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Effect of Chlorination Treatment on Gram Negative Bacterial Composition of Recycled Wastewater

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Abstract: In order to assess the quality of recovered wastewater from the treatment plant of Mutah University, analysis was performed on samples collected from influent, polishing pool, chlorination tank and the ultimately disposal site at the university campus. In this assessment following parameters were used: temperature, BOD5, COD, effect of chlorination treatment, total bacterial counts (TBC), type of bacterial species isolated and the development of antibiotic-resistant bacteria during the treatment. Though BOD5 and COD values of treated water were within the acceptable range of Jordanian standards, the bacterial counts suggested that the efficiency of applied chlorination treatment was inconsistent. This discrepancy was particularly noticed on the water sample collected from polishing pool. Chlorine treatment of wastewater was not an efficient disinfectant method, besides it's potential to promote the production of antibiotic resistant bacteria. Some of the coliform species isolated from influent and effluent samples were found to be chlorine resistant. Such resistance seams to be species specific. Also an unexpected bacterial growth profile was observed where the total bacterial counts on some selective media were higher than the counts detected on enriched media. A re-evaluation for the current method of wastewater treatment is recommended.

Key words: Waste water, chlorination, bacterial identification

INTRODUCTION

Due to water scarcity in Jordan, large amounts of wastewater are retreated to be used for irrigation purposes. Wastewater represents an extremely complex mixture of organic and inorganic materials as well as the harboring of complex ecosystem (Horan, 1993). Such system may contains variety of microorganisms including: bacteria, fungi, protozoa and nematodes (Williams and Baun, 2003).

A substance is considered pollutant because it is perceived to have an adverse effect on the environment and some times may indirectly influence the human health (Vesilind *et al*., 1990). Pathogenic microorganisms are routinely encountered in wastewater. When the water source is contaminated with fecal material there is a high health risk predictable from the associated pathogens (Horan, 1993). The most common member of this contaminating group is the organism *Escherichia coli* (faecal coliform), which has been widely adopted as a fecal pollution indicator (Horan, 1993).

In Mutah University a local treatment plant receives water influent primarily from the campus facility services for students, residential areas, in addition to a secondary water influent received from the medical center as well as the laboratories of science college. The sources of microorganisms that cause infectious diseases are enormously variable and usually referred to as reservoirs of infection. Therefore water must be effectively treated and the response of pathogens to treatment procedures should be carefully evaluated. Several factors such as construction material, the initial sewage composition and the treatment procedures may influence the final sewage composition (Andersen, 1993). The disinfections of wastewater is considered as one of the primary public health protection to avoid the transmitting of water-borne infectious diseases. The three commonly used methods for disinfections are: chlorination, ozonation and ultraviolet (UV) disinfection (Solomon *et al*., 1998). Although it is under scruting, chlorine is widely used as chemical disinfectant for the treatment of wastewater discharges in many countries including Jordan (Jordanian standard 893/2002). It may be applied as chlorine gas, hypochlorite solution and other chlorine compounds in solid or liquid form to destroy the target organisms by oxidation of the cellular materials (Solomon *et al*., 1998). Different microorganisms and sometimes-different strains of the same species may show

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variations in their response to the chlorine disinfectant (Taylor *et al*., 2000).

Despite a disinfectant efficiency, sometimes the bacteria are able to escape and protect themselves from different environmental stress including the presence of killing agents by entering a dormant-like state called viable but non culturable (VBNC) phase (Gauthier, 2000). Under these conditions the bacteria loose the ability to grow on bacteriological media but maintain their viability as well as pathogenicity and may revert to a regular division upon restoration of normal growth condition.

As the use of antibiotic for clinical and veterinary purposes has become widespread, their continuous contact with bacteria probably has major influence on the development of antimicrobial resistance. A particular example is the tetracycline resistance gene which increased from about 30% to more than 80% of bacteroides species over the past decades in United States (Shoemaker *et al*., 2001). Furthermore, a high multiple antibiotic resistances were significantly reported in bacterial population regrown after chlorination treatment than in the influent sample (Murray *et al*., 1984). In the present study we selected our university was tewater plant as a model for the efficiency of treatment and reutilization of wastewater. In this campus a special treatment plant unit usually handles the drained wastewater before it is distributed for irrigation purposes. Our pilot study aimed mainly to determine the microbial quantity and quality of the wastewater at three sites of the university campus, firstly before treatment, secondly after chlorination and thirdly at the field area where the treated water is used for irrigation. To investigate the efficiency of wastewater treatment plant, specially the disinfection process that suppose to eradicate the contaminated microbes. Further objective would be to evaluate the quality of treated wastewater by determining the BOD5 and COD analytical parameters.

MATERIALS AND METHODS

Description of the wastewater treatment plant: Wastewater analysis was carried out through out the period 2003/2004. This mechanical/biological treatment plant has a design load = 800 m^3 day^{-1} and applied load 1000 m^3 day^{-1}. Three types of tanks were used in the treatment process including two aeration tanks that act by fluctuation, two polishing pools and one chlorination tank. As the raw sewage enter the plants it passes through hard screen, followed by fine screen and then through motorize gate valves before it is directed towards one of the two aeration tanks. In these tanks the working cycle lasts for 4 h according to the following procedure: 2 h of aeration where all the mechanical units functionally working (mixer and pumps), followed by 1 h of sedimentation where all mechanical units functionally stop and then terminated by 1 h of decanting. After this phase the sewage is directed to the polishing pool which is basically a physical treatment aiming to remove pathogenic microorganisms such as the bacteria and helminths. This step usually lasts for 4 days and achieved by providing a retention time long enough to reduce the microorganism numbers to a standard level. Finally the treated water is directed to the chlorination tank where the retention time last for 1h. The chlorinated water directed out of the treatment plant to reach reservoir about 2 km from the plant where the treated water is used later for the irrigation of University agriculture fields.

Waste water samples were obtained from a municipal treatment plant at Mu`tah University located in the Karak province, south of Jordan and serving a population of 30,000. The wastewater in this facility is treated via an activated sludge process, followed by disinfection by injection of chlorine gas, which is neutralized with sulfur dioxide gas before release. The initial chlorine concentration in the contact basin is 3.5 mg L^{-1} and has a contact time of 1 h. Raw wastewater samples used in this study were taken from the effluent of the final clarifiers prior to chlorination. Chlorinated samples were collected from the effluent from the chlorine contact basin. One-liter volumes of secondary treated wastewater were shaken at 200 rpm at 30°C on a GFL Model 3032 shaker. Sodium hypochlorite was added to achieve 3.5 mg of available chlorine per liter. After I h, sodium thiosulfate was added (15 mg L^{-1}) to neutralize the remaining free chlorine. Nutrient supplementations were accomplished by addition of 0.1% trypton followed neutralization.

Sample collection: Weekly water samples were collected from all sites to ensure proper functioning of the pilot system. Raw and treated (effluent and field water) samples were collected at 10-12 a.m by plant personnel and transported within 10 min to the microbiology laboratory. The bacterial populations were isolated from collected samples over the period of December 2003 through May 2004.

BOD and COD tests: Water sampling bottles used for BOD5 and COD analyses were filled fully, while those samples used for microbiological analyses were kept in unfilled bottles to allow, small space for aeration and mixing (APHA., 1992). BOD5 and COD were analyzed using WTW Oxymeter and K_2CrO_4 respectively (Jiries, 2001).

Isolation of influent, effluent and field water populations: The one liter volume samples were shaken mechanically at 200 rpm in a GFL Model 3032 shaker to disrupt clumps of some material exist. Serial dilutions of all samples were made with chlorine-free saline water. To determine the bacterial type and counting, four different kinds of media were used by spreading 0.1 ml from each serial dilution in triplicate: (1) Nutrient agar (NA), as a general purpose medium (Fluka). (2) Eosin methylen blue (EMB), medium for the isolation and enumeration of coliform organisms. (3) Desoxycholate citrate agar (DCA), a selective differential plating medium for the isolation of intestinal pathogens (Biolife). (4) Hektoen enteric agar (HEA), selective differential agar for the isolation of *Salmonella* and *Shigella* from food and clinical specimens.

Identification of isolated bacteria: Selection of bacterial culture for identification of isolated colonies was made according to the procedure described by Claus and Balkwill (1989). The microbial identification system (REMEL) was utilized in this study to identify a number of organisms so that population changes in influent and effluent can be determined (chlorination tank, polishing pool and field water). For this purpose two kits were used; (1) REMEL S RapID™ ONE System: a qualitative micromethod employing conventional and chromogenic substrates for the identification of medically important enterobacteriaceae and other selected oxidase-negative, gram-negative bacilli isolated from human clinical specimens.(2) REMEL S RapID™ NF Plus System: A qualitative micromethod employing conventional and chromogenic substrates for the identification of medically important glucose non-fermenting, gram-negative bacteria. Also the identification involved other selected lactose-fermenting bacteria not belonging to the family Enterobacteriaceae, which have been isolated from human clinical specimens. Each isolated bacterium was tested for oxidase and gram stained. The Gram negative, oxidase negative samples were tested by the first kit (REMEL S RapIDTM ONE System); while the gram negative, oxidase positive were tested by the Second kit (REMEL, S RapIDTM NF Plus System). *E. coli* ATCC number 25922 was obtained from the WHO and used as a control.

Oxidase test (cytochrome c): This test was made according to the method of Claus and Balkwill, (1989).

Antibiotic susceptibility test: Nine different antibiotics were obtained from either OXOID or BBL™ Company tested against the isolated bacteria. These were mentioned in the legend of Table 4. The interpretation of their interactions was analyzed according to the manufacture's guidelines.

RESULTS

BOD_5 and COD Tests: BOD5 and COD of the plant (polishing pool and effluent) as well as the field showed significant decrease compared to that of the raw wastewater. The BOD5 and COD values for the samples of influent were 746.9 (±89) and 2042 (±143) mg L^{-1}, polishing pool were 14 (±3) and 59 (±8) mg L^{-1}, effluent were 15 and 84.4 (±12) mg L^{-1}, the field were 14.4 (±3) and 85.4 (±13) mg L^{-1} respectively. The overall treatment efficiency for BOD5 and COD was 97 and 96%, respectively. The values of treated wastewater in plant and field were within the acceptance range of the Jordanian standard (300 for BOD_5 and 500 mg L^{-1} for COD) (Jordanian standard 893/2002).

Bacterial counts on NA, EMB, DCA and HEA media from raw and treated sewage: The average total bacterial counts (TBC) on NA medium of the raw sewage was 10^6 cfu mL^{-1}. TBC decreased to approximately 10^3 cfu mL^{-1} after chlorination treatment. The relative reduction in the total bacterial counts was estimated to be 0.014% of that in the raw sewage. The average number of coliform bacteria grown on EMB medium was about 10^6 cfu mL^{-1} when the source was raw sewage and 10^3 cfu mL^{-1} when the source was treated sewage. The total coliform counts was approximately 0.05% of that in the raw sewage. The coliform population cultured on EMB plates constituted 27.3% of the total number in raw sewage cultured on NA plates while it constituted 96% of the total number in treated sewage cultured on NA plates.

Using DCA medium the average intestinal pathogen estimated in raw sewage was 10^5 cfu mL^{-1} whereas in treated sewage only two samples showed a detectable growth. In the raw sewage the total number of intestinal pathogens grown on DCA plates was 6.9% of that grown on NA plates.

In a sample from raw sewage the number of *Salmonella* and *Shigella* species cultured on HEA medium was 10^6 cfu mL^{-1}, which was reduced to zero in a sample from treated sewage.

Effect of chlorination on bacterial counts using different culture media: To examine the effect of chlorination on bacterial counts, the samples from polishing pool and chlorination tank were cultured on NA, EMB, DCA and HEA culture media. Table 1 shows a high bacterial counts in some samples collected from the polishing pool (10^5, 10^6, 10^7 and very dense cfu) reflecting an incomplete chlorination treatment. However when the population size in polishing pool was low (10^2,10^3 cfu) the chlorination treatment appeared to be more efficient than that in the previous samples.

Bacterial counts in field samples grown on NA, EMB, DCA and HEA media: The effect of wastewater treatment was also evaluated on the field water using the same cultivated media that were previously utilized for the cultivation of treatment plant sample (Table 1). Surprisingly the average total bacterial count on NA plates in the field was 5.6×10^3 cfu mL^{-1}, which represents 127 fold of the counts, appeared in the sample from chlorination tank. Also the number of bacteria cultivated on EMB (coliform) was about 5.4×10^4 cfu mL^{-1}, which represents 5.4×10^4 fold of the counts seen on the chlorination tank sample grown on the same media. When the field sample was cultured on DCA plates (intestine pathogens) the average bacterial counts was 5.6×10^3 cfu mL^{-1}. In contrast only one out of all samples from the chlorination tank had a significant bacterial growth apparently suggesting an efficient treatment process. Using the HEA plates (*Salmonella* and *Shigella*) the total counts were almost 3.7×10^3 cfu mL^{-1} in the field sample compared with zero counts in the chlorination tank sample. The above results confirmed that the total bacterial counts had significant increase in the field water source compared with that in the chlorination tank.

Based on different sampling dates there was inconsistency appeared in species composition from day to day. This may reflect either variations in the type of bacterial species or the limitation of the media for growing whole cells present in samples. To clarify this point we made further evaluations presented in the following data: (1) Figure 1a shows that only NA and EMB plates had significant growth but NA plates relatively possessed higher bacterial growth than EMB; (2) Figure 1b indicates that only NA plates produced significant growth; (3) Figure 1c indicates that the NA plates had higher bacterial counts than EMB, which is usually expected whereas the HEA plates (*Salmonella* and *Shigella*) gave higher total bacterial counts than the DCA plates (intestine pathogens); (4) Figure 1d shows unexpected growth profile. For example, the total bacterial counts on EMB (coliform) represented 84% of the total combined growth on NA, EMB, DCA and HEA, knowing that NA is a general-purpose media. Furthermore, the total bacterial counts on HEA (specified for *Salmonella* and *Shigella*) were higher than the total bacterial counts on DCA, which is a specific medium for intestinal pathogens; (5) In another sampling date (Fig. 1e) though little growth appeared on NA, EMB, HEA media a similar growth that supposed to be seen on DCA as a supporting medium for the growth of intestine pathogens, was not actually observed; (6) In the last sampling dates (Fig. 1f) the problem even gets much more complicated where the total bacterial growth measurements on EMB>NA and on HEA>DCA, as well as the growth on HEA were higher than the growth observed on NA plates.

Table 1: Effect of chlorination on bacterial counts using four different types of media (NA, EMB, DCA and HEA)

EMB (cfu)		NA (cfu)		
Field	Chlorination tank/plant	Field	Chlorination tank/plant	Date
1.1×10^2	2.8×10^3	5.6×10^3	4.4×10^2	9/12/03
No growth	No growth	2.6×10^2	3.1×10^3	30/12/03
5.4×10^4	No growth	7.3×10^3	No growth	10/3/04
3.7×10^2	3.1×10^3	6.4×10^2	No growth	31/3/04
ND	No growth	ND	No growth	28/4/04
3.1×10^3	3.1×10^2	2.4×10^3	2.9×10^3	13/5/04
HEA (cfu)		**DCA(cfu)**		
ND	ND	5.6×10^3	No growth	9/12/03
No growth	No growth	2.6×10^2	No growth	30/12/03
3.7×10^3	No growth	2.4×10^3	1.3×10^2	10/3/04
6.5×10^2	ND	2.5×10^2	3.4×10^5	31/3/04
ND	No growth	No growth	No growth	28/4/04
2.3×10^3	No growth	2.2×10^3	No growth	13/5/04

ND, Not determined

Table 2: Identified bacterial strains that were isolated from university plant influent on EMB medium

Isolation source	Gram stain	Oxidase test	Identified strains
IE1	-	-	Shigella *sonnei*
IE2	-	-	*Klebsiella rhinoscleromatis*
IE3	-	-	*Pantoea agglomerans*
IE4	-	-	Kit can't identify this isolate[a]
IE5	-	-	*Serratia odorifera* 1 and 2
IE6	-	+	ND
IE7	-	-	*Enterobacter aerogenes*
IE8	-	-	*Yersinia enterocolitica*
IE9	-	-	*Pantoea agglomerans*
IE10	-	-	*Klebsiella penumoniae*
IE11	-	-	*Citrobacter koseri*
IE12	-	-	*Leminorella grimontii* (EG-57)
IE13	-	-	Kit result show overlap between two species[b]
IE14	-	-	*Klebsiella oxytoca*

I: Means Influent; E: EMB medium; 1,2,3...14, Isolate numbers [a]*Sphingomonas paucimobilis* (85.28)% overlap with *Stenotrophomonas maltophilia* (14.72) [b]*Providencia oryzihabitans* (86.19)% overlape with yersinia pseudotuberculosis (10.10)%

Table 3: Identified bacterial strains that were isolated from chlorinated plant effluent water on EMB medium

Isolation source	Gram stain	Oxidase test	Identified strain
EE1	-	-	*Acinetobacter calcoaceticus*
EE2	-	-	*Pantoea agglomerans*
EE3	-	-	*Klebsiella ozaenae*
EE4	-	-	*Citrobacter koseri*
EE5	-	-	*Acinetobacter calcoaceticus*
EE6	-	-	*Ewingella americana*

EE1.2.3...6: E: Effluent; E: EMB medium; 1.2.3 ...6: Isolate number

Identification of the bacteria isolated from raw and treated sewage influent of the University treatment plant: Table 3 shows the data obtained from testing 14 different morphological characteristics to identify the bacteria isolated from the sample grown on EMB plates. A total of 14 isolates were collected, 11 of them were already identified (as mentioned in experimental procedure). The

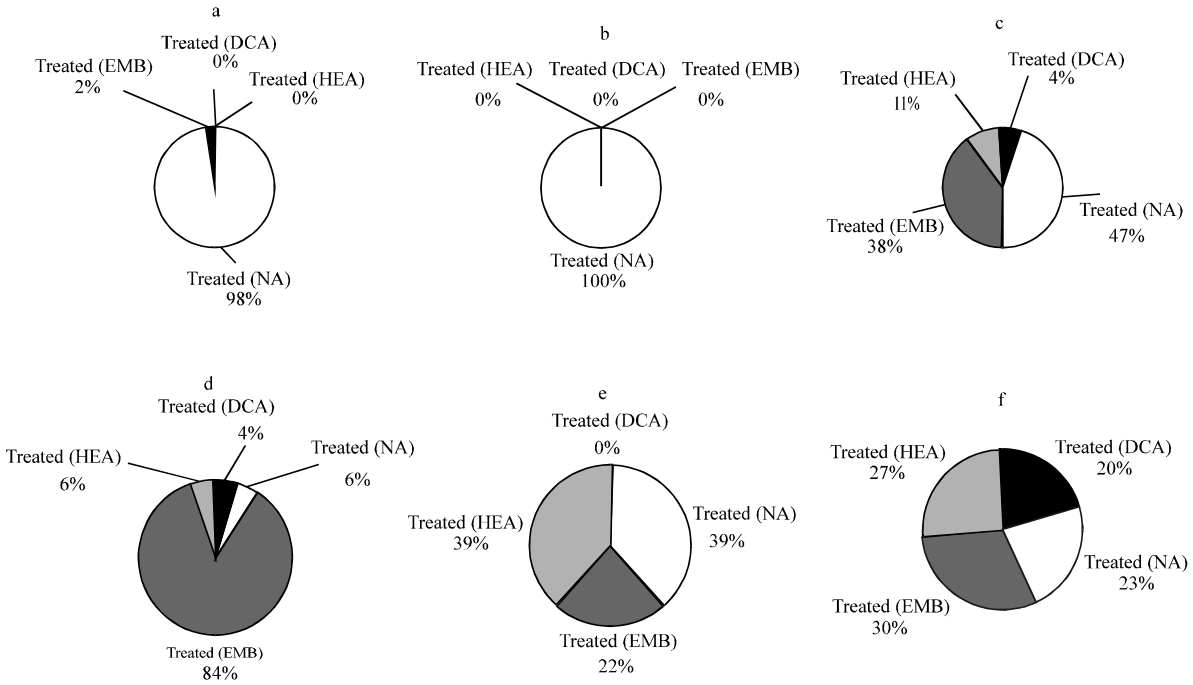

Fig. 1: Recovery of bacteria grown on NA, EMB, DCA and HEA media collected from university field recycled water in different sampling dates. (a) in 9/12/2003; (b) 30/12/2003; (c) 21/1/2004; (d) 10/3/2004; (e) 31/3/2004; and (f) 13/5/2004. Treated EMB (■); Treated/NA (□); Treated HEA (■); Treated DCA (■)

Table 4: Comparison between three bacterial species isolated from both raw (influent) and treated (effluent + field) wastewater versus their susceptibility to nine different antibiotics

	Serratia 1 and 2 *odoriferal*		*Pantoea agglomerans*		*Citrobacter koseri*	
Antibiotic code	Influent (inhibition zone +susceptibility)	Field (inhibition zone +susceptibility)	Influent (inhibition zone +susceptibility)	Field (inhibition zone +susceptibility)	Influent (inhibition zone +susceptibility)	Field (inhibition zone +susceptibility)
TE	25, S	29, S	21, S	22, S	19, S	21, S
S	22, S	25, S	21, S	24, S	23, S	31, S
OFX	30, S	20, S	27, S	21, S	31, S	30, S
CAZ	28, S	30, S	27, S	28, S	29, S	33, S
NA	21, S	0.0, R	20, S	0.0, R	22, S	28, S
CF	12, R	0.0, R	0.0, R	13, R	0.0, R	11, R
AML	0.0, R	0.0, R	0.0, R	0.0, R	0.0, R	0.0, R
CL	22, S	25, S	23, S	24, S	23, S	28, S
AMP	0.0, R	0.0, R	0.0, R	0.0, R	0.0, R	0.0, R

TE, Tetracyclin; S, Streptomycin; CF, Cephlothin; NA, Nalidixic acid; CAZ, Ceftazidime; OFX, Ofloxacin; AML, Amoxycillin; CL, Cephalexin; AMP, Ampicillin

three unknown isolates might be the result of an overlapping between two species (Table 2) or they may represent an obscured species that were not picked up by our standard morphological tests. The 11 identified isolates were: *Shigella sonnei*, *Klebsiella rhinoscleromatis*, *Pantoea agglomerans*, *Serratia odorifera* 1 and 2, *Enterobacter aerogenes*, *Yersinia enterocolitica*, *pantoea agglomerans*, *Klebsiella penumoniae*, *Citrobacter koseri*, *Leminorella grimontii*(EG-57) and *Klebsilla oxytoca.*

Effluent from the University treatment plant: Morphologically 8 isolates were obtained from the chlorination tank sample after cultivation on EMB medium. Two of them were lost during the identification procedure and the remaining six isolates were identified (Table 3). These isolates were resistant to the chlorination treatment. They compose the following genera: *Acinetobacter calcoaceticus*, *Pantoea agglomerans*, *Klebsiella ozaenae*, *Citrobacter koseri*, *Acinetobacter calcoaceticus* and *Ewingella americana.*

By using NA plates for cultivating the same sample from the chlorination tank only three morphological isolates were observed. These isolates were characterized as Streptococcobacilli, cocci and staphylococci. All of them were identified as gram positive but one strain was found to be oxidase positive.

Field of the university campus: The water sample from the University campus field produced 3 isolates on EMB medium. These were identified as *Neisseria weaveri/elongata, Shigella* sp. and *Serratia odorifera* 1 and 2 (data not shown). When NA medium was used for the same cultivation also 3 different isolates were observed (one Streptobacilli and two bacilli). Two of them were oxidase positive and gram negative, while the third was oxidase negative and gram positive. One of them was suspected to be either *Moraxella osloensis* or *Alcaligenes faecalis*, whereas a second isolate could not be identified. The third was oxidase negative as well as gram positive and therefore could not be identified by the available tests.

Effect of different antibiotics on the isolated species from raw and treated sewage: Generally all isolates were resistant to at least one of the antibiotics being used (data not shown). The multiple antibiotics resistant (three or more) appeared in eleven of the fourteen isolates from the water influent. Six isolates were obtained from the effluent samples that were resistant to at least three antibiotics. Also, two isolates from the field sample were subjected to similar tests and found resistant to at least three types of antibiotics. Furthermore, two isolates from either effluent or field sample cultivated on NA media were found resistant to at least three types of antibiotics.

There are three bacterial species isolated from the influent sample, which also detected in either the chlorination tank or field samples. These species are *Serratia odorifera* 1 and 2 isolated from the influent and field; *Pantoea agglomerans* and *Citrobacter koseri* were isolated from influent and chlorination tank. These species showed differences in the inhibition zone before and after treatment without changing their interpretive standard values (Table 4). Exceptions were found in *Serratia odorifera* 1 and 2 and *Pantoea agglomerans* behavior towards nalidixic acid, which were sensitive before treatment (21 and 20 mm respectively) and became completely resistant after treatment. Whereas *Citrobacter koseri* was slightly sensitive before treatment and developed more sensitivity towards Nalidixic Acid after treatment.

DISCUSSION

The generation of hazardous biological and chemical wastes from various sources including wastewater treatment plants, chemical industries, laboratories and hospitals is a worldwide problem. About 80% of the total wastes on land posses the risk of contaminating ground water and vegetation causing adverse health effect (Orteigo *et al*., 1995). Jordan has set certain standards for the evaluation of treated wastewater. Generally, these standards depend on outside information, as little data are available regarding the wastewater evaluation in Jordan. Therefore, this study represents an attempt to evaluate treated water as well as the validity of the local standards for this evaluation.

In raw waste water, it was observed that both the level of microorganisms (10^6 cfu) and the concentration of substrate (615-995 mg L^{-1}) were elevated. As the chlorination treatment processes progressively continue, the aerobic and facultative anaerobic bacteria cooperatively manage to decrease the level of BOD5. According to the Jordanian standards of BOD5 and COD, the efficiency of treatment plant at the University campus is apparently acceptable. In raw water and in chlorination tank, the relation between total bacterial counts, BOD5 and COD show some agreement (data not shown). However, in the polishing pool, the situation is quite different, where BOD5 and COD had low values, while the total bacterial counts were fluctuating (Table 2). It is possible that the substrate availability is not the only limiting factor for total bacterial counts, but there might be an involvement of species variations. So that certain bacterial species are capable of growing for long term while others lack such capability, especially under starvation conditions (Horan, 1993). Some workers found that *Vibrio parahaemolyticus* did not injured as a result of starvation (Jiang and Chai, 1996). Their data showed that when these bacteria were cultured on selective and non-selective media they produced the same TBC regardless of how long the cells were starved. Our data on the high TBC in polishing pool could be partly attributed to the absence or lowering of amoeba and ciliated protozoa that feed on bacteria (Horan, 1993).

Beyond the exhaustion of substrate existed in wastewater by microorganisms, it is necessary to get rid of microorganisms themselves (some are pathogens) prior to the diversion of treated water back to the environment. Our data suggest that the efficiency of chlorination treatment did not always show the same quality. The outcome of chlorination treatment seems to be satisfactory but still there were occasions, where the

efficiency of chlorination was variable from day to day (Fig. 1a-c). It's notable that the species composition of the sample has certain affect on the efficiency of chlorination treatment. This may explain the reason why the sample from field (Fig. 1d) had TBC on EMB (coliform) more than that of NA (general media). In contrast little growth appeared on DCA (for intestinal pathogens) and on HEA media (for *Salmonella* and *Shigella*) indicating that high number of bacteria growing on EMB media was resistant to chlorination treatment. In other samples (Fig. 1e and f), all bacteria were capable of growing on EMB, DCA and HEA were either killed or deactivated by chlorination treatment. In contrast, those species of bacteria, which grew on NA medium (probably gram positive) showed resistant to chlorination process. These data are in agreement with the finding of Cheryl *et al.* (2000) as well as the observation of Horan (1993) who reported fluctuation in bacterial species composition of wastewater from day to day and found a species-specific resistant to chlorine. The high TBC observed in field water compared to effluent water samples could be attributed to a residual remaining of alive bacteria. Alternatively, it is possible that some bacteria became viable but non-culturable (VBNC) in response to the chlorine treatment (Arana *et al.*, 1999) which was activated later during subsequent incubation (Rockabrand *et al.*, 1999).

In alignment with other findings (Taormina and Beuchat, 2000; Rockabrand *et al.*, 1999) the present data suggest that chlorine might not be an efficient disinfectant. Besides, it might have a risk of producing carcinogenic compounds in water (APHA., 1992). *Salmonella* is a prone food poisoning agent, which can be transmitted to some vegetables like tomato through irrigated water (Guo *et al.*, 2001). Our data suggest that the growth of *Salmonella* and *Shigella* was undetectable on HEA selective medium when the sample was obtained from the chlorination tank, though significant growth was detected in four of the six field water samples. Meanwhile, further culturing of the same samples on medium (selective for intestine pathogens) yielded a significant bacterial growth. It seems therefore that those pathogenic bacterial species, which were able to grow on DCA and HEA managed to escape the plant chlorination treatment probably by entering VBNC state before being resuscitated in the field reservoir or distribution system. This may raise a serious pollution problem because the field treated water is usually used for irrigation of human-consumed vegetables at the University campus.

Three species of bacteria were persistently collected from all water sites (influent, chlorination tank and field). These bacterial species were *Serratia odorifera* 1 and 2, *Pantoea agglomerancs* and *Citrobacter koseri*. The TBC of the coliform on EMB relative to that on NA was 27.3%, whereas in treated sewage water the same group gave relative TBC of 96%. The reasons behind these variations in species composition and relative growth may reflect differences in the conditions of water ponds (Horan, 1993). Moreover, this variation may reflect differences in survival abilities similar to that reported for *E. coli* (Andersen, 1993). On the other hand, the isolation of *Klebsiella*, from both raw and treated water samples may suggest low treatment efficiency, which agrees with the observation of Andersen (1993).

The antibiotic effect on the flora of sewage water can be categorized into three groups: the first group of antibiotics include TE, S, OFX and CAZ which produced high inhibitory action on the bacteria isolated from raw and treated sewage. The second group includes Amp, AML, CF that showed little effect on these bacteria. The third group includes NA, CL that exhibited variation in their effect on bacterial species (some are resistant and some are sensitive).

Resistance to certain antibiotics appeared to be linked, where 18 of the 26 isolates have the following pattern of resistance Amp^r Cep^r $Amox^r$. The phenomenon of linked multiple drug resistance may reflect, to some extent, the microfloras continuous exposure to antibiotics (Harwood *et al.*, 2000; Murray *et al.*, 1984; Oppegaard *et al.*, 2001). In alignment with other observations (Murray *et al.*, 1984; Harwood *et al.*, 2000; Oppegaard *et al.*, 2001) our isolates were mostly resistant to Amp (22 of 26) and amoxycilline. In contrast the high susceptibility of our isolates to tetracycline is in disagreement with other findings (Shoemaker *et al.*, 2001; Murray *et al.*, 1984; Rhodes *et al.*, 2000). Tetracycline was found to be plasmid encoded, which may explain the increased resistance to this antibiotic in many societies through an exchange of resistance genes between different species (Rhodes *et al.*, 2000; Shoemaker *et al.*, 2001). However, the high resistance of our isolates to ampicillin and cephalothine may represent a chromosomal rather than plasmid coded resistance (Walsh *et al.*, 1995). Both ampicillin and cephalothin are inactivated by chromosomal beta–lactamase produced by many enterobacteria (Goni-Urriza *et al.*, 2000). The isolates *Serratia odorifera* 1 and 2 obtained from influent and field water were sensitive to tetracycline (25 and 29 mm respectively) which does not go along with the data of Livermore, (1996) who found that the *Serratia* are intrinsically resistant to tetracycline. Antibiotic-resistance bacteria are possibly discharged to the environment as a result of increasing antibiotic use in medical, agriculture

and veterinary practice (Guardabassi *et al*., 1998). The possibility exists that the chlorination treatment might increase the percentage of antibiotic resistant bacteria (Murray *et al*., 1984). This may explain why *Serratia odorifera* 1 and 2 and *Pantoea agglomerance* were sensitive to nalidixic acid before treatment while both species showed full resistance to this antibiotic after treatment.

In conclusion, the present investigation suggests inconsistency in the chlorination method and indicates that chlorine treatment is an insufficient disinfection method and it may enhance the growth of antibiotic-resistant bacteria. Further studies on this issue are recommended to evaluate other parameters such as total nitrogen, total phosphorus, total dissolved solid, cations and anions.

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