Original article

The study of influence of some physicochemical agents in resistance pattern of *Escherichia coli* and *Pseudomonas aeruginosa*

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Abstract

Introduction and objective: Nowadays the microbial resistance to antibiotics and other physicochemical agents has caused various problems in the process of disinfecting devices and surfaces of the hospitals and in treatment of the patients**.** Thus, the aim of this study has been to examine resistance patterns of some penicillin resistant bacteria to physiochemical agents (UV and gamma radiation, cetrimide, and some of heavy metals).

Materials and methods: In the present study, strains of penicillin resistant bacteria isolated from Alzahra hospital of Esfahan, including 39 strains of *Escherichia coli* and 23 strains of *Pseudomonas aeruginosa* were studied. To screen for cetrimide and cadmium, mercury and arsenate resistance was measured using micro titer plates, steer's replicator and serial dilution for determining minimal inhibitory concentration (MIC).

Results: The most MIC determined in *E. coli* and *P. aeruginosa* strains respectively were 50 and 400µg/ml (Cd and Hg). MIC of metals was determined in adaptation condition for some of resistant bacteria. Results showed that in the most cases bacteria were selected and radiated with intensity of $0.25J/m^2s$. Mean of cfu/0.1ml after 240s radiation in *E. coli* and *P. aeruginosa* were, respectively 4.7 and 3.6. Gamma radiation was performed with intensity of 20Gray /min (GY/min) in different times in these resistance bacteria. Mean of cfu/ml for *E. coli* and *P. aeruginosa* strains in 1000Gray dose were, respectively 2.4×10^2 and 8.2×10^2.

Conclusion: *Pseudomonas aeruginosa* strains showed higher resistance than *E. coli*. But there was no significant difference in MIC of arsenate between these bacteria.

Significance and impact of the study: The impact of this study is to find *E. coli* and *P. aeruginosa* strains that are multi resistant to physicochemical agents; and to suggest a solution for destroying these specific strains.

Keywords: Resistance; Penicillin; Cetrimide; Heavy metal; Radiation

Introduction

Regarding the importance of the bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* in man pathogenesis, it is critically necessary to fight and annihilate such bacteria under disease conditions. Due to the over consumption of penicillin in the recent decade's, considerable resistance against this group of antibiotics has emerged. In many cases, the index of resistance against penicillin is similar to the indices of resistance against ammonium tetravalent compounds such as cetrimide and also some of heavy metals including cadmium, mercury and arsenic [l,2].

The study of simultaneous resistance related to the above mentioned factors is thus important. *P. aeruginosa* is an opportunistic human pathogen exhibiting innate resistance to multiple antimicrobial agents [3]. This intrinsic multi drug resistance is caused by synergy between a low permeability outer membrane and expression of a number of broadly specific multidrug efflux (Mex) systems, including Mexab-oprM and MexYY-oprM. In addition to this intrinsic resistance, these and three additional systems, MexCD-oprJ, MexxEF-oprJ and MexJK-oprM promote acquired multi drug resistance as a consequence of hyper-expression of the efflux genes by mutational event. In addition to antibiotics, these pumps export biocides, dyes, detergents, metabolic inhibitors, organic solvents and molecules involved in bacterial cell to cell communication [4,5].

In *P. aeruginosa*, the MexAB-OprM multidrug efflux system exports a number of antimicrobial compounds, such as ß-lactms and it is largely responsible for high levels of intrinsic resistance of this microorganism to antibiotics [5]. In *E. coli*, the RND pump AcrAB is induced by stress [5-7]. The effectiveness of UV light in biological inactivation arises primarily from the fact that DNA molecules absorb UV photons between 200 and 300nm with peak absorption at 260nm. This absorption creates DNA strand. This damage occurs particularly between pyrimidine bases. If the damage goes unrepaired, DNA replication is blocked and this ultimately results in cell death [8,9].

UV ray is sometimes used to sterilize the operation room space, utensils and drinking water treatment. Also gamma ray is used to sterilize some medical instruments such as disposable syringes and to reduce pathogens in a variety of foods [9-11]. If resistance emerges against the above-mentioned factors, fighting microbes by these factors will encounter more problems. This research is aimed at studying the existence against some of these physicochemical factor and double and multifold resistances. In this connection therefore, the resistance of pathogenic bacteria isolated from different infections were studied.

Materials and methods

Test microorganisms

In the present study, standard bacterial strains of *E. coli* ATCC 8739 and *E. coli* ATCC 25922 (PTCC1431) were used for measuring antimicrobial materials and to study their resistance against UV ray. In addition, *P. aeruginosa* ATCC 9027 and *P. aeruginosa* ATCC 27853 (PTCC1399) were used for the measurement of antimicrobial materials and study of resistance against UV ray, respectively.

Culture media

The culture media for bacteria were media such as Nutrient agar (NA, Merck, Germany), Nutrient broth (NB, Merck, Germany), Trypitcase-soy agar (TSA, Merck, Germany), Trypitcase-soy broth (TSB, Merck, Germany), Muller Hinton, agar (MHA, Merck, Germany), Eousin

methylene blue (EMB, Merck, Germany), MacConkeys agar (Merck, Germany), base culture for metals (PHG-II), biochemical culture media for the identification of isolated bacteria, cadmium nitrate, sodium arsenate, mercury nitrate and cetrimide, for screening resistant bacteria.

Methods of determining bacterial sensitivity to antimicrobial materials

Disk diffusion by Kirby Bauer methods were used for determining bacterial sensitivity to antimicrobial materials and isolating resistant bacteria [12]. Broth dilution and agar dilution methods were used to determine Minimum Inhibitory Concentration (MIC). The method of agar on plate was used to determine at least one concentration of antimicrobial materials, which have the capacity to annihilate 99.9% of the microorganisms.

For screening the bacteria resistant against cetrimide, replica-plating method was used by steer's replicator. MIC and Minimum Bactericidal Concentration (MBC) were determined by using agar dilution method [8-14]. About 55°C, heavy metal solution with specific concentration was added to the medium and then, the pH adjusted and medium was poured into the plates and 0.1ml of microbial suspension in log-phase was spread on PHG-II plates. All samples were incubated at 35°C for 24-72h [15].

Heavy metal concentrations were as follows: Cadmium nitrate: 0.037, 0.075, 0.15, 0.31, 0.62, 1.23, 2.46, 4.93 (mg/ml), Mercury nitrate: 0.12, 0.25, 0.50, 1.00, 2.00, 4.00 (mg/ml), Sodium arsenate: 2, 4, 8, 16, 32, 64, 128 (g/l). The influence of UV ray (UV -C) on the growth of bacteria under hood of laminator flow (slee mains VLFS 636, Swiss) was measured by photon-meter, (Hausatech Quavtum Sensor Q SPAR, Japan)). The bacteria were grown in TSB for 24h on shaker at 30rpm. Log phase cultures were diluted to approximately 1.5×10^3 bacteria per ml, and 0.1ml was spread evenly on TSA plates. The bacterial culture was exposed to the UV radiation during different times (0, 30, 60,120 and 240S) at intensity of $0.25J/m^2s$. All samples were incubated at 35°C for 24h before scoring. The following equation was used to determine the coefficient of sensitivity to UV ray: S_{UV}= Ln [(CFU) d/ (CFU) O]/d where (CFU) 0= the number of bacteria in a certain volume of control sample, (CFU) d: the number of bacteria in the same volume of sample after UV irradiation and d: the rate of dose in terms of J/m^2 [8].

In this study, Iridium 192 source projector, sentinel 660, was used as a gamma ray generator. The bacteria were grown in tubes containing 5m1 of TSB medium at 35°C on shaker at 30rpm to reach 0.5 McFarland standard tubes. Then tubes were exposed to the gamma radiation in different doses (0.50, 0.750 and 1000 Gy). After radiation 0.1ml of samples were spread on TSA plates. The samples were incubated at 35°C for 24-72h [11].

Results

The percentages of bacteria grown at different concentrations of cetrimide were shown in table 1. A considerable difference has been observed between resistances to cetrimide in different microbial groups. *P. aeruginosa* imparts more resistance to cetrimide. In the study, it was revealed that the greatest MIC and MBC levels were respectively 50μg/ml and 200μg/ml in *E. coli* and 400μg/ml and 800μg/ml in *P. aeruginosa*. MIC and MBC of the strains of *E. coli* with *P. aeruginosa* were significant but this difference was not significant.

The most common metal resistance in the whole bacteria (100%), was against arsenate, followed by cadmium (50%) and mercury (46.72%) (Table 2). Double metal resistances were 40.32%, 66.12% and

40.77% for Cd-Hg, Cd-As and Hg-As respectively. Triple resistance (Cd-Hg-As) against metals was 40.32%. The study of the MIC of three metals of cadmium, mercury and arsenate is indicative of the fact that in general, the level of *P. aeruginosa* resistance against cadmium and mercury is higher than that of the *E. coli*. However a significant difference in the level of resistance against arsenate is not observed in the two groups of bacteria.

Table 1: The percentage of the grown bacteria in various concentration of cetrimide

Bacteria	Concentration (µg/ml)											
	1.25	4.16	5.21	6.25	9.37	10.41	14.58	20.83	26.04	208.33	260.41	312.5
E. coli	100	100	100	89.74	61.54	30.77	19.33	19.53	12.82	0.0	0.0	0.0
P. aeruginosa	100	100	100	100	100	100	100	100	100	100	69.56	60.86

Table 2: General pattern of the resistance against metals

Bacteria	No strains	The rate of resistant bacteria						
		Cd	Hg	As	Cd-Hg	Cd-As	Hg-As	Cd-Hg-As
E. coli	39	18 (46.15%)	10 (25.64%)	39 (100%)	6 (15.38%)	18 (46.15%)	10 (25.64%)	6 (15.38%)
P. aeruginosa	23	23 (100%)	19 (82.61%)	23 (100%)	19 (82.61%)	23 (100%)	19 (82.61%)	19 (82.61%)
Total	62	31 (50%)	29 (46.77%)	62 (100%)	25 (40.32%)	41 (66.12%)	29 (46.77%)	25 (40.32%)

Figures 1 and 2 respectively show the comparison of coefficient of sensitivity to UV rays for the strains of *E. coli* and *P. aeruginosa.* The study the resistance of bacterial to UV ray, the most important strains relative to heavy metals and cetrimide which have been selected have been under UV ray influence at intensity of 0.25J/m^2s in 0, 30, 60, 120 and 240 second time spans (Figs. 3 and 4). The mean number of the colonies grown in the culture medium after 240 seconds of UV radiation was 4.7 in the strains of *E. coli*, and 3.6 in *P. aeruginosa*. The mean numbers of the colonies after 120 seconds of UV irradiation were, respectively 16.2 and 13.5 in the strains of *E. coli* and *P. aeruginosa*.

The comparison of coefficient of sensitivity to UV ray (S_{UV}) in Figs (1 and 2) shows that the strains of *E. coli* and *P. aeruginosa* have, respectively mean sensitivity coefficient of -0.466 and -0.476. The colony mean number in all of the strains under study was equal to 17.68 and 5.46 in 60, 120 and 140 second time spans. Statistical calculations show that the difference between the colony number in 60, 120 and 240 seconds of UV radiation in every two groups of bacteria are not significant at the confidence level of 95%.

In the comparison between standard strains in each group with other strains in 60 seconds, the difference is not significant in *E. coli* and *P. aeruginosa*. In 120 seconds, the difference was not significant in none of the groups. In 240 seconds, the difference was significant in *P. aeruginosa*, but not significant in *E. coli*. In general, the overall comparison between the bacteria under study showed that strains of *E. coli* had a higher resistance than *P. aeruginosa*.

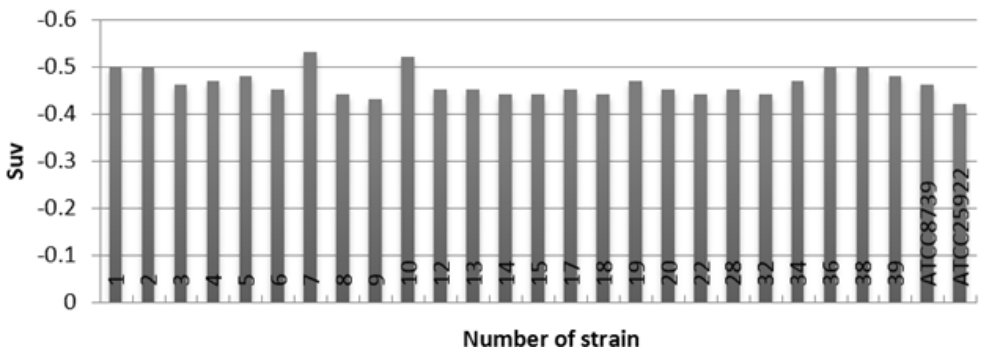

Fig. 1: Comparison of coefficient of sensitivity to UV ray (Suv) for strains of *E. coli*

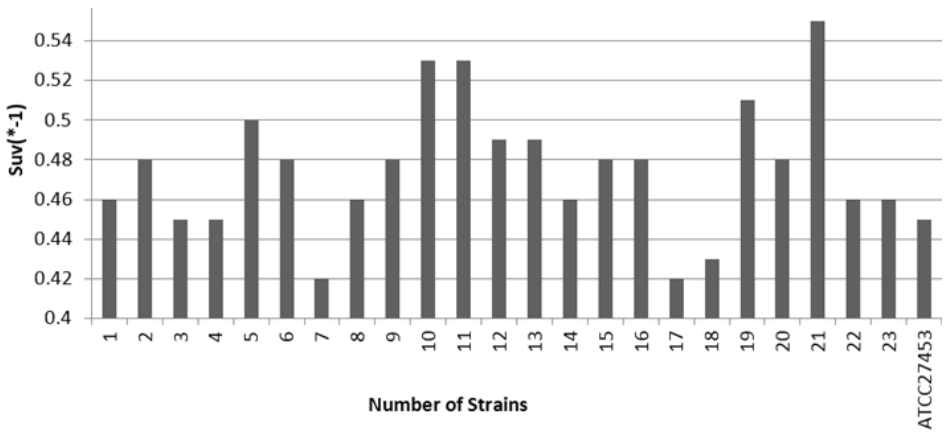

Fig. 2: Comparison of coefficient of sensitivity to UV ray (Suv) for strains of *P. aeruginosa*

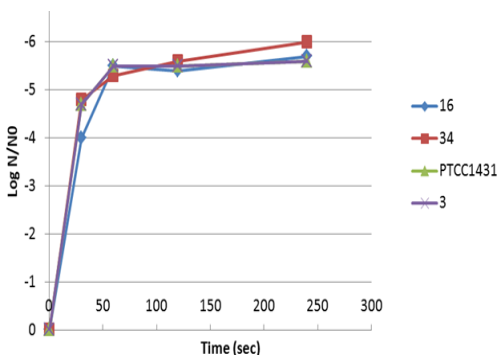

Fig. 3: Comparison of survival curves of some *E. coli* strains after irradiation by UV with 0.25 J/m^2S^{-1}

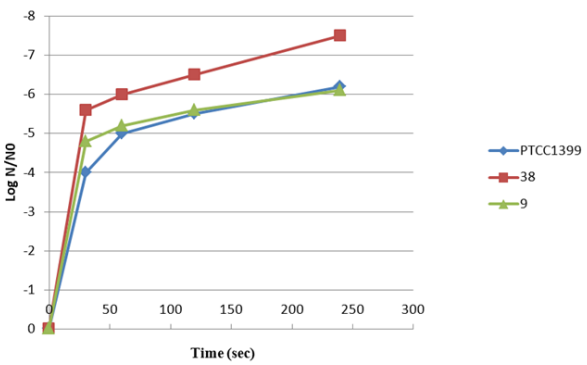

Fig. 4: Comparison of survival curves of some *P. aeruginosa* strains after irradiation by UV with 0.25 J/m^2S^{-1}

Gamma irradiation at the intensity of 20 Gy/min was performed in different times to attain 500, 750 and 1000 Gray. Regarding tables 3 and 4, it is observed that with the doses of 500, 750 and 1000 Gray, the difference between the number of *E. coli* and *P. aeruginosa* was not significant. In the comparison of standard strains between every group and other strains in the 500 Gray dose, this difference was not significant. In 750 and 1000 Gray doses, the difference between the standard strain of *E. coli* and *P. aeruginosa* and other strains of these two groups was not significant.

In general, the level of *P. aeruginosa* strains resistance was higher than the resistance of *E. coli* strains. In tables 5 and 6, total resistances of the bacteria under experiment to all of physicochemical factors are presented. Resistance strains regarding any physical or chemical factors are based on the following relations.

Table 3: The total count of colonies/ml after exposed to the gamma rays for strains of *E. coli*

Strain's number	Dose (Gy)		
	500	750	1000
3	2.1×10^5	2.0×10^4	3.0×10^2
5	2.2×10^6	4.5×10^5	4.5×10^3
13	1.2×10^6	2.5×10^4	4.6×10^3
16	1.5×10^6	3.0×10^4	5.0×10^3
28	4.1×10^6	2.3×10^5	4.6×10^3
32	13.6×10^5	2.1×10^4	5.0×10^3
34	4.0×10^6	6.2×10^4	8.0×10^3
38	7.0×10^5	3.1×10^4	4.0×10^2
39	6.1×10^5	4.3×10^4	2.1×10^2
ATCC25922	2.9×10^6	9.8×10^3	1.1×10^4

Table 4: The total count of colonies/ml after exposed to the gamma rays for *P. aeruginosa*

Strain's number	Dose (Gy)		
	500	750	1000
1	1.0×10^4	1.0×10^3	10.0
2	8.4×10^5	4.5×10^6	7.2×10^3
6	3.7×10^4	1.2×10^2	0.0
7	1.0×10^4	6.0×10^2	3.2×10^2
8	2.9×10^4	5.3×10^3	1.3×10^2
14	3.1×10^4	6.0×10^3	3.0×10^3
15	1.6×10^4	3.0×10^3	12.4×10^2
16	4.7×10^4	1.0×10^3	2.9×10^2
17	2.3×10^4	3.7×10^3	2.0×10^2
13	1.3×10^4	3.4×10^3	1.1×10^2
19	1.8×10^4	1.2×10^2	2.0×10^1
23	7.2×10^4	9.6×10^3	1.9×10^2
ATCC278 53	2.0×10^6	3.1×10^4	3.8×10^2

Discussion

In the work of Russell *et al*. [15] the level of cetrimide MIC for *P. aeruginosa* respectively has been obtained 64-128 and 0.4μg/ml in agar dilution procedure. In the work of Burke *et al*. [1] and Pumels *et al*. [16] the level of heavy metal as cadmium, mercury and arsenic for these bacteria respectively has been obtained 0.62μg/ml, 1μg/ml and 4μg/ml but in our result the MIC of these metals were more than their results.

All known organisms have multidrug resistance pumps that can extrude chemically unrelated antimicrobials from the cell. Gram-negative bacteria tend to be more resistant to lipophilic and amphiphilic inhibitors than Gram-positive bacteria. Such inhibitors include dyes, detergents, free fatty acids, antibiotics, and other chemotherapeutic agents. Many lipophilic antibiotics, such as penicillin G, are much less active against most Gram-negative bacteria. In fact, a survey of recently reported antibiotics of natural origin showed that, among those compounds that showed activity against Gram-positive bacteria, more than 90% lacked activity at a useful level against *E. coli* [17].

This intrinsic resistance of Gram-negative bacteria has often been attributed entirely to the presence of the outer membrane barrier. This barrier does contribute to the resistance, as the narrow porin channels slow down the penetration of even small hydrophilic solute, and the low fluidity of the lipopolysaccharide they say leaflet decreases the rate of transmembrane diffusion of lipophilic solutes. However the outer membrane barrier cannot be the whole explanation, even with species such as *P. aeruginosa* which produces an outer membrane of exceptionally low permeability [6].

Table 5: Resistance pattern of *E. coli* strains to all of physicochemical agents

Strain's number	Kind of agents					
	Cetrimide	Cadmium	Mercury	Arsenate	UV ray	γ ray
1	-	-	+	+	-	ND
2	-	-	+	+	-	ND
3	-	-	-	+	-	-
4	-	+	-	+	-	ND
5	-	+	-	+	-	-
6	+	-	+	+	-	ND
7	-	-	-	+	-	ND
8	-	-	-	+	-	ND
9	+	-	-	+	-	ND
10	-	-	-	+	-	ND
11	-	-	-	+	ND	ND
12	-	+	-	+	-	ND
13	-	+	-	+	-	-
14	+	+	-	+	-	ND
15	-	+	-	+	-	ND
16	-	-	-	+	ND	-
17	+	+	+	+	-	ND
18	+	+	-	+	-	ND
19	-	-	+	+	-	ND
20	+	+	-	+	-	ND
21	-	-	-	+	ND	ND
22	+	+	+	+	-	ND
23	-	-	-	+	ND	ND
24	-	-	-	+	ND	ND
25	-	-	-	+	ND	ND
26	-	-	-	+	ND	ND
27	-	-	-	+	ND	ND
28	-	+	+	+	-	-
29	+	-	-	+	ND	ND
30	-	-	-	+	ND	ND
31	-	-	-	+	ND	ND
32	-	+	-	+	-	-
33	-	-	-	+	ND	ND
34	-	+	-	+	-	-
35	-	-	-	+	ND	ND
36	-	-	+	+	-	ND
37	-	-	-	+	ND	ND
38	-	-	+	+	-	-
39	-	+	+	+	-	-

(ND) not determined, (+) resistant strain, (-) sensitive strain

Table 6: Resistances pattern of *P. aeruginosa* strains to all of physicochemical agents

Strain's number	Kind of agents					
	Cetrimide	Cadmium	Mercury	Arsenate	UV ray	γ ray
1	-	+	+	+	-	-
2	+	+	-	+	-	ND
3	-	+	-	+	-	ND
4	-	+	-	+	-	+
5	+	+	+	+	-	ND
6	-	+	+	+	-	-
7	+	+	+	+	+	-
8	-	+	+	+	-	-
9	+	+	+	+	-	ND
10	+	+	+	+	-	ND
11	+	+	-	+	-	ND
12	-	+	+	+	-	ND
13	-	+	+	+	-	ND
14	+	+	+	+	-	-
15	-	+	+	+	-	-
16	+	+	+	+	-	-
17	+	+	+	+	+	-
18	-	+	+	+	+	-
19	-	+	+	+	-	-
20	-	+	+	+	-	ND
21	+	+	+	+	-	ND
22	-	+	+	+	-	ND
23	-	+	+	+	-	-

(ND) not determined, (+) resistant strain, (-) sensitive strain

This is seen from the fact that equilibration across the outer membrane is achieved very rapidly, in part because the surface- to-volume ratio is very large in a small bacterial cell. Thus, the periplasmic concentrations of many antibiotics are expected to reach 50% of their external concentrations in 10 to 30 sec in *P. aeruginosa* and in a much shorter time period in *E. coli*. Additional mechanisms are therefore needed to explain the level of intrinsic resistance.

With the earlier ß-lactam compound, this second contributing factor is the hydrolysis by the periplasmic ß-lactamases that are encoded by chromosomal genes in many Gram-negative bacteria, and the level of resistance can be explained quantitatively, in many cases, by the synergy between the outer membrane barrier and ß-lactamases. However, recent studies showed that multiple drug efflux pumps, many with unusually broad specificities, play a major role in the intrinsic resistance of Gram-negative bacteria [6,18,19].

In the research conducted by Bank *et al.* [8] the standard strains were subjected to UV irradiation with the wavelength of 254nm for 60s and this caused six to seven log unit decrease in number of viable bacteria which is consistent with the above-mentioned. Snyder and Poland, [19] fatal dose required for *E. coli* 1000-2300 Gray and for *P. aeruginosa*1600-2300 Gray. The results of this study are considerably similar to these data.

Conclusion

The tested *P. aeruginosa* strains were more resistant than the tested *E. coli* strains to physicochemical agents used in this research.

Conflict of interest statement: All authors declare that they have no conflict of interest.

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References

1) Burke E, Wing K, Pfister RM. Cadmium sorption by bacteria and fresh water sediment. *J Ind Microbiol*. 1991; 8: 201-8.
2) Foster TJ. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. *Microbiol Rev*. 1983; 47: 361-409. PMID: 6355806
3) Teitzel GM, Parsek MR. Heavy metal resistance of biofilm and planctonic *Pseudomanas aeruginosa*. *Appl Environ Microbiol*. 2003; 69: 2313-20. PMID: 12676715
4) Schweizer HP. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. *Genet Mol Res*. 2003; 1: 48-62. PMID: 12917802
5) Poole K. Multidrug efflux pumps and antimicrobial resistance in *Pseudomanas aeruginos*a and related organisms. *J Mol Microbiol Biotechnol*. 2001; 3: 255-64. PMID: 11321581
6) Nikaido H. Multidrug efflux pumps of Gram-negative bacteria. *J Bacteriol*. 1996; 178: 5853-9. PMID: 8830678
7) Silver S, Phung LT. Bacterial heavy metal resistance: new surprises. *Annu Rev Microbial*. 1996; 50: 753-89. PMID: 8905098.
8) Bank HL, John J, Schemehl MK, Dratch RJ. Bacteriocidal effectiveness of modulated UV light. *Appl Environ Microbial*. 1990; 56: 3888-9. PMID: 2128016.
9) Block SS. Disinfection sterilization and preservation. 4th ed, Philadelphia, Lea & Febiger. 1990: 729-747, 823-853.
10) Zimmer JL, Slawson RM. Potential repair of *Eschericha coli* DNA following exposure to UV radiation from both medium and low pressure UV sources used in drinking water treatment. *Appl Environ Microbiol.* 2002; 68: 3293-9. PMID: 12089006
11) Billi D, Friedman EI, Hofer KG, Caiola MG, Ocampo-Friedmann R. Ionizing-radiation resistance in the desiccation-tolerent *Cyanobactrium chroococcidiopis*. *Appl Environ Microbiol*. 2000; 66: 1489-92. PMID: 10742231
12) Performance standards for antimicrobial disk susceptibility test. Approved standard ed. 9. January 2006. Vol. 23. No. I.
13) Kasra Kermanshahi R. Kazemi MJ, Taherzadeh M. Laboratory exercises in microbiology. Vol. 1. Iran, Azad University of Yazd publication. 2010: 61-72, 274.
14) Beson HJ. Microbiological applications: laboratory manual in general microbiology. USA, McGraw Hill publication. 2004; 478, 485.
15) Russell AD, Hammond SA, Morgan JR. Bacterial resistance to antiseptics and disinfectants. *J Hosp Infect*. 1986; 7: 213-25. PMID: 2873167
16) Pumles T, Pernfub B, Pigher B, Diols, Svinner F. A rapid screening method for the isolation of metal accumulating microorganisms. *J Ind Microbiol*. 1995; 14: 213-7.
17) Vaara M. Antibiotic super susceptible mutant of *Escherichia coli* and *Salmonella typhimurium*. *Antimicrob Agents Chemother*. 1993; 37: 2255-60. PMID: 8285603
18) Nikaido H. Outer membrane barrier as a mechanism of antimicrobial resistance. *Antimicrob Agents Chemother*. 1989; 33: 1831-6. PMID: 2692513
19) Snyder OP, Poland M. Food irradiation today. Hospitality institute of technology and management. 1995; 250-62. (www.hi-tm.com/Documents/Irrad.html)