

Incidence of Heavy Metal & Antibiotic Resistant Coliforms in Coastal Waters & Sediments of Kuwait City

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Received 29 October 1984

Water and sediment samples from 4 stations along the coast of Kuwait city are analysed. Coliforms resistant to Cd, Ni and Fe are more prevalent at all stations compared to Hg resistant. Percentage of heavy metal resistant coliforms are higher in sediment than in water samples. Ampicillin, streptomycin and chloramphenicol resistant coliforms are more common in the total coliform population. *Escherichia coli* are most abundant among coliforms resistant to majority of heavy metals and antibiotics.

Though metals can be toxic to bacteria¹, some have the capacity to develop tolerance to initially toxic concentrations. Organisms having or developing natural tolerance to one metal may also be tolerant to more than one metal². Presence of antibiotic resistant coliforms in Kuwait marine environment and their ability to transmit their resistance to other bacteria through R⁺ plasmids are reported earlier³. Kuwait coastal waters are polluted with fecal coliforms^{3,4} and heavy metals such as Hg, Pb, Fe, Ni and Cd, etc.⁵. This study has been undertaken to determine the incidence of heavy metal and antibiotic resistant coliforms in the coastal waters and sediments of Kuwait city.

Four stations known to be polluted with raw sewage⁴ and also with heavy metals⁵ were selected along the coast of Kuwait city. Water and sediment samples were collected 10 m away from the outfalls, 5 times, between Sept. 1983 and Feb. 1984. Water samples were collected in sterile bottles and sediments with Van Veen bottom grab sampler.

Samples were analyzed for coliforms by the membrane filtration (MF) technique⁶. Water samples diluted with normal saline water were passed through membrane filters (size 0.45 μ m) and filters were placed on McConkey agar plates. Total number of coliforms were counted after 24 h of incubation at 37°C. Similarly, 10 g of sediment were suspended in 10 ml of sterile normal saline, mixed thoroughly and left for 10 min. Dilutions were prepared from the supernatant and processed by MF technique as described for water samples.

Isolated coliforms were examined for resistance to 4 heavy metals (Cd, Ni, Fe and Hg). The metals were used as CdCl₂, NiCl₂, FeCl₃ and HgCl₂. Stock solutions of the metal salts were filter sterilized through millipore filters (0.45 μ m) and added to molten McConkey agar (cooled at 45°C) to give final heavy

metal concentration of 15 μ g.ml⁻¹ of agar medium. Colonies from the master plate were replicated onto each metal containing McConkey agar plate, using replica plating technique. Unsupplemented plate was considered as control. Colonies growing on the supplemented plates were considered resistant.

Antibiotic resistance patterns were determined similarly as above using antibiotic solutions instead metal salts. Antibiotics used with the concentrations (μ g.ml⁻¹) were kanamycin (30, K), ampicillin (10, A), streptomycin (10, S), chloramphenicol (30, C), tetracycline (30, T) and gentamycin (10, G).

Selected isolates were identified up to species level using commercially available API 20E identification system⁷ and checked for their multi metal and antibiotic resistance.

A total of 80 water and sediment samples were collected from 4 stations. Percentages of heavy metal and antibiotic resistant coliforms in water and sediment samples are presented in Table 1. The incidence of heavy metal resistance in isolates from all stations was almost similar. Coliforms resistant to Cd, Ni, and Fe were more prevalent compared to Hg resistant. At each of the stations, metal resistance ranking was Ni > Cd > Fe > Hg. Percentages of resistance to heavy metals were higher in sediments than in water samples at all stations. However the incidence of heavy metal resistance in sediment isolates from all stations was similar to that of water isolates. The frequency of heavy metal resistance in sediment was Fe > Ni > Cd > Hg.

Isolates were also tested for their resistance to 6 commonly used antibiotics. Percentage of coliforms resistant to each antibiotic are presented in Table 1. Ampicillin, chloramphenicol and streptomycin resistant were most common among coliforms from water and sediment samples at all stations. This could be due to the wide usage of these antibiotics in Kuwait

Table 1—Heavy Metal and Antibiotic Resistant Coliforms in Water and Sediment Samples

Sts	Total No. of coliforms [10 ³ .(100ml) ⁻¹]	Metal resistance (%)				Antibiotic resistance (%)					
		Cd	Ni	Fe	Hg	K	A	S	C	T	G
		Water									
1	13.5	42.2	46.6	39.2	34.0	Nil	27.2	24.2	24.2	3.0	3.0
2	16.1	41.0	45.3	41.6	32.9	3.4	34.4	20.6	31.0	6.9	Nil
3	13.1	51.1	38.1	48.1	42.0	4.7	57.1	42.8	38.0	9.5	Nil
4	5.0	50.0	70.0	52.0	22.0	Nil	38.4	15.3	30.7	Nil	7.7
Mean		46.0	50.0	45.0	32.7	2.0	39.2	25.7	30.9	4.8	2.7
Sediment											
1	387	60.0	62.7	80.0	51.4	Nil	14.2	14.2	42.8	28.5	14.2
2	540	78.5	85.1	76.5	28.3	11.2	11.2	33.1	22.0	11.6	11.2
3	7467	44.6	55.2	47.6	40.4	Nil	80.0	40.0	20.0	40.0	30.0
4	620	83.2	79.3	86.1	70.1	Nil	16.6	33.3	16.6	18.0	16.0
Mean		66.5	70.5	72.5	47.5	2.8	30.5	30.1	25.3	24.5	17.8

K = kanamycin, A = ampicillin, S = streptomycin, C = chloramphenicol, T = tetracycline, G = gentamycin

Table 2—Heavy Metal and Antibiotic Resistance Patterns of Different Isolates

Species	No. of isolates	Resistance pattern
<i>Escherichia coli</i>	22	Cd, Ni, Fe, Hg, A, S, C, T, G
<i>Enterobacter cloacae</i>	7	Cd, Ni, Fe, A, S, C, T
<i>Enterobacter</i> sp.	5	Cd, Ni, Fe, K, A, S, C
<i>Klebsiella pneumoniae</i>	6	Ni, Fe, K, A, S, C, T
<i>Klebsiella</i> sp.	5	Cd, Fe, K, A, T
<i>Citrobacter</i> sp.	5	Cd, Ni, Fe, Hg, A, C, T, G

(Personal communication). Similar results were observed in Kuwait marine environment by Al-Mossawi *et al.*³.

Fifty colonies were selected randomly and checked for their combined heavy metal - antibiotic resistance patterns (Table 2). The most common resistance pattern observed among isolates was Cd, Ni, Fe and ASC. Among the isolates *Escherichia coli* was resistant to almost all metals and antibiotics. *E. coli* with

multiple antibiotic and heavy metal resistances were common in sediments of sewage dumping area.

The source of antibiotic resistant coliforms could be the sewage sludge which acquires metal resistance after dumping into the sea. However, further study is required on transfer of R + plasmid to support this statement.

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