for gentamicin was 4 mg/L in the present study which in similar studies, the MIC for *Bacillus* spp and *Pseudomonas aeruginosa* isolates were 0.5-32 µg/mL and 20 mg/L, respectively also the MIC 256 mg/L determined [4, 15, 20] The result shows the type and source of isolated bacteria are effective in their antibiotic resistance patterns [4](. The MIC penicillin G for two isolates (*Acinetobacter* strain SR2 *and Aeromonas*

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hydrophila strain (SR4) were 10 mg/L [3]. But, the MIC in the present study show variable range 1-256 mg/L. This antibiotic is used extensively as well produced by many soil bacteria[22]. The MIC for the most of isolates from agricultural soils was higher than MIC for the isolates from industrial soils probably, in the manure-amended soils or irrigated by river water exposed with antibiotics [19].

The MAR frequently determined among the bacterial community. The highest antibiotic resistance determined by triple-resistance pattern and the most abundant type of resistance pattern observed among four antibiotics include erythromycin, vancomycin, penicillin G, and co-trimoxazole. In several studies, the range of MDR was 56-95% of isolates that resistance to two or more antibiotics was approved [4, 12, 14]. The study done in Mexico showed a lot of bacteria were resistant to more than six antibiotics that the population of gram-positive bacteria such as *Bacillus* sp were higher than gram-negative species. The endospore-forming ability prepares a long-term environmental survival strategy for resistance under stressful conditions[13]. The most presence of resistant bacteria in irrigated soils determined in depth of 15-30 cm [13, 14]. Many multidrug resistance cases reported from poultry dropping soil, and sediment from fish ponds that sometimes >90% of isolates were resistant to antibiotics [14]. Efficient efflux proteins, impermeable outer membrane, enzyme inactivation of drugs and mutation are some mechanisms that increased the antibiotic-resistant among bacteria [1, 3]. Some studies demonstrated that the anthropogenic factors such as irrigation with untreated water, wastewater or livestock enhance the frequency and distribution of environmental multi-drug resistance [1]. The most of multidrug-resistant isolates obtained from soils that irrigated by Zayanderud River. The hospital, municipal and agricultural wastewater are sources of antibiotics and as well the resistant bacteria in the aquatic environment directly affected to other ecosystems. Humans attacked by MDR environmental bacteria through consumption of contaminated water or farm products. Antibiotic resistance genes easily transferred to the pathogenic bacteria and increased their pathogenicity.

Refrences

[1] Esiobu, N., Armenta, L. and Ike, J.(2002). Antibiotic resistance in soil and water environments. International Journal of Environmental Health Research. 12(2), 133-144.

[2] Kümmerer, K. (2004). Resistance in the environment. Journal of Antimicrobial Chemotherapy. 54(2), 311-320.

[3] Rajkishore, G.B., et al. (2015). Characterization of drug resistant bacteria, conjugal transfer efficiency and their growth kinetics against Cassia plants leaf extract. Current Research in Bacteriology. 8, 1-10.

[4] DebMandal, M., Mandal, S. and Pal, N.K.(2011). Antibiotic resistance prevalence and pattern in environmental bacterial isolates. The Open Antimicrobial Agents Journal.3, 45-52.

[5] Knapp, C.W., et al. (2017). Relationship between antibiotic resistance genes and metals in residential soil samples from Western Australia. Environmental Science and Pollution Research. 24(3), 2484-2494.

[6] Pümpel, T., et al.(1995). A rapid screening method for the isolation of metal-accumulating microorganisms. Journal of industrial Microbiology. 14(3-4), 213-217.

[7] Krishna, M., et al.(2014). Multiple antibiotic resistance of environmental bacteria isolated from heavy metal polluted industrial region. Trakia Journal of Sciences. 2, 109-113.

[8] Garrity, G.M., Bell, J.A. and Lilburn, T.G. (2004).Taxonomic outline of the prokaryotes. Bergey's manual of systematic bacteriology. Springer, New York, Berlin, Heidelberg.

[9] Blair, J.M., et al.(2015). Molecular mechanisms of antibiotic resistance. Nature reviews microbiology. 13(1), 45-51.

[10] Balouiri, M., Sadiki, M., and Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 6(2), 71-79.

[11] Im, H., et al.(2016). Functional metagenome mining of soil for a novel Gentamicin resistance gene. Journal of Microbiology and Biotechnology. 26**,** 521-529.

[12] Mwaikono, K.S., Maina, S. and Gwakisa, P.(2015). Prevalence and antimicrobial resistance phenotype of enteric bacteria from a municipal dumpsite. Journal of Applied & Environmental Microbiology. 3(3), 82-94.

[13] Palacios, O.A., et al.(2017). Monitoring of indicator and multidrug resistant bacteria in agricultural soils under different irrigation patterns. Agricultural water management. 184, 19-27.

[14] Walsh, F. and Duffy, B.(2013). The culturable soil antibiotic resistome: a community of multi-drug resistant bacteria. PLoS One. 8(6), e65567.

[15] Adelowo, O.O., Fagade, O.E. and Agersø, Y.(2014). Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. The Journal of Infection in Developing Countries. 8(09), 1103-1112.

[16] Marti, R., et al.(2013). Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Applied and Environmental Microbiology. 79(18), 5701-5709.

[17] Ernervik, A. (2011). A risk analysis of the potential harm on the soil environment caused by antibiotics in biosolids.

[18] Tang, P., et al.(2003). Investigation of *Staphylococcus aureus* isolates identified as erythromycin intermediate by the Vitek-1 system: comparison with results obtained with the Vitek-2 and Phoenix systems. Journal of clinical microbiology. 41(10), 4823-4825.

[19] Popowska, M., et al. (2012). Influence of soil use on prevalence of tetracycline, streptomycin, and erythromycin resistance and associated resistance genes. Antimicrobial Agents and Chemotherapy. 56(3), 1434-1443.

[20] Novais, C., et al. (2005). Environmental contamination with vancomycin-resistant enterococci from hospital sewage in Portugal. Applied and Environmental Microbiology. 71(6), 3364-3368.

[21] Alkhaleefah, F.(2015). Isolation and characterisation of imipenem-resistant bacteria from natural environments and clinical settings. University of Hull.

[22] Schmieder, R. and Edwards, R. (2012). Insights into antibiotic resistance through metagenomic approaches. Future Microbiology. 7(1), 73-89.