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Vol. 8(42), pp. 3639-3649, 15 October, 2014
DOI: 10.5897/AJMR2014.6892
Article Number: 171DC5648181
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Metal and antibiotic resistance among heterotrophic bacteria inhabiting hospital waste water and polluted sea water

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Received 8 May, 2014; Accepted 12 September, 2014

The pollution of marine environment with heavy metals, antibiotics, dyes and surfactants may possess a serious threat to human life and the other organisms because of their toxicity and ability to persist for long time. The metals and antibiotics resistance of 48 heterotrophic bacterial isolates from hospital waste water and polluted sea water were studied. The minimum inhibitory concentrations (MICs) of heavy metals were different for each isolate. All isolates showed high resistance to nickel, chromium, barium and copper. On the other hand, mercury was the highest toxic metal against all the isolates. The frequencies of resistance for all isolates to each metal ion tested were as follows: Ni, 88%; Ba, 88%; Cr, 75%; Cu, 38% and Hg, 13% for hospital waste water isolates, and Ni, 88%; Ba, 75%; Cr, 50%; Cu, 50% and Hg, 0% for seawater bacteria. The response of the isolates to five antibiotics was tested; it ranged from complete resistance to total sensitivity. The hospital waste water isolates showed resistance for 4 of 5 antibiotics with percentages that ranged from 13 to 100%. On the other hand, 88% of seawater isolates resisted only one antibiotic. Multiple-metal resistance was exhibited by 100% of heterotrophic bacteria, while penta-metal-resistant bacteria was observed only in hospital waste water isolates. Multiple-antibiotic resistance was exhibited by 63% of the hospital waste water bacteria. The highest incidence of metal-antibiotic double resistance was observed in hospital waste water isolates compared with sea water isolates. Moreover, all isolates exhibited multiple resistances for different dyes as crystal violet, iodine and sufranine and other chemicals such as sodium lurayl sulfate. The bacterial isolates from hospital waste water showed higher resistance to dyes and surfactant than those isolated from polluted seawater. The composition of bacterial communities which were resistant to different chemicals ranged from 0.02 to 0.33% for the bacterial pathogens and from 1.95 to 15.0% for the fecal bacteria in the hospital waste water whereas, in seawater samples, the percentages of bacterial pathogens ranged from 0.04 to 0.12% and the percentages fecal bacteria ranged from 0.95 to 29.10%. This finding suggests that the discharge of hospital waste water without preliminary treatment is an important source for the spread of new phenotype bacteria with multiple-resistance in natural habitats which can pose a public health risk.

Key words: Metal resistance, antibiotic resistance, heterotrophic bacteria, hospital waste water, polluted sea water.

INTRODUCTION

The problem of hospital waste is becoming increasingly important. These effluents represent a major threat to the receiving environment and to human health. Scientists wonder about the long term effects they can have on human health and the environment; taken in consideration the nature and significance of specific substances they contain (Acharya and Singh, 2000; Kümmerer, 2001; Askarian et al., 2004). From a microbiological view, these effluents contain microbes' specific to the hospital medium. These microbes are often polyresistant to antibiotics and responsible for many infections (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, etc...) and fecal coliform bacteria (*Escherichia sp.* and fecal *streptococci*) (Ibekwe et al., 2003; Ivnitsky et al., 2007). From the chemical point of view, the hospital effluents contain organic compounds, heavy metals such as mercury, silver, nickel, chromium and cobalt (Acharya and Singh, 2000; Kümmerer, 2001; Mandal and Dutta, 2009; Mathur et al., 2012). Also, effluents contain dyes that serve as principal source of water pollution (Hassan et al., 2013). Most of these dyes are potentially toxic to aquatic Life. Furthermore, color of the dyestuff interrupts the aquatic environment (Khadijah et al., 2009; Hassan et al., 2013). Pollutants discharges in natural environments may alter the natural composition of microbial communities resulting in poor taxonomical diversity and allowing the survival of species resistant or able to degrade the pollutants. In chronically polluted environments microbial communities can acquire specific traits (such as heavy metal or antibiotic resistance), but in many cases these changes cannot be detected easily because they are sometimes unapparent. Complex adaptative mechanisms are initiated in microbial cell during the chronic exposure to pollutants that results in specific and discernable traits. Appearance of these traits is dependent on the presence of unfavorable factors for a long period of time necessary in stabilizing the metabolic pathways involved in conversion of pollutants (Aonofriesei, 2003). Much of the antibiotic used in humans and animals remains un-metabolized and thus a significant amount is added to the environment via excretion. This ultimately contributes to the residues of antibiotics in recipient waters. Antibiotics might also be added to the environment from pharmaceutical plants and as a result of the dumping of unused antibiotics. Also, antibiotics are entering the aquatic environment through hospital effluent. Besides antibiotics, resistant bacteria also enter into the aquatic environment (Diwan et al., 2010).

Until recently, the majority of study has been carried out on clinical material and thus, little is known about bacterial resistance to antibiotics in the natural environment. Hence, the role of antibiotic substances secreted into the natural environment has not been recognized in a comprehensive way and ever since has been one of the most controversial issues of microorganisms' ecology (Mudryk, 2002). Antibiotic resistant organisms are selected in the natural environments not only in presence of antibiotics but also in presence of some non-antibiotic substances including heavy metals. Genes conferring antibiotic resistance and genes conferring heavy metal resistance are most often found to be located on the same plasmid. That is why, if heavy metals are present in an environment as pollutant, many bacteria that survive in presence of them are found to be resistant not only to heavy metals but also to antibiotics. Thus antibiotic resistant organisms are selected to flourish even in absence of antibiotics.

A vast body of information is available in the literature on co-occurrence of antibiotic and heavy metal resistance in various types of natural isolates of bacteria (Chattopadhyay and Grossart, 2011). It has been for a long time; heavy metal pollution has become one of the most serious environmental problems. The pollution of environment with toxic heavy metals is spreading throughout the world along with industrial progress. So, presence of heavy metals even in traces is toxic and detrimental to all living organisms (Pandit et al., 2013). Presence of these heavy metals in the marine environment may pose a serious threat to the environment because of their ability to persist for several decades (Kamala-Kannan and Lee, 2008; Matyar, 2012). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, chromium, mercury and arsenic (Nithya et al., 2011; Kacar and Kocyigit, 2013). Some metals were toxic often at low concentration, and microorganisms were influenced by this toxicity, that concerns their diversity and activities and consequently their sustainability (Karbasizaed et al., 2003; Habi and Daba, 2009). Microorganisms play an important role in the environmental fate of toxic heavy metals with a multiplicity of mechanisms (Chatterjee et al., 2014).

The present study aimed to investigate the heavy metal and antibiotic resistance in heterotrophic bacterial community inhabiting the hospital waste water and polluted seawater. The study extended to investigate the multiple resistance of these bacteria, and they resistance to other inhibitors such as dyes and surfactant.

MATERIALS AND METHODS

Site description

The Eastern Harbor of Alexandria (Egypt), selected as the study area, is a semi-enclosed, protected and shallow bay (2-12 m). The circular basin covers an area of about 2.8 km^2 and occupies the central part of Alexandria coast. One major and 11 minor outfalls discharge large amounts (exceeding than 230000 m^3d^{-1}) of untreated waste waters into the Eastern Harbor water (Sabry et al., 1997). Poorly untreated industrial waste, domestic sewage, shipping industry and agricultural runoff are being released to the coast (Frihy et al., 1996).

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Table 1. Minimal inhibitory concentrations of various heavy metals (ppm) to the heterotrophic bacterial isolates.

Bacterial isolate	Metal ions (ppm*)				
	Hg	Ni	Cr	Ba	Cu
Waste water					
H1	1	30	45	45	5
H2	1	10	50	45	40
H3	10	70	55	50	5
H4	1	30	40	50	5
H5	1	30	40	40	5
H6	1	30	40	40	5
H7	1	30	40	40	40
H8	1	30	60	45	40
Sea water					
S1	1	30	50	45	5
S2	1	10	40	40	5
S3	1	10	40	45	40
S4	1	40	60	50	40
S5	1	30	40	40	20
S6	1	30	45	45	20
S7	1	30	45	45	20
S8	1	5	40	40	20

Samples collections

A total of 48 water samples were collected during Autumn 2013.Eight samples were collected weekly in triplicate from the hospital waste water discharge (H), El-Meery Hospital, Egypt. Other eight sea water samples (S) were collected weekly in triplicate from Alexandria Eastern Harbor, Egypt. Sampling was performed according to WHO (1995) and Clesceri et al. (2012).

Bacteriological examination

Total viable heterotrophic bacterial counts (THVC) were detected on Zobell agar medium (Oxoid LTD, England). Decimal dilutions of water samples were prepared and the pour plate technique was used (Clesceri et al., 2012).

Staphylococcus sp, and *Vibrio* ssp. were detected on manitol salt agar and thiosulphate citrate bile salt sucrose agar (TCBS), (Oxoid LTD, England), respectively (Atlas, 1997). Total coliforms (TC), *Escherichia coli* (EC) and fecal streptococci (FS) were enumerated on m-endoles agar medium, mFC agar and m-enterococcus agar medium, (Difco, Detroit, MI), respectively. The membrane filtration technique was used according to ISO 7899/2 (1984) and ISO 9308/1 (1990). Three replicates for each sample were used and the final counts were estimated as colony forming units (CFU/100 ml).

Heavy metals toxicity test

To examine the ability of heterotrophic bacterial isolates to resist heavy metal, cells of overnight grown cultures (10^7 CFU/ml) were inoculated on nutrient agar plates supplemented with different concentrations (10-700 ppm) of heavy metals (Sterilized stock solutions of each metal salt: $HgCl_2$, $NiSO_4$, $K_2Cr_2O_7$, $BaCl_2$ and $CuSO_4$ were used). Cultures were incubated at 30°C for 24-48 h and the cell growth was observed. Plates containing media without metal were inoculated in the same way to act as controls (Hookoom and Puchooa, 2013).

Antibiotics resistance test

Resistance of heterotrophic bacterial isolates from water samples were tested against different antibiotics by the disc diffusion method according to Bauer et al. (1966). The following five antibiotics were tested with their amounts: levofloxacin (25 mg), ceftriaxone (20 mg), vancomycin (5 mg), imipenem (30 mg) and amikacin (30 mg). The antibiotic discs (Oxoid Company, England) were applied to the surface of the seeded medium. The degree of resistance of the bacterial isolates was determined on the basis of the measurements of lightened zones (mm) around the disc. However, the multiple antibiotic resistances (MAR) index of isolates against tested antibiotics was calculated based on the following formula: MAR index = X / (Y x Z); X is the total of bacteria resistant to antibiotics; Y is the total of antibiotics used in the study; while Z is the total of isolates (Lee et al., 2009).

Dyes and surfactant resistance test

The ability heterotrophic bacterial isolates to grow in the presence of dyes (crystal violet, iodine and sufranine) at different concentrations (0.001, 0.01, 0.1 and 1%); and surfactant (sodium lurayl sulfate, SLS) at concentrations 50, 100, 200, 300, 500 and 700 ppm were investigated, individually. Cells of overnight grown cultures (10^7 CFU/ml) were inoculated on nutrient agar plates using pour technique method. Surfactant and dye stock solutions were prepared and sterilizes. A range of potential inhibitors at diagnostic concentrations were added to nutrient agar medium after sterilization. The growth was detected after incubation at 30°C for 24-48 h. Plates containing media without inhibitors were inoculated in the same way to act as controls (Williams et al., 1989).

RESULTS

Response to heavy metals

The minimum inhibitory concentrations (MICs) of heavy metals were different for each isolate. All isolates showed high resistance to Ni, Cr, Ba and Cu. On the other hand, mercury was the highest toxic metal against all the isolates; they showed 100% no growth in 1 ppm concentration for sea water isolates and 88% of waste water isolates exhibited no growth at the same concentration. The toxic effects of these metals increased with increasing concentration. The bacterial isolate (H3) from hospital waste water exhibited the highest resistance to most metal tested, Its MIC reached 70, 55, 50, 40 and 10 ppm for Ni, Cr, Ba, Cu and Hg, respectively . The isolate (S2) from sea water was the highest sensitive to metals; it exhibited absence of growth in 40, 40, 10, 5 and 1 ppm of Cr, Ba, Ni, Cu and Hg, respectively (Table 1).

The frequencies of resistance for all isolates to each metal ion tested were as follows: Ni, 88%; Ba, 88%; Cr, 75%; Cu, 38% and Hg, 13% for hospital waste water isolates, and Ni, 88%; Ba, 75%; Cr, 50%; Cu, 50% and Hg, 0% for seawater bacteria (Table 2).

Multiple metals resistance

Multiple metals resistance (MMR) is presented in Table 3.

Table 2. The frequencies of the heterotrophic bacterial isolates resistant to various concentrations of metals ions.

Metal ion	Cumulative % of isolates resistant at the following concentrations (ppm)								
	1	3	5	10	20	30	40	50	70
Waste water									
Hg	13	13	13	13	0	0	0	0	0
Ni	100	100	100	88	88	13	13	13	0
Cr	100	88	75	75	75	63	50	38	0
Ba	100	100	100	100	88	88	75	25	0
Cu	63	50	38	38	38	13	0	0	0
Sea water									
Hg	0	0	0	0	0	0	0	0	0
Ni	100	88	88	63	63	0	0	0	0
Cr	100	100	100	100	88	50	50	25	0
Ba	100	100	100	100	75	75	75	25	0
Cu	75	63	50	50	25	25	0	0	0

Table 3. The multiple metal resistances of the heterotrophic bacteria inhabiting the hospital waste water and sea water.

Bacterial isolate	No. of metals to which isolates were resistant at different concentrations (ppm)							
	1	5	10	20	30	40	50	70
Waste water								
H1	3	3	3	3	2	2	1	0
H2	4	4	3	3	3	2	1	0
H3	5	4	4	3	3	3	2	0
H4	4	3	3	3	2	1	1	0
H5	4	3	3	3	0	0	0	0
H6	3	3	3	2	0	0	0	0
H7	4	4	4	4	1	1	0	0
H8	4	4	4	4	2	2	1	0
Sea water								
S1	4	3	3	3	2	2	1	0
S2	4	4	2	1	0	0	0	0
S3	4	4	3	3	3	1	0	0
S4	4	4	4	4	3	2	1	0
S5	3	3	3	1	0	0	0	0
S6	4	4	4	3	2	2	1	0
S7	4	4	4	3	2	2	1	0
S8	3	2	2	2	2	1	0	0

All bacterial isolates were resistant to more than one metal ions (multiple metals resistances) at 1, 5 and 10 ppm of metal ions. The bacterial isolates H3 were resistant to the five metals ions tested (penta-metal-resistant); these penta-metal-resistant bacteria (represented 13%) were exhibited only in hospital waste water isolates. At 1 ppm, 63% of hospital waste water isolates were tertra-metal-resistant and 25% of isolates were Tri-metal-resistant. Sea water isolates were either tertra-metal-resistant (75%) or tri-metal-resistant (25%). At 50 ppm, only one isolates (H3) from waste water were double-resistant and 50% of heterotrophic bacterial isolates were mono-resistant.

Antibiotics resistance

The resistance of heterotrophic bacteria isolated from sea water and hospital waste water samples against selected antibiotics was examined. As shown in Table 4, the patterns

Table 4. Inhibition zones (mm) of bacterial isolates against selected antibiotics.

Bacterial isolate	Inhibition zones* (mm)				
	Levofloxacin (25 mg)	Ceftriaxone (20 mg)	Vancomycin (5 mg)	Imipenem (30 mg)	Amikacin (30 mg)
Waste water					
H1	22±0.92	0	0	38±0.34	12±0.76
H2	8±0.60	0	8±0.25	28±0.90	9±0.08
H3	0	0	15±0.18	25±0.16	10±0.15
H4	0	0	21±0.15	17±0.87	0
H5	16±0.78	0	0	30±0.42	8±0.15
H6	8±0.24	0	10±0.32	24±0.70	9±0.20
H7	8±0.32	0	8±0.70	10±0.64	8±0.15
H8	0	0	0	15±0.18	11±0.15
Sea water					
S1	23±0.90	19±0.15	0	30±0.68	10±0.22
S2	25±0.62	19±0.25	0	43±0.90	13±0.19
S3	11±0.52	10±0.47	0	36±0.26	10±0.18
S4	30±0.42	15±0.18	0	42±1.09	18±0.35
S5	49±1.06	40±0.80	0	30±0.3 5	21±0.65
S6	9±0.28	15±0.16	0	26±0.75	9±0.35
S7	17±0.52	13±0.28	0	38±0.45	11±0.08
S8	26±0.30	49±0.35	12±0.24	31±0.28	34±0.40

*Values are average ± standard deviation (n=3).

of resistances of bacteria to various antibiotics were considerably differed. Bacteria inhabiting hospital waste water were more resistant to ceftriaxone, at the same time 100, 88, 63 and 63% of isolates were sensitive to imipenem, amikacin, levofloxacin and vancomycin, respectively with different degrees. The maximum range of inhibition zones were exhibited toward imipenem (10-38 mm).

On the other hand, isolates from seawater were resistant to vancomycin (except S8) and sensitive to other antibiotics with different degrees. The maximum ranges of inhibition zones were exhibited toward levofloxacin (9-49 mm) and ceftriaxone (10-49 mm) and the minimum range of inhibition zones were toward amikacin (9-34 mm).

The heterotrophic bacteria inhabiting hospital waste water were resistant to all antibiotics with different percentages (ranged from 13 to 100%), except imipenem that inhibited all isolates from hospital waste water. On the other hand, the heterotrophic bacteria inhabiting seawater were sensitive to all tested antibiotics except vancomycin.

Multiple antibiotics resistance

The percentage of multiple antibiotics resistances (MAR) were calculated. As shown in Figure 1, all heterotrophic bacteria inhabiting hospital waste water were resistant to at least one antibiotic; the majority showed resistance to two or three of the five tested antibiotics, then the percentages of MAR ranged from 20 to 60%. Most bacterial isolates from sea water resisted one antibiotic only, then the percentage of resistance frequencies to antibiotics did not exceed than 20%.

The multiple antibiotics resistance index of bacterial communities inhabiting hospital waste water was 0.38 while the MAR index for bacterial communities inhabiting sea water was 0.18. The MAR indexes indicated that there was a difference between the heterotrophic bacteria inhabiting hospital waste water and sea water in their resistance to the antibiotics used in this study.

Correlation between antibiotics and metals resistance

The percentage of metal and antibiotic double-resistant isolates was calculated by dividing the number of isolates resistant to a certain antibiotic and simultaneously to a specific metal ion by the total number of isolates resistant to this particular antibiotic x 100 and is represented in Table 5. The highest incidence (100%) of metal-antibiotic double resistance in waste water isolates exists between: nickel and ceftriaxone, vancomycine and amikacin; chromium and levofloxancin and amikacin; and finally barium and all antibiotic except imipenem. Moreover, the double resistance among seawater isolates exists between nickel, chromium and barium and only one antibiotic (vacomycin).

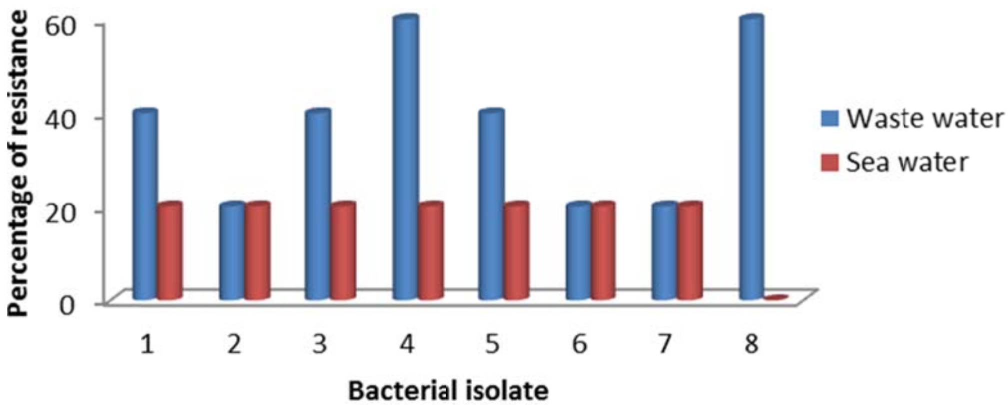

Figure 1. The percentage of multiple antibiotic resistances of bacterial isolates from hospital waste water and sea water.

Table 5. Correlation between antibiotic and metal resistance.

Antibiotic		Mercury			Nickel			Chromium			Barium			Copper		
	TNo.	No.	%	T%	No.	%	T%	No.	%	T%	No.	%	T%	No.	%	T%
Waste water																
Levofloxacin	3	1	33	13	2	67	25	3	100	38	3	100	38	1	34	13
Ceftriaxone	8	1	13	13	8	100	100	6	75	75	8	100	100	3	38	38
Vancomycin	3	0	0	0	3	100	38	2	67	25	3	100	38	1	34	34
Imipenem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amikacin	1	0	0	0	1	100	13	1	100	13	1	100	13	0	0	0
Sea water																
Levofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceftriaxone	0	0	0		0	0	0	0	0	0	0	0	0	0		0
Vancomycin	7	0	0	0	7	100	88	7	100	88	7	100	88	3	43	38
Imipenem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amikacin	0	0	0	0	0	0	0	0	0	0	0		0	0		0

TNo, Total number of isolates resistant to the particular antibiotic; No., number of isolates resistant to metal and antibiotic; %, percent of total number of isolates resistant to the particular antibiotic; T%, per cent of total number of isolates (8 for waste water and 8 for sea water).

Resistance to different dyes

The average counts of bacterial isolates from hospital waste water showed higher resistance to crystal violet and iodine than those isolated from polluted seawater. The order of resistance to the dyes was found to be as crystal violet > iodine > sufranine in hospital waste water. The order of dyes resistance was found to be crystal violet > sufranine > iodine in seawater (Figure 2).

Multiple dyes resistance

Figure 3 shows that the heterotrophic bacteria isolated from hospital waste water and sea water resist the three dyes tested (the percentage of multiple dye resistance was 100%), except isolates H5 and H6 from hospital discharge water resist only two dyes (resist 66% of dyes). As well also, isolates 2S and 5S from seawater resisted only 33% of dyes.

Resistance to SLS surfactant

All the isolates were highly resistant to surfactant. Bacterial isolates from hospital waste water (H2, H3, and H4) and sea water (S2, S3 and S4) showed no growth in 200, 200 and >700 ppm of surfactant, respectively. Two bacterial isolates (H4 and H8) from hospital waste water were capable of tolerating higher concentration of

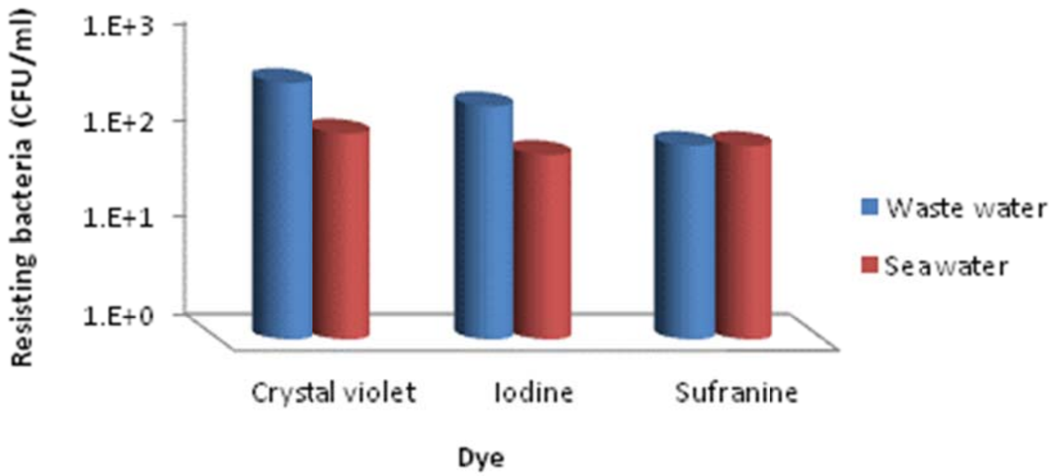

Figure 2. The average counts of heterotrophic bacteria resisting to 1% of different dyes, individually, in hospital waste water and sea water (control, 10^7 CFU/ml).

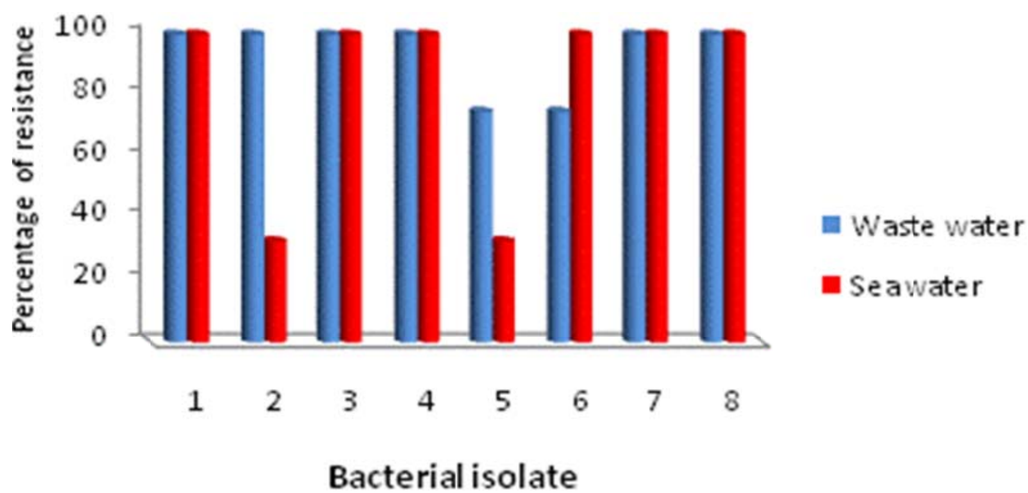

Figure 3. The percentage of dye resistance (at 1% of each dye, individually) of heterotrophic bacterial isolated from hospital waste water and sea water.

Table 6. Minimum inhibitory concentrations of SLS surfactant (ppm).

Waste water		Seawater	
isolate	MIC	Isolate	MIC
H1	200	S1	500
H2	200	S2	200
H3	200	S3	200
H4	> 700	S4	> 700
H5	100	S5	200
H6	200	S6	700
H7	500	S7	300
H8	> 700	S8	300

MIC, Minimum inhibitory concentrations.

surfactant more than 700 ppm compared to one bacterial isolates (S4) from sea water (Table 6).

Composition of the bacterial community

The variation in densities of pathogens and coliforms populations in hospital waste water samples was conducted. *Staphylococcus* sp. and *Vibrio* sp. were detected in all water samples collected during the period of study from the first to the eighth week. *Staphylococcus* sp. present in low counts ranged from 0.7×10^1 to 4.7×10^1 CFU/100 ml. *Vibrio* sp. ranged from 1.4×10^1 to 7.3×10^1 CFU/100ml (Figure 4a).

Hospital waste water samples had high densities of

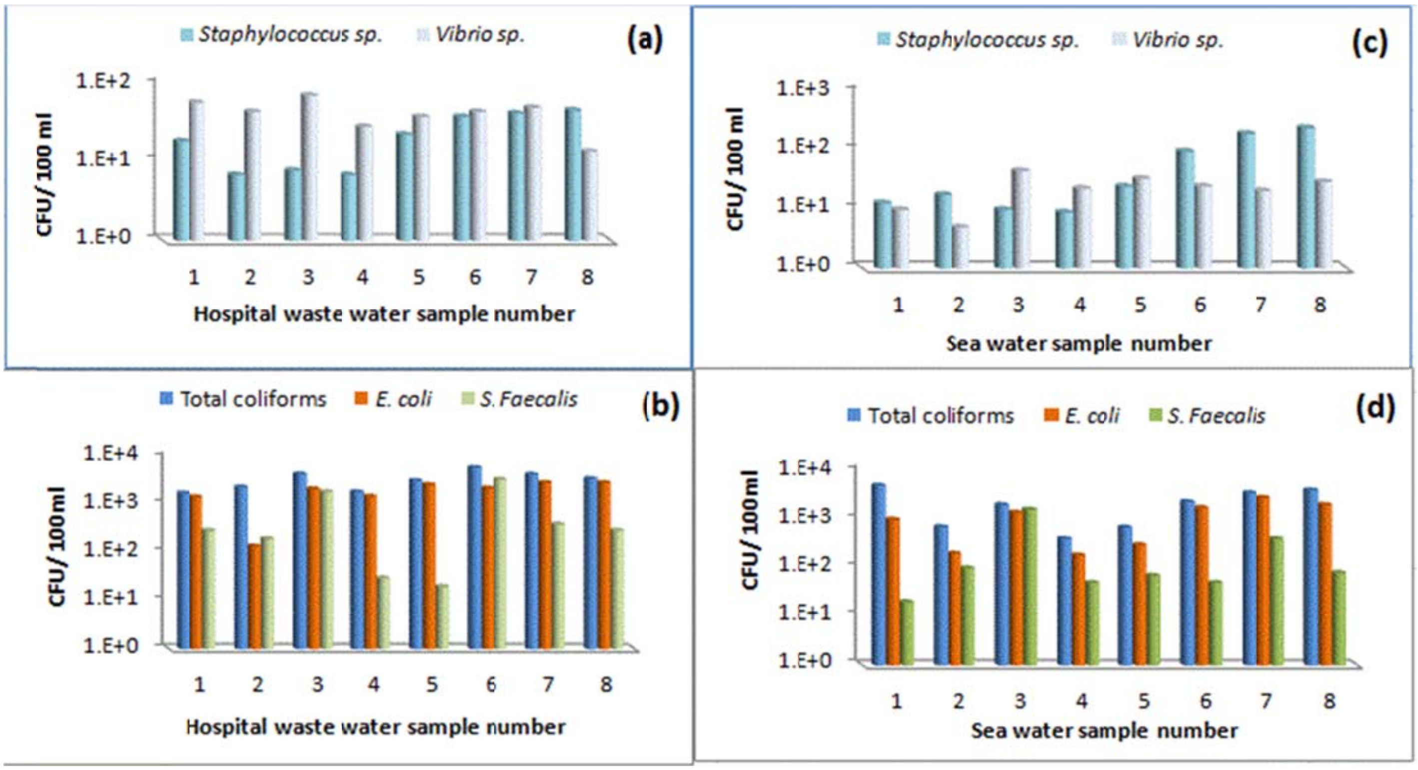

Figure 4. Viable count of pathogens (a) and fecal coliforms (b) in sea water samples, and also pathogens (c) coliforms (d) in hospital waste water samples. Values are average (n=3).

fecal indicators. The counts of total coliforms (TC), *Escherichia coli* (EC) and fecal *Streptococci* (FS) fluctuated between $1.8x10^3$ and $6.2x10^3$ CFU/100 ml for TC, $1.4x10^2$ to $3.0x10^3$ CFU/100 ml for EC and 2.0 $x10^1$ to $3.5x10^3$ CFU/100 ml for (FS), (Figure 4b).

The general bacteriological picture in sea water samples showed that the density of *Staphylococcus* sp. ranged from $0.9x10^1$ to $2.5x10^2$ CFU/100 ml and *Vibrio* sp. was recovered in fewer counts (from $0.5x10^1$ to $4.6x10^1$ CFU/100 ml), (Figure 4c).

Total coliforms, *Escherichia coli* and fecal *Streptococci* were detected in 100% of sea water samples with high densities. The counts fluctuated between $4.0x10^2$ to $5.0x10^3$ CFU/100 ml for TC, between $1.9x10^2$ to $2.9x10^3$ CFU/100 ml for EC, and from $2.0x10^1$ to $1.6x10^3$ CFU/100 ml for FS, (Figure 4d).

The composition of bacteria resistance to different chemicals (heavy metals, antibiotics, dyes and surfactant) was examined in natural bacterial communities from hospital waste water and sea water. The occurrence percentages of pathogens with respect to the total heterotrophic counts in hospital waste water ranged from 0.02 to 0.33%, while coliforms represented in higher percentages ranged from 1.95 to 15.0%. In seawater samples the occurrence percentages of pathogens ranged from 0.04 to 0.12% and coliforms ranged from 0.95 to 29.10%.

The average count of total viable heterotrophic bacteria in hospital waste water was 1.4 x 10^5 and in sea water was 1.5 x 10^5 CFU/100 ml. The occurrence percentages of total pathogens and fecal coliforms in hospital waste water (0.11 and 7.8%, respectively) were higher than that in sea water samples (0.08 and 6.09%), (Table 7).

DISCUSSION

Most bacteria have multiple routes to resistance to any drug and, once resistant, can rapidly give rise to vast numbers of resistant progeny (Livermore, 2003). Antibiotic and metal resistance is frequently associated with each other and often strongly correlated. Antibiotic resistant and metal tolerant organisms seem to be present due to the exposure to environment contaminated with metals that causes co-incidental selection for antibiotics and heavy metals resistance factors (Ansari et al., 2014).

Concern for environmental quality has stimulated scientific studies on the biological effects of metal contamination on the marine environment. From the standpoint of environmental pollution, heavy metals and metalloids are extremely toxic because of their relative accessibility to biological systems. Yet, in response to toxic concentrations of heavy metals, many aquatic organisms,

Table 7. The occurrence percentages (%) of pathogens and coliforms with respect to total heterotrophic counts in hospital waste water and sea water samples.

Sample	Waste water			Sea water		
Number	THVC	Pathogen	Fecal coliforms	THVC	Pathogen	Fecal coliforms
1	2.4×10^4	0.33	15.0	2.0×10^4	0.12	29.10
2	2.8×10^4	0.19	9.78	2.1×10^4	0.11	4.81
3	8.0×10^4	0.10	10.78	7.1×10^4	0.08	7.03
4	4.5×10^4	0.08	7.82	6.7×10^4	0.05	0.95
5	5.8×10^4	0.11	10.35	5.9×10^4	0.10	1.79
6	2.8×10^5	0.03	4.26	3.1×10^5	0.04	1.33
7	3.2×10^5	0.03	2.45	3.3×10^5	0.07	2.03
8	3.4×10^5	0.02	1.95	3.6×10^5	0.08	1.69
Average	1.4×10^5	0.11	7.80	1.5×10^5	0.08	6.09

THVC, Average count of total viable heterotrophic bacteria (CFU/100 ml).

including microorganisms, can develop resistance and the ability to degrade or utilize these substances (Sabry et al., 1997; Hookoom and Puchooa, 2013).

It can be assessed that most bacterial isolates were sensitive to Hg but they were highly resistant to Cr, Ba, Ni and Cu. Although some of them act as essential micro nutrients for living beings, at higher concentrations they can lead to severe poisoning (Hookoom and Puchooa, 2013).

Multiple metal resistances also seemed to be the rule rather than the exception. All bacterial isolates were resistant to more than one metal ions but penta-metal-resistant bacteria were exhibited only in hospital waste water isolates. Similar observations were previously reported (Sabry et al., 1997; De Souza et al., 2006; Kacar and Kocyigit, 2013).

Emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used, but occurrence of antibiotic resistance bacteria is also increasing in marine environment. This is why many studies have been recently carried out to determine the distribution of antibiotic resistance bacteria in freshwater basins, estuaries, municipal drinking water and sewage waters. However, there is relatively very few studies on antibiotic resistant bacteria in marine water (Manivasagan et al., 2011).

Antibiotic resistance of heterotrophic bacteria isolated from the surface and subsurface water of estuarine was determined. The levels of resistance of bacteria to various antibiotics differed considerably (Mudryk, 2002).

The current study has displayed that bacteria inhabiting the hospital waste water were much more resistant to antibiotics than those isolated from polluted sea water. In hospital waste water, the response of the heterotrophic bacteria to five tested antibiotics ranged from complete resistance to total sensitivity, where, the bacterial resistance was noted in the cases of ceftriaxone, levofloxacin vancomycin, and amikacin, while at the same time the bacteria were most sensitive to imipenem while in sea water a high level of bacterial resistance to vancomycin was noted with a simultaneous high sensitivity to ceftriaxone, levofloxacin, amikacin and imipenem.

Similarly, the bacterial resistance was reported by Sabry et al. (1997) which showed that the response of the sea water isolates to 11 tested antibiotics ranged from complete resistance to total sensitivity and multiple antibiotic resistance was exhibited by 70.4% of the total isolated population The highest incidence of metal-antibiotic double resistance existed between lead and all antibiotics (100%), copper and penicillin (95%) and nickel and ampicillin (83.3%).

Microorganisms resistant to both metals and antibiotics have been isolated frequently from different environments and clinical samples (Sabry et al., 1997; Kacar and Kocyigit, 2013). As a result new phenotypes are inherited in communities which develop under the influence of pollutants. The presence of metal and/or antibiotic-resistant bacteria in natural habitats can pose a public health risk (Sabry et al., 1997).

Most antibiotic resistant microbes emerge as a result of genetic change and subsequent selection processes by antibiotics. The resistance factor may be chromosomal, that developed as a result of spontaneous mutations and extrachromosomal resistance (plasmid resistance) (Selim et al., 2013).

Also, to face heavy metals profusion in the environment, bacteria have evolved several resistance mechanisms that lead to persist or/and to grow are in several cases plasmid-borne. These plasmid mediated resistance to heavy metals can also carry genes coding for antimicrobial resistance (Karbasizaed et al., 2003; Habi and Daba, 2009).

The present results have proven that, the incidence of multiple resistances either to metal or antibiotics was observed in heterotrophic bacteria inhabiting hospital waste water, while heterotrophic bacteria inhabiting sea water were characterized by single antibiotic resistance and multiple metal resistances. In hospital waste water, most of the bacteria were resistant to 2-3 antibiotics and

3-5 metals (at 1 ppm). Moreover, in seawater samples, most of the bacteria were resistant to only one antibiotic and 3-4 metals (at 1 ppm).

One environmental concern related with hospital effluents is their discharge without preliminary treatment. The presence of antibiotics may enhance the effectiveness of multi-drugs resisting bacteria (Almeida et al., 2014). Multidrug-resistant strains are critical to the total accumulation of resistance (*Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus*) (Livermore, 2003).

Bacteria inhabiting many water basins are characterized by multiple antibiotic and metal resistance (Mudryk, 2002; Ansari et al., 2014). Multiple resistance is a phenomenon whose mechanisms have not yet been well recognised. Multiple resistances may be coded on plasmids, mutational events or on even smaller and mobile genetic elements called transposons. Transposons are able to move between plasmids and bacterial chromosomes. The bacterial strains inhabiting the waters of lake are characterised by multiple antibiotic resistance. This indicates that estuarine bacteria are perfectly capable of detoxicating these antibacterial substances (Mudryk, 2002).

Most of heterotrophic bacterial isolates have high incidence of metal-antibiotic double resistance, also these isolates exhibited multiple resistance to different dyes as crystal violet, iodine and sufranine and other chemicals as SLS surfactant. In the same trend, De Souza et al. (2006) reported that, mercury resistant bacteria are also resistant to many antibiotics and other toxic chemicals. In addition, it has long been reported that bacteria inhabits in industrial effluents utilizing its constituents as their source of energy (Hassan et al., 2013). The microorganisms used in most of these studies were *Staphylococcus sp., E. coli, Bacillus sp., Clostridium* sp., and *Pseudomonas* sp. in bacteria (Marimuthu et al., 2013).

Adams (1967) confirmed that crystal violet has an antibacterial action against *E. coli, S. aureus, S. faecalis* and *B. subtilis*. The effect of the dye was measured as minimum inhibitory concentration or retardation of growth. On the other side, Lachapelle et al. (2013) concluded that antiseptics have broader spectrums of antimicrobial activity than antibiotics.

Povidone iodine has the broadest spectrum of antimicrobial activity. Corwin et al. (1971) stated that the sensitivity to SLS of *Shigella flexneri* and *E. coli* is determined by at least three genes. A major difference between the two strains is their relative resistance to the anionic detergent, sodium lauryl sulfate.

The composition of bacteria resistance to different chemicals (heavy metals, antibiotics, dyes and surfacetant) was examined in natural bacterial communities from hospital waste water and sea water. Obviously, the occurrence percentages of total patho-gens and fecal coliforms in hospital waste water (0.11 and 6.31%, respectively) were higher than that in sea water samples (0.08 and 4.64%).

Species composition of water samples could affect antibiotic resistance pattern. Significant differences to some antibiotics of *Enterobacteriaceae* strains isolated from different sites were not related to fecal pollution level. Also, global resistance to metals was not influenced by fecal pollution (Habi and Daba, 2009). In recent decades, the antimicrobial resistance of bacteria isolated from hospital increased. Gram negative bacterial strains are the most frequent bacterial strains (Selim et al., 2013).

Conclusion

Depending on the metal and antibiotic resistance of heterotrophic bacteria inhabiting hospital waste water and polluted seawater, the current study observed high incidence antibiotics-metals double resistance among heterotrophic bacteria inhabiting hospital waste water comparing with polluted seawater. Also these isolates exhibited multiple resistances to different dyes as crystal violet, iodine and sufranine and other chemicals as SLS surfactant.

As a result new phenotypes are inherited in communities which develop under the influence of pollutants. One environmental concern related with hospital effluents is their discharge without preliminary treatment. The finding suggests that hospital waste water is an important source for the spread of new phenotype bacteria with mutiple-resistance. The presence of new phenotype bacteria with mutiple-resistance in natural habitats can pose a public health risk.

Conflict of interest

The author(s) have not declared any conflict of interests.

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