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 $ZnS0_4$.

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 $ZnS0_4$, and 6400-25600 µg/ml of $Pb(CH_3\text{-}C00)_2$), 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 $Na2AsO_4$ 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

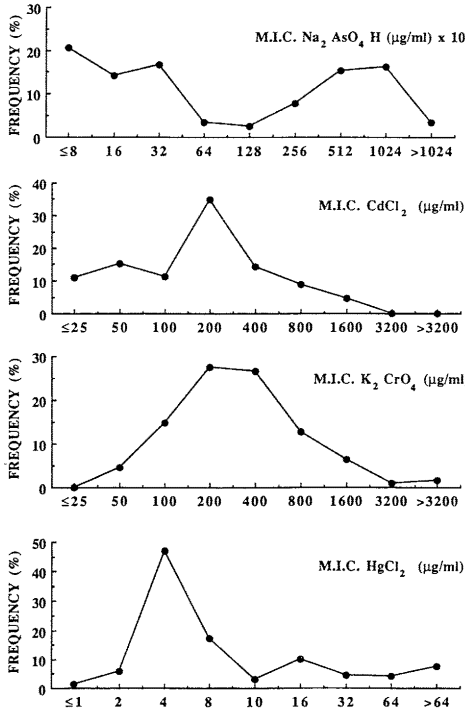

Fig. 1.- M.I.C. distribution of heavy metals observed in bacterial strains isolated from marine environments

REFERENCES

AUSTIN B., ALLEN D.A., MILES A.L. & COLWELL R.R. , 1977. - *Can. J. Microbiol.*, 23: 1433-1447.

GAUTHIER M.J., BREITTMAYER J.P., BREITTMAYER V.A., CLEMENT R.L. & FLATAU G.N., 1986. - *Environ. Technol. Lett.*, 7: 335-340.

TIMONEY J.F., PORT J.G., GILES J. & SPANIER J. , 1978. - *Appl. Environ. Microbiol.* 36:465-472.

WASHINTONG, J.A. & SUTTER V.L., 1982.- *In:* : Manual de Microbiologia Clinica. Panamericana.