Heavy metal resistance of bacteria isolated from marine environments - Critical concentrations to determine resistance or sensitivity -

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Introduction.

Bacterial strains resistant to heavy metals have been isolated from different marine environments. Resistance or sensitivity of microorganisms to heavy metals are clearly determined by the assayed concentration of metals, but there are not generally accepted criteria for selecting these concentrations. The purpose of this study is to propose appropriate critical concentrations of metals to determine the threshold of resistance or sensitivity of microorganisms isolated from marine environments.

Material and Methods.

Bacterial strains were isolated from samples of water, sediments and shellfish. These samples were collected from the marine area near the Guadalhorce river mouth and on beaches affected by sewage discharges in Malaga (Spain). The resistance to heavy metals was studied in isolates of coliforms, fecal streptococci, *Pseudomonas aeruginosa*, *Salmonella* serotypes, *Aeromonas hydrophila*, *Vibrio* spp. and *Staphylococcus* spp. by using the agar dilution method (4). The minimal inhibitory concentration (MIC) was determined by testing twofold serial dilutions of $HgCl_2$, Na_2AsO_4H, K_2CrO_4, $CdCl_2$, $CuCl_2$, $NiCl_2$, $Pb(CH_3\text{-}COO)_2$) and $ZnSO_4$.

Results and Discussion.

The distribution of MIC for Cu, Ni, Zn, and Pb are very narrow (800-1600 µg/ml of $CuCl_2$, $NiCl_2$, and $ZnSO_4$, and 6400-25600 µg/ml of $Pb(CH_3\text{-}COO)_2$), nearly all the strains studied present the same two or three MIC values. However, the distribution of MIC values for As, Cd, Cr, and Hg show a wider range (Figure 1). A bimodal distribution for the MIC values of As and Hg is observed, so 12800 µg/ml of Na_2AsO_4 and 10 µg/ml of $HgCl_2$ are the critical concentrations proposed to determine the resistance or sensitivity to As and Hg, respectively. On the other hand, MIC distributions with only one peak are observed for Cd and Cr; for this, it is difficult to establish the critical concentration. However, 400-800 µg/ml of $CdCl_2$ and 800 µg/ml of K_2CrO_4 could be appropriate values, because at those MICs a decrease in the frequency of resistant strains is observed. In Table 1, the critical concentrations proposed in this work are compared with those proposed by other authors.

Table 1. Critical concentrations (µg/ml) of metals to determine resistance/sensitivity of bacteria isolated from marine environment.

Na_2AsO_4H	$CdCl_2$	K_2CrO_4	$HgCl_2$	Reference
	100	100	10	Austin *et al.*, 1977
-	11	-	5	Timoney *et al.*, 1978
-	10-60	-	-	Gauthier *et al.*, 1986
12800	400-800	800	10	This study

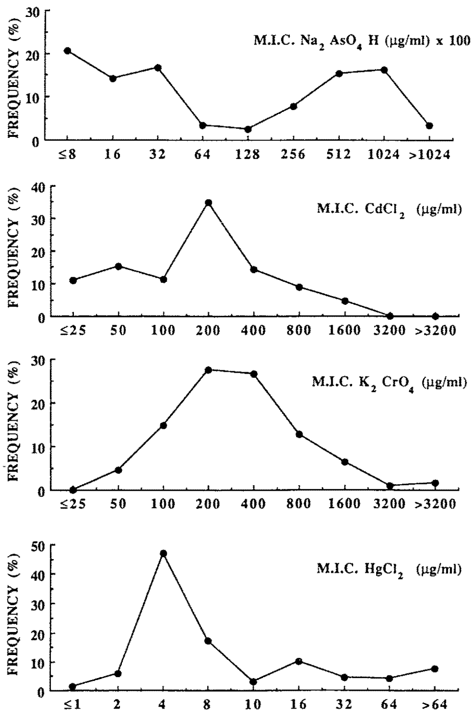

Fig. 1.- M.I.C. distribution of heavy metals observed in bacterial strains isolated from marine environments

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Distribution of bacterial plasmids in marine environments

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Introduction.

Plasmid carriage is an adaptative advantage for microorganisms, so the percentage of plasmid carrying microorganisms is expected to be higher in polluted environments (3).

Likewise, it is largely accepted that bacterial strains harbouring plasmids are more frequent in environments exposed to certain pollutants or stress factors (4,5).

Material and Methods.

Samples of water, shellfish and sediments were collected in the marine area near the Gualdalhorce river mouth in Malaga (Spain). These samples showed three different ranges of heavy metal concentrations (1) with the highest levels in sediments and the lowest in water. The microorganisms studied belong to two groups: fecal pollution indicators (Coliforms and Fecal Streptococci) and pathogens (*Pseudomonas aeruginosa*, *Salmonella* serotypes, *Aeromonas hydrophila*, *Vibrio* spp. and *Staphylococcus* spp.). The culture media employed for the isolation of microorganisms were: Endo agar, KF agar, Cetrimide agar, XLD agar, mA agar, TCBS agar and Mannitol salt agar for each microbial group, respectively. Plasmid content was analyzed using a modified alkaline-SDS procedure and electrophoresis on agarose gels (6).

Results and Discussion.

The results obtained are summarized in Tables 1 and 2, which show the frequencies of plasmid detection in relation to fecal pollution levels and to the isolation sources, respectively. A statistical test of the difference in proportions of bacteria containing plasmids in each environment was performed (3).

Globally, higher detection frequencies were observed in fecally polluted zones (A and C) with regard to those wich show the lowest levels of pollution (B) (Table 1). *A. hydrophila* and *Vibrio* show higher frequencies of plasmid harbouring strains in the areas more highly polluted; however, a clear relation of the fecal pollution level and the occurrence of plasmid carrying microorganisms is not observed, when the other bacterial groups are considered. These results are in disagreement with those reported by other authors, who have observed higher frequencies of strains of *P. aeruginosa* and coliforms with plasmids in highly polluted zones (2,3).

The highest frequencies of plasmid harbouring strains were observed in shellfish samples and the lowest in the isolates from seawater. Although significant differences were obtained for the plasmid occurrence with relation to the isolation source of microorganisms (Table 2), these differences were not related to the concentrations of heavy metals. These results are in accordance with BURTON *et al.* (3), who studied the plasmid content of heterotrophic microorganisms isolated from clean and polluted environments.

Table 1. Frequency of plasmid detection related to the fecal pollution level.

	Zone B		Zone C		Zone A		TOTAL	
	n	%	n	%	n	%	n	%
P. aeruginosa	1	100	6	33.3	11	45.4	18	44.4
Coliforms	5	80.0	7	100	9	77.8	21	85.7
Salmonella	1	100	1	0.0	3	33.3	5	40.0
A. hydrophila	1	0.0	5	60.0	12	75.0	18	66.7
Vibrio	4	0.0	-	-	11	45.5	15	33.3
Staphylococcus	1	0.0	3	66.7	2	50.0	6	50.0
Fecal streptococci	7	71.4	3	33.3	10	70.0	20	65.0
TOTAL	20	55.0	25	60.0	58	60.3	103	59.2

Table 2. Frequency of plasmid detection related to the isolation source.

	Water		Shellfish		Sediment		TOTAL	
	n	%	n	%	n	%	n	%
P. aeruginosa	11	45.5	5	60.0	2	0.0	18	44.4
Coliforms	9	88.9	8	87.5	4	75.0	21	85.7
Salmonella	3	33.3	1	100	1	0.0	5	40.0
A. hydrophila	6	50.0	7	71.4	5	80.0	18	66.7
Vibrio	3	0.0	4	50.0	8	37.5	15	33.3
Staphylococcus	5	60.0	1	0.0	-	-	6	50.0
Fecal streptococci	5	40.0	5	60.0	10	80.0	20	65.0
TOTAL	42	52.4	31	67.7	30	60.0	103	59.2

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