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Plasmid Incidence, Antibiotic and Metal Resistance among Enterobacteriaceae Isolated from Algerian Streams

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Abstract: Enterobacteriaceae isolates from surface water were examined to assess impact of feacal and/or metal pollution on heavy metal, antibiotics resistance and plasmid incidence. A bi-modal CMI distribution was noted for cadmium and mercury. On the other hand, modal distribution was observed for Pb. Critical metal concentration were >8, >32, ≥4096 μg mL^{-1} for mercury, cadmium and lead, respectively. High resistance to Pb and low resistance to Cd were remarked in stream water polluted with heavy metal. Resistance to antibiotics was most frequent to erythromycin (45.45-68.8%), tetracyclin family (14-61.11%), streptomycin (16-24%) and furan (8.16-24.1%). Bacterial resistance to some antibiotics (kanamycin, tetracyclin, doxycyclin, furan and chloramphenicol) was significantly different ($p<0.05$) between streams water. Analysis of antibiotic resistance by principal component analysis showed a clear difference between fresh water and urban waste water for two principal components (1, 2) and the difference between principal component scores of antibiotic could not be related to the faecal pollution level. No difference was found between stream water subjected or not to contamination from metallic or poultry waste. The frequency of strains carrying plasmids was higher in urban waste water than metal and/or low faecal polluted stream water. No correlation was observed between plasmid and metal resistance.

Key words: Plasmid, antibiotic, heavy metal, enterobacteria, water

INTRODUCTION

Currently, sustainable development has gained a great importance in political development. Therefore, it is incompatible with an environment highly polluted by toxic compounds as heavy metals.

Heavy metals are stable and persistent environmental contaminants; they can be accumulated and transferred to higher organisms of food web (DeForest *et al*., 2007; Croteau *et al*., 2005) leading serious ecological and health problem.

Some metals were toxic often at low concentration (Mills and Colwell, 1977) and microorganisms were the first trophic web organism influenced by this toxicity (Giller *et al*., 1998). These compounds may have deleterious effects on microorganisms as increasing lag-phases (Morozzi *et al*., 1982), inhibition enzymatic activities (Nweke *et al*., 2007) damage the structure of DNA (Bruins *et al*., 2000) modifying composition and genetic structure of microbial populations (Kozdrój and van Elsas, 2001; Satchanska *et al*., 2005) and reducing microbial diversity (Anne *et al*., 1999). Since, bacteria play a key role in the environment, thus factors that concern their diversity and activities threat fertility of ecosystems and consequently their sustainability.

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 (Silver, 1996). These plasmid mediated resistance to heavy metals can also carry genes coding for antimicrobial resistance (Karbasizaed *et al*., 2003). As these resistance traits are generally associated with transmissible plasmids (Karbasizaed *et al*., 2003; Ünaldi Coral *et al*., 2005; Ghosh *et al*., 2000), their dissemination requires a survey. Spreading of heavy metal resistance represented an ecological advantage for bacteria especially in heavy metals polluted environments. Proliferation of antibiotic resistant bacteria, by direct (antibiotic usage) or indirect (heavy metal pollution) selection, present a potential health hazard because its represent therapeutic failure sources.

Impact of dilution fluid (fresh water or urban waste water) on toxicity of metallic effluent was few explored. In Setif (Algeria), batteries manufacture plant discharge effluent from lime neutralized process into sewer. No information about waste environmental risk is available.

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Moreover, level of faecal pollution or origin of water on bacterial heavy metal and antibiotic resistance has received little importance.

This study has as objectives:

- To determine the critical metal concentration that permit to differentiate sensitive and resistant bacteria
- To compare antibiotic and heavy metal resistance of bacteria isolated from urban waste water, metal and/or faecal polluted fresh water
- To determine the distribution of plasmid in various streams water, urban waste water and relationship between metal resistance and plasmid

MATERIALS AND METHODS

Sampling sites and samples collection: Streams of setif region are characterized globally by flood in winter and a partial or total drought in summer. Four sampling sites, localized on 4 streams have been chosen (Fig. 1).

Level of faecal pollution, pollution type (organic, metallic) and origin of water (fresh or used) were criteria which governed sites selection. Site 1 (4'24.82"N latitude, 5°25'34.26"E longitude) was on the Ouricia stream. It was polluted by urban used water of the city Ouricia. Site 2 (36°12'50.03"N latitude, 5°22'58.50"E longitude), located 3 km downstream the begining of Bousselam stream which was the main stream bordering Setif city. It was contaminated by brut urban effluent coming from Ouricia, Fermatou towns and neighboring localities, as well as sewage mill. Site 3 (36°9'48.66"N latitude and 5°25'34.26"E) was located on Echouk stream. This stream in an open sewer and have received urban effluent from North-East and South of Setif, as well as industrial sewages: mineral, metallic (Pb) and organic. Water was sampled 25 m downstream from the metallic effluent discharge point after input of industrial effluent. Site 4 was on El-Malah stream at 36°3'42.59" N latitude and 5°25'34.26"E longitude; It was located approximately 100 m from the confluence of Bousselam and El-Malah streams. The last stream was resulted from several streams that browsed salty soils and received urban used water from Guellal and Ouled Gassem localities, as well as industrial effluent coming from Echouk stream. Bousselam and El-Malah streams constitute the main water suppliers for barrage Ain Zada. Sites sampling are classified as following:

- **Site 1 (Ouricia stream):** Low faecal polluted water
- **Site 2 (Bousselam stream):** Moderate faecal polluted water
- **Site 3 (Echouk stream):** High faecal and industrial polluted water
- **Site 4 (El Mallah stream):** Low faecal and metal (Pb) polluted water

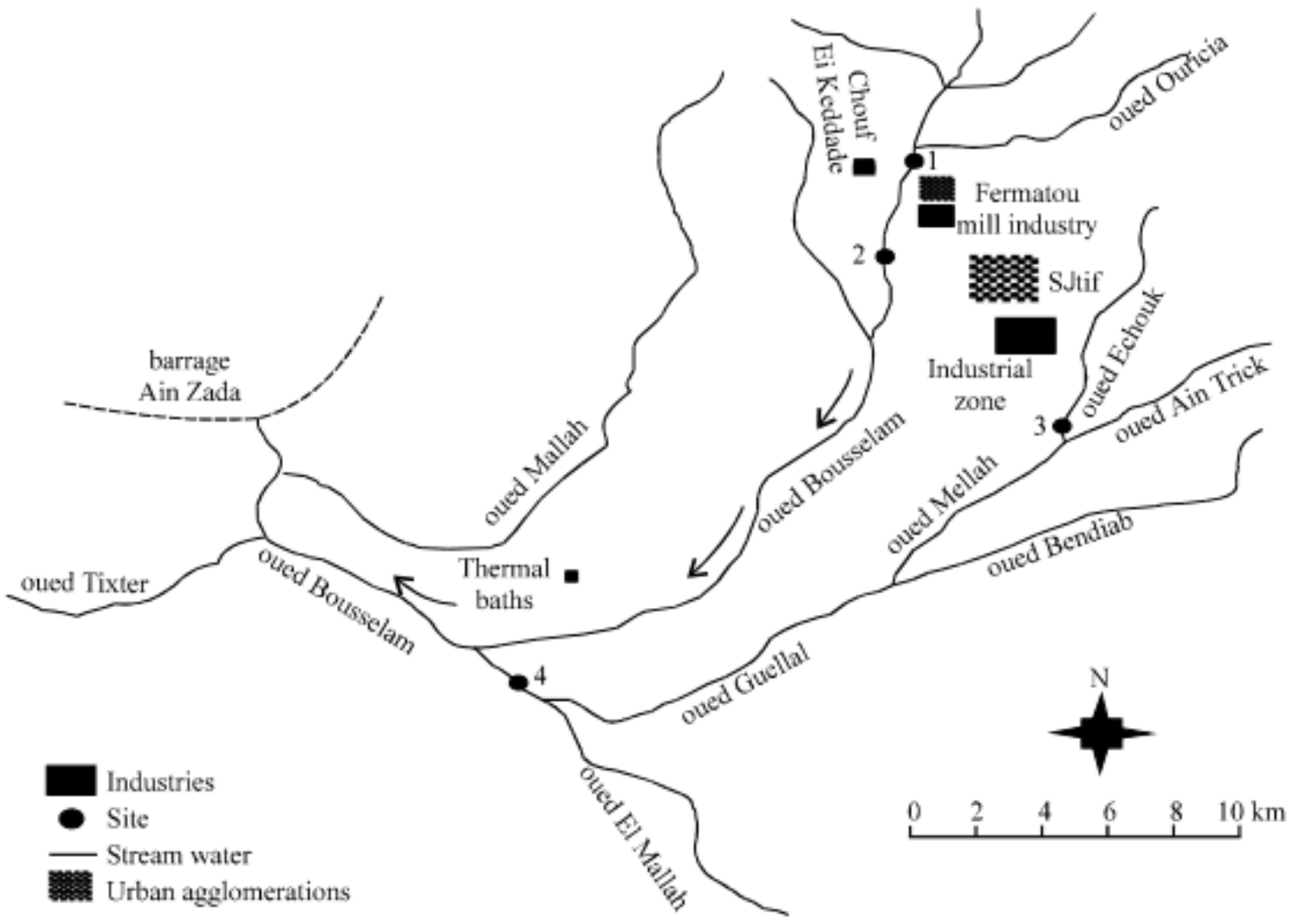

Fig. 1: Map of study area and site locations

Ouricia stream and El Mallah streams have browsed some poultry farming zones. Water samples were collected under the stream surface in sterilized glass bottles and stored on ice for up to 6 h from the time of collection for transport and subsequent analysis in the laboratory. For Echouk stream, water was sampled at 30 m after metallic effluent input. A total 10 samples by site have been harvested between January 1998 and May 2002.

Isolation and identification of enterobacteria: Samples of raw water and/or its dilutions were plated on Mac Conckey agar media. After incubation (18-24 h, 37°C), 10 to 12 lactose positive colonies, by sample of water, were purified on nutrient agar. Preliminary identification of strains obtained in pure culture was based on general characteristics of *Enterobacteriaceae* family in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). After confirmation of negative Gram reaction, negative oxidase test, facultative aero-anaerobic respiratory type and respiratory-fermentative type of metabolism, isolates were identified by API bacterial identification system (API 20E, Bio-Merieux). After identification, isolates were placed in a -70°C freezer.

Determination of resistance to heavy metals: The Minimal Inhibitory Concentration (MIC) of metals has been tested by two-fold serial dilution in Mueller-Hinton broth according to protocols described by Lennette *et al*. (1985). Three stocks solutions (10x) of heavy metals salts ($HgCl_2$, Pb $(NO3)_2$, $CdCl_2$ 2.5 H_2O) were prepared in distilled water and sterilized by membrane filtration (0.2 µm). A set of metallic solutions (12) has been prepared, for each selected heavy metals salts, by two-fold serial dilution of the stock solutions, in sterile distilled water (10 mL). The range of concentration of metallic salts solutions was between 2.5 and 5120 µg mL^{-1} for $HgCl_2$, 20 and 40960 µg mL^{-1} for $Pb(NO_3)_2$ or $CdCl_2$, 2.5 H_2O. The metallic solutions were then diluted at 1/10 in Mueller-Hinton broth. Finally 200 µL of each Mueller Hinton broth supplemented with metallic solution were transferred separately in a 96-well microtiter plate rounded format and then inoculated with 10 µL of a bacterial suspension (10^7 cells mL^{-1}). After incubation (37°, 18-24 h) without shaking and observation of microtiter plate, using a reversed mirror, MICs were recorded in the lowest metallic concentration which prevented visible turbidity.

Determination of resistance to antibiotics: The antimicrobial resistance patterns of 208 strains, isolated from four sites, were tested by single disc diffusion method using Muller-Hinton agar against the following antibiotics (Sanofi Diagnostics Pasteur): chloramphenicol (Cm, 30 µg), trimethoprim-sulphamethoxazole (Sxt 1.25/23.75 µg), amikacin (Ak, 30 µg), kanamycin (Km, 30 UI), tetracycline (Te, 30 µg), oxytetracyclin (Ot, 30 µg), doxycyclin (Do, 30 UI), streptomycin (S, 10 µg), colistin (Co, 50 µg), furan (Ft, 300 µg), erythromycin (E, 15 UI), tobramycin (Tm, 10 µg). Overnight broth cultures were diluted on saline solutions (9 mL). Bacterial saline suspensions (1:1000) were spread over Mueller-Hinton agar plates and plates were dried at 37°C for 30 min. Antibiotic discs were placed using disk distributor. The plates were incubated for 24 h at 37°C and organisms were classified as sensitive, or resistant. Intermediate susceptibility organism was scored as resistant. The tests were performed following National Committee for Clinical Laboratory Standards (1984) recommendations, including *Escherichia coli* ATCC 25922 as a control strain.

Detection of plasmids: Seventy three randomly *E. coli* strains isolated from the four sampling sites have been submitted to plasmid search. These strains have been divided in 2 distinct groups (first with 38 and second with 35). Two DNA markers (I and II) were kept as reference molecular weight marker to determine plasmid size. First marker was taken as standard for one group and second for other group. Marker I is a mixture of 5 DNA fragments (56, 33, 10.5, 4, 2.96 kb, Laboratoire de Microbiologie pharmaceutique, Rennes I, France) and Marker II (VII, 0.08-8.57 kb, Roche). Plasmids DNA was extracted from each strain after overnight growth at 37°C in Trypticase soja broth and prepared by alkaline lysis method modified by the addition of lysozyme (Walker, 1984; Grinsted and Bennett, 1988). Agarose gel 0.8% (w/v) was prepared and 12 µL of DNA preparation was loaded into each well. Electrophoresis was conducted for 2-3 h at 75-100 V and gel was stained with 0.5 µg mL^{-1} ethidium bromide (Grinsted and Bennett, 1988). Plasmid DNA band was observed with UV transilluminator and photographed with a Polaroid MP4 camera equipped with red filter and type 667 Polaroid film (Grinsted and Bennett, 1988).

Statistical analysis: Data derived from antibiotic and heavy metal susceptibility testing of *Enterobacteriaceae* isolates were converted to binary code. Statistical analysis of metal tolerance data were performed by a comparison of proportions by the Z-test, with confidence levels of 5% being considered significant. All pairwise comparison of proportions of antibiotic resistance, plasmid and factor scores were made by Mann-Whitney test and multiple comparisons of principal

component scores procedure were analyzed by Kruskal-Wallis test with confidence levels of 5% being considered significant. Statistica software was used to analyze all data.

RESULTS

Identification: On a total of 373 isolated enterobacteria strains, 340 were identified. The strains were distributed according to following species: *E. coli*, *C.* sp., *En.* sp., *K.* sp., *S.* sp., NI (Table 1). The most frequent genera isolated from 1, 2, 4 sites were *Escherichia*, *Citrobacter*, *Klebsiella*. At site 3, only two genera *Escherichia*, *Klebsiella* were dominant.

Heavy metal resistance

Metal resistance level: Distribution CMIs value to lead, cadmium and mercury (Fig. 2) showed large range and varied between 64-8192, 2-512 and 0.25-168 μg mL^{-1}, respectively. A bi-modal CMIs distribution was noted for cadmium and mercury. On the other hand modal distribution was observed for Pb. Otherwise 8 μg mL^{-1} of mercury and 32 μg mL^{-1} of cadmium were proposed as critical values to distinguish resistant and sensitive strains. While for Pb, it was difficult to establish critical value. However, 4096 μg mL^{-1} could be proposed, because of curve decrease of strains percentage at this value. According to CMIs values, metal toxicity gradient was found in the following order: Hg>Cd>Pb.

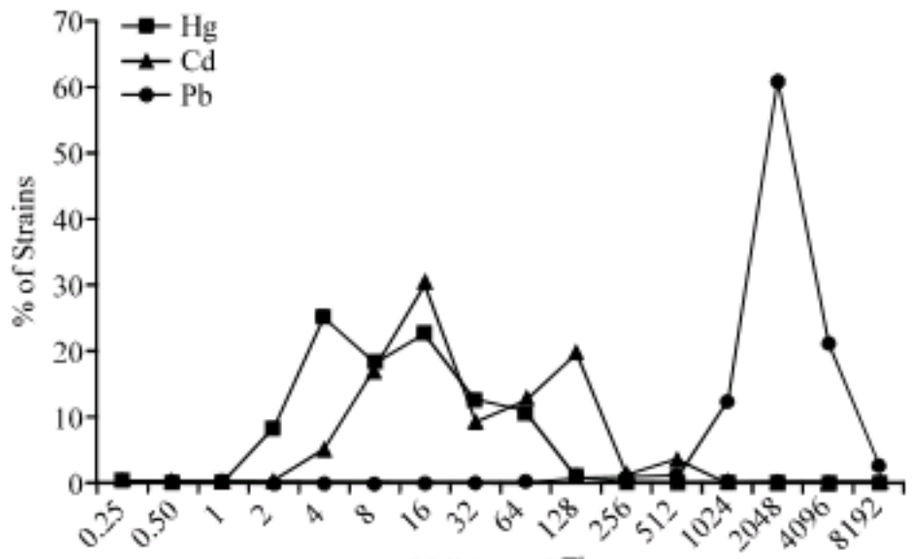

Fig. 2: The minimal inhibitory concentration (MIC) distribution of resistance to mercury, cadmium and lead among 373 enterobacteria strains.

Table 1: Distribution of the enterobacteria species found in different sites

	Percentage of strains			
Species	Ouricia (95)	Bousselam (96)	Echouk (98)	El-Malah (84)
Citrobacter sp.	22.11	17.70	9.18	13.10
Escherichia coli	50.53	33.30	45.90	53.57
Enterobacter sp.	3.16	8.33	5.10	3.57
Klebsiella sp.	13.68	27.10	26.50	19.05
Serratia sp.	1.05	6.25	5.10	0.00
Ni[a]	9.47	7.29	8.16	10.71

a : unidentified

Metal resistance pattern: Globally the most frequent phenotypes, summarized in Table 2, were represented by Hg, Pb, HgCd and HgCdPb and varied between sites. Resistance to mercury was almost always associated to cadmium resistance. For resistance to single metal, percentage of strains with Pb phenotype (19.05%) were significantly higher in site 4 compared to strains isolated from site 1 or 2 (6.12-6.32%). Also, marked frequency to Hg phenotype was shown in site 4 (21.43%) than in site 3 (10.2%). HgCd phenotype was more frequent among strains isolated from site 1 or 2 (30.21-32.63%) than those from site 4 (14.29%). Whereas HgCdPb phenotype, higher frequency predominate among strains isolated from site 3 (14.29%) than strains of other sites (1.04- 3.16%).

Mono, multiple and cumulative resistance to metals: Except values found in some streams, globally resistance to 2 metals, as shown in Table 3, was more frequent than resistance to one or 3 metals . Proportion of sensitive strains was less frequent in site 4 than in site1. Resistance to one metal was more common among strains isolated from site 4 than those other sites. Resistance to 2 metals was expressed by significantly greater proportions of

Table 2: Distribution of heavy metal resistance pattern of enterobacteria strains at different

	Percentage of resistants strains			
Resistance pattern	Ouricia (95)	Bousselam (96)	Echouk (98)	El-Malah (84)
S	43.16[a]	34.38	35.71	27.38[a]
Hg	14.74	12.50	10.20[a]	21.43[a]
Cd	1.05	4.17	6.12	3.57
Pb	6.32[a]	6.25[b]	6.12[c]	19.05[abc]
HgCd	29.47[a]	30.21[b]	19.39	14.29[ab]
HgPb	1.05	4.17	3.06	4.76
CdPb	1.05[ab]	7.29[a]	5.10	7.14[b]
HgCdPb	3.16[a]	1.04[b]	14.29[abc]	2.38[c]

( ) No. of strains. [a-c]Difference is statistically significant ($p<0.05$) in sites having same letter in the same line

Table 3: Frequency of mono and multi-resistance to heavy metals among enterobacteria strains isolated from different sites

	Percentage of resistants strains			
No. of resistance pattern	Ouricia (95)	Bousselam (96)	Echouk (98)	El-Malah (84)
0	43.16[a]	34.38	35.71	27.38[a]
1	22.10[a]	22.92[b]	22.45[c]	44.05[abc]
2	31.58	41.67[ab]	27.55[a]	26.19[b]
3	3.16[a]	1.04[b]	14.29[abc]	2.38[c]
Global resistance to metals	56.84[a]	65.62	64.29	72.62[a]

( ) No. of strains, [a-d]Difference is statistically significant ($p<0.05$) in sites having same letter in the same line

site 2 isolates than site 3 or 4 isolates. Resistance to three metals was prevalent amongst site 3 isolates. A higher incidence of global resistance to metals was found among site 4 isolates than those in site 1 isolates. When cumulative metal resistance frequency, summarized in Table 4, was compared between sites subjected or not to heavy metal pollution, no marked difference was observed for mercury (42.86-47.92%). For cadmium, significant difference was found between site 4 (27.38%) and site 2 or 3 (42.71-44.9%). Whereas for lead higher difference was noted between site 3 (28.57%) and 1 (11.58%) or between site 4 (33.33%) and 1 or 2 (11.58-18.75%).

Antibiotic resistance: A panel of nine antibiotics was used to test antibiotic resistance of strains isolated from stream polluted with the mixed effluent (urban and industrial), faecal polluted stream or urban used water. The antibiotic resistances of 208 enterobacteria strains are shown in Table 5. The most frequent resistance was found for tetracyclin family (14-61.11%), erythromycin (45.45-68.8%), streptomycin (16-24%) and furan (8.16-24.1%). Resistance to kanamycin (5.45-12.96%),trimethroprim-sulphametaxazole(8-14.29%) and chloramphenicol (2-12.24%) was relatively low. Resistance to colistin, tobramycin and amikacin was absent. The difference between sites was significant ($p<0.05$) for family tetracyclin, chloramphenicol, erythromycin and furan. Without introducing discrimination variable of site, all pairwise comparison of antibiotic resistance between freshwater streams and/or used water has not given a clear difference (Table 5). Principal Component Analysis (PCA) could be another method to make an approach of antibiotic resistance between stream water when variable was high. Discrimination of site against source of water, faecal pollution level, metal pollution, or poultry farming waste input was taken into account when antibiotic resistance was analyzed by PCA. Antibiotic resistance data of strains isolated from three streams and used water were combined and all variables were coded. Correlation matrix was generated, based on all coded variable. Two components were extracted; component 1 and 2 accounts for 31.54, 14. 4 % respectively of the variance of antibiotic resistance of strains isolated from four sites. First component was represented by doxycyclin, streptomycin and oxytetracyclin; these antibiotics contributed to about 50% of this axis. The second component was represented by erythromycin and contributes to about of 45% of this axis. Spearman correlation coefficients between principal component 1, 2 scores and variable were shown in Table 6. Significant difference (Table 7) of factor 1 and 2 were found with faecal pollution and origin of water or with factor1 and site. With faecal level, multiple comparison of p-values have shown that significant difference occur between high and moderate level for principal component 1 or between low and high level for principal component 2. Otherwise for site, multiple comparisons of p-values have shown that significant difference occur between 3 and 2. No significant difference was seen between streams water subjected or not to metal pollution and/or to poultry waste.

Table 4: Frequency of cumulative resistance to heavy metals among enterobacteria strains isolated from different sites

	Percentage of resistants strains			
Metal	Ouricia (95)	Bousselam (96)	Echouk (98)	El-Malah (84)
Hg	46.31	47.92	46.94	42.86
Cd	34.74	42.71^{a}	44.90^{b}	27.38^{ab}
Pb	11.58^{ca}	18.75^{b}	28.57^{c}	33.33^{ab}

( ) No. of strains. $^{a-c}$ Difference is statistically significant ($p<0.05$) in sites having same letter in the same line

Plasmids analysis

Plasmid distribution: When taking DNA size marker I (≤56 kb) (Table 8), frequency of strains carrying plasmids between sites was not significant. On the other hand with the DNA size marker 2 (≤8.57 kb), significant difference ($p<0.05$) was noted between strains isolated from site 3 and sites 1, 4.

Table 5: Incidence of antibiotic resistance among enterobacteria strains isolated from different sites

	Percentage of strains resistant to antibiotic			
Antibiotic	Ouricia	Bousselam	Echouk	El-Malah
Metal	(95)	(96)	(98)	(84)
S	24.07	16	20.00	20.41
K	12.96	8	5.45	12.24
Te	61.11^{a}	34^{ab}	56.36^{b}	44.90
Ot	33.33^{a}	30^{b}	50.91^{ab}	40.82
Do	35.19^{a}	14^{abc}	41.82^{b}	32.65^{c}
Sxt	11.10	8	9.09	14.29
F	24.10^{a}	10	18.18	8.16^{a}
E	68.50^{a}	62	45.45^{a}	57.14
C	11.10	2^{a}	10.91	12.24^{a}

( ) No. of strains. $^{a-c}$Difference is statistically significant ($p<0.05$) in sites having same letter in the same line

Table 6: Sperman correlation coefficients between scores and variable on principal component axes 1 and 2

Variables	Principal component 1	Principal component 2
S	0.655*	NS
K	0.411*	0.188*
Te	0.522*	-0.520*
Ot	0.787*	-0.221*
Do	0.788*	NS
Sxt	0.410*	NS
F	0.159*	0.443*
E	NS	0.797*
C	0.470*	0.165*

NS: Not significant. *$p<0.05$

Table 7: Statistical test difference for principal component 1, 2 scores and variable

Variables	PCA axes	Rank[1] of site characteristic	Test
Fæcal pollution level	Principal component 1	High > moderate	K.W.[2] P = 0.0000
Origin of water		Fresh water > Urban used water	M.W.[3] P = 0.0309
Site		3>2	K.W P = 0.0174
Fæcal pollution level	Principal component 2	Low>high	K.W. P = 0.0343
Origin of water		Fresh water> Urban used water	M.W. P = 0.0095

[1]:Ranking of site characteristic was conducted with multiple comparisons of p-values. [2]:K. W.: Kruskal-Wallis test. [3]: M. W.: Mann-Whitney test

Table 8: Frequency of plasmid distribution among enterobacteria strains by DNA size marker and sampling site

	Percentage of strains with plasmid	
Site	DNA size marker 1 (≤56 kb)	DNA size marker 2 (≤8,57 kb)
Ouricia	50.00 (10)	30[a] (10)
Bousselam	33.33 (9)	71.43 (7)
Echouk	30.00 (10)	100[ab] (10)
El-Mallah	33.33 (9)	62.5[b] (8)

( ) No. of strains. [a,b]Difference is statistically significant ($p<0.05$) in sites having same letter in the same column

Table 9: Plasmid heavy metal correlation

No. of *E. coli* tested	Presence or absence of plasmids	Resistance to Hg	Cd	Pb
3	-	-	-	-
1	-	+	-	-
23	-	+	+	-
2	-	-	+	+
1	-	+	-	+
7	-	+	+	+
4	+	-	+	-
2	+	+	-	-
18	+	+	+	-
1	+	+	-	+
3	+	-	+	+
5	+	+	+	+
3	+	-	-	-

Plasmid and metal resistance correlation: No correlation was found between plasmid and heavy metal resistance (Table 9).

DISCUSSION

$HgCl_2$, of $Pb(NO_3)_2$ and $CdCl_2$ concentrations determined according to the CMI curves and allowing to differentiate sensitive from resistance strains was in agreement with the studies of Grewal and Tiwari (1990), Antai, (1987) and Groves and Young (1975). On the other hand these proposed values differ from those others authors (Jones *et al*., 1986; Niemi *et al*., 1983). The difference in the determination of the critical concentrations can have several explanations: (1) method used, (2) type of medium, (3) nature of metal salt used, (4) type of bacteria tested have greater influence on metal toxicity. At sites 1 and 2, higher frequency of HgCd phenotype was probably linked to: (1) generic and species composition of sites, (2) specific conditions in these sites that favors proliferation of this phenotype, (3) no human strains origin, (4) conditions presents in sites 3 and 4 repress this phenotype. Survey or testing metal CMI from randomly isolated strains is an excellent method for detecting heavy metal pollution or discriminating between sites with or without heavy metal pollution. High percentage of Pb phenotype or Pb resistant strains observed in site 4 (El Mallah) could explane the metallic pollution (Pb) induced by the battery manufacture effluent. Earlier studies have noted that higher number of metal resistance bacteria in given site was attributed to selective pressure by heavy metal (Al-Jebouri, 1985; Zelibor *et al*., 1987; Diaz-Ravina *et al*., 1994). But, we are unable to explain the high percentage of Hg phenotype observed in strains isolated from site 4, since identifiable or characterized mercury pollution source do not exist. Low percentage of cadmium resistant strains observed in site 4 could probably be related to higher salinity of soil and probably interfering with Cd efflux mechanism. Global resistance to metals was not influenced by faecal pollution level. In this study, streams water (sites 1, 2 for Pb, Cd, Hg and 3 for Hg) without direct or historical metal pollution contain heavy metal resistant bacteria suggesting the existence of other factors, than direct selection, for promoting heavy metal resistance.

The present study has displayed that significant difference to some antibiotics of *Enterobacteriaceae* strains isolated from different sites were not related to faecal pollution level. Miranda and Castillo (1998) were found similar results when testing sensitivity to some antibiotics of *Aeromonas* isolates from different sources with varied level of faecal pollution. Comparison of antibiotic resistance with similar studies can be made while taking into account origin of the strains and the studied bacterial groups. Antibiotic resistance of *Enterobacteriaceae* strains for fresh water has reported by Goni-Urriza *et al*. (2000), Antai (1987), Obi *et al*. (2004) and Al-Jebouri (1985). The values of these studies range from 9.1-70%, 0-17%, 1.8-17.8%, 1.8-80%, 50-80% for tetracycline, kanamycin, chloramphenicol, streptomycin and erythromycin, respectively. Present results correspond fairly well to the results from these studies. Whereas for strains isolated from sewage effluent, values found in this study was comparable for resistance to streptomycin (1-55%), tetracycline (5-75%), erythromycin (30-60%), kanamycin (5.6-25%) reported by Al-Jebouri (1985), Jones *et al*. (1986), Silva *et al*. (2006) and Olayemi

and Opaleye (1990). Otherwise observed frequency to chloramphenicol and kanamycin resistance among sewage strains was markedly lower than those demonstrated in similar studies (Silva *et al*., 2006; Olayemi and Opaleye, 1990). On the contrary the rate of resistance to tetracyclin was higher compared to others findings (Jones *et al*., 1986; Niemi *et al*., 1983; Al-Jebouri, 1985; Watkinson *et al*., 2007). With principal component analysis no clear difference was seen with faecal pollution level. In the same way no significant difference were seen with sample water subjected or not to metal pollution and/or to poultry waste, but marked difference was noted between fresh and urban used water. These mitigated findings of antibiotic resistance were probably linked to insufficiency sampling. Indeed Miranda and Castillo (1998) have shown that moderately polluted waters showed lower antibiotic multiresistance and metal susceptibility than unpolluted and highly polluted ones. In the same way Tuckfield and McArthur (2008) have found when heavy metal concentration increase, the prevalence of antibiotic resistance decrease. Difference in antibiotic resistance between fresh water and urban used water could have several explanations: generic and species composition, distance between sampling site and contamination with urban used water, transfer of antibiotic resistance. In fact several authors (Boon and Cattanach, 1999; Goni-Urriza *et al*., 2000; McArthur and Tuckfield, 2000) have shown that the antibiotic resistance increase downstream from the polluted discharge. In the same way transfer of antibiotic resistance between strains (Silva *et al*., 2006) in stream water could be favoured by distance between sampling site and input of the contamination by urban used water. Also, species composition of water sample could affect antibiotic resistance pattern (Niemi *et al*., 1983).

Numerous studies have examined the relationship between plasmid incidence and the presence of environmental contaminants at a given site. Generally higher plasmid incidence was observed in polluted sites (Baya *et al*., 1986; Bell *et al*., 1983; Burton *et al*., 1982; Hada and Sizemore, 1981). However, with regard to level of faecal pollution, consensus was not observed when a plasmid frequency between sites was examined. Significance difference in frequency of plasmid was noted between sites with low and high faecal pollution when examining *Pseudomonas*-like isolates (Burton *et al*., 1982) or *Aeromans/Vibrio* group. But no difference was noted with non *Pseudomonas*-like isolates (Burton *et al*., 1982) or other bacterial group. Our data indicate difference between urban used water and low faecal polluted streams when examined relatively low size plasmid frequency among enterobacteria. The relatively preponderance of small plasmids showing in this study contrast with the results reported by Glassman and McNicol (1981). Higher incidence of enterobacteria strains harbouring these smaller plasmids probably have human origin and agree the findings of Al-Bahry (2000) which report that most human strains have relatively much smaller plasmids. Absence of correlation between presence of plasmid and resistance to mercury, cadmium and lead was in general agreement with that reported by Fredrickson *et al*. (1988) and Karbasizaed *et al*. (2003), suggesting that these resistance characters were chromosomally coded. In fact antibiotic and metal resistance can be governed by genes carried by the chromosome (Silver, 1996; Witte *et al*., 1986) or by transposons (Lett *et al*., 1985).

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