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Relation between densities of indicator organisms and microbial pathogens in sea water

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This research programm was carried out to examine possible relations among the densities and the species of indicator organisms on the one hand and the presence and numbers (if possible) for organisms pathogenic for man on the other. This investigation was planned to include sea areas with different degrees of pollution and to search for significant correlations between densities of microbe indicators and patho genic organisms.

Samples of sea water were collected from 5 areas of the Saronic Gulf, with different grades of faecal contamination, from May 1984 to Novenber 1986 at 5 day intervals. Each sample was examined for the following: 1) Plate colony count/100ml at 37°C for 48h. 2) Coliform count/100ml 3) E.coli count/100ml 4) Enterococci count/100ml 5) Staphylococci for the first and second year in 0.5ml and afterwords in 100ml with modified technique 6) Salmonellae in 1L 7) Yearsiniae in 1L 8) V.cholera and V.parahaemolyticus in 1L 9) Yeasts in 50ml 10) Campylobacter in 200ml.

For Campylobacter isolation we tested different methods but finally selected the following technique: Enrichment of 200 ml in double strength Preston broth at 43°C/24h (semianaerobic conditions) and plating on Skirrow agar incubated under the same conditions for 48h.

Attempts to isolate Staphylococci by direct plating on selective media come up against a number of difficulties as a higher number of other cocci (Planococci, Micrococci) and even rods grow and mask or inhibit the growth of S.aureus. We tested different methods but finally used the MPN technique. m-staphylococcus broth was modified in order not to produce precipitation with sea water and to support the growth of S.aureus. For plating Vogel and Johnson agar (DIFCO) was used.

The comparison of Rappaport-Vassiliadis medium and Muller-Kauffmann's tetrathionate broth for salmonella isolation after a common preenrichment step shows clearly the great superiority of R-V medium, both in the number of isolations and also the variety of serotypes (P 0.0001).

Statistical analysis of the densities of microbe indicators in relation to the pre sence of Salmonella shows a significant correlation between Salmonella and coliforms, E.coli, Enterococci and plate count colonies (TABLE 1). There was no significant association between plate count colonies Enterococci and Salmonella in moderately polluted areas. There was no significant association between the microbe indicators and Salmonella when M-K broth was used nor between Campylobacter and the microbe indicators or between S.aureus and Salmonellae. Vibrios and Y.enterocolitica were not isolated from any of the samples. More work is necessary for species identification and epidemiological correlation with their origin.

TABLE 1

Association between microbe indicators and presence of Salmonella and Campylobacter level of significance, p, from Mann-Whitney test comparing microbe densities between samples with and without Salmonella or Campylobacter.

		Keratsini (heavily polluted)	N. Faliron (moderately polluted)
Col-	R-V *	.006	.007
	M-K **	.80	.24
	Salmonella ***	.008	.015
	Campylobacter	.73	.13
E.coli-	R-V *	.007	.002
	M-K **	.54	.37
	Salmonella ***	.005	.017
	Campylobacter	.51	.27
Ent-	R-V *	.005	.044
	M-K **	.72	.77
	Salmonella ***	.010	.22
	Campylobacter	.56	.34
PCA	R-V *	.001	.022
	M-K **	.36	.42
	Salmonella ***	.001	.028
	Campylobacter	.74	.60
Saph. - aureus	R-V *	.66	.34
	M-K **	.67	.28
	Salmonella ***	.38	.82
	Campylobacter	.06	.98

* Salmonella isolation when Rappaport-Vassiliadis medium was used

** Salmonella isolation when Muller-Kauffmann's tetrathyonate broth was used

***Salmonella isolation when either of the media was used

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Relationship between the persistence of Pseudomonas aeruginosa in seawater and its resistance to antibiotics and heavy metals

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Introduction.

Detection of *Pseudomonas aeruginosa* in natural waters is usually associated with pollution produced by sewage discharges. The high resistance of *P. aeruginosa* to heavy metals, antibiotics, and other environmental factors could be an important aspect in relation to the persistence and selection of strains in the marine environment (3,4). The purpose of the present study is to determine the relationship between some characteristics of *P. aeruginosa* strains (pyocines production and antibiotics and heavy metals resistance) isolated from seawater and their survival ability in this environment.

Material and Methods.

One hundred and eighty eight isolates of *P. aeruginosa* on mPA-E agar (5) were studied. Water samples were collected from two areas near Málaga (Spain): a sewage-polluted beach and the Guadalhorce river and the coastal area influenced by the river mouth. In both areas, three sample groups have been considered: the pollution sources (sewage and river), mixing area (<250 m from the outfall and estuary), and the marine area influenced by these pollution sources (>500 m from the sewage outfall in the beach and the coastal area of 1000 m around the river mouth).

The antibiotic resistance patterns were studied by disk diffusion method, and the heavy metal resistance was evaluated by agar dilution method (6). The pyocin types were investigated by scrape and streak method (6).

Results and Discussion.

Among antimicrobial agents assayed, variable results were only observed for gentamicin, sulfadiazine, mercury, arsenic, and chromium, being the resistance to these agents used as markers for the studied strains.

In Table 1 it can be observed that the highest frequencies of resistant strains, except for chromium, are detected in the farthest areas from the pollution sources, especially for mercury and arsenic, so the frequencies of resistant microorganisms to these agents are twofold higher than the frequencies observed in the river and sewage. Likewise a higher frequencies of multiresistant strains in seawater is obtained.

When it is considered the distribution of pyocin types in the different areas, higher percentages of pyocin types 13B and 12A are observed in the strains isolated in the farthest areas from the pollution source (seawater) respect to the frequency in the nearest areas. On the contrary, the frequency of the other pyocin types decreases at longer distances from the pollution sources (Table 2). The mentioned pyocin types are associated with noticeable heavy metals resistance characteristics (Table 3), thus, the 13B strains have the highest percentages of resistance to arsenic, and all 12A strains are mercury resistant and they have the maximum frequencies of resistance to gentamicin and sulfadiazine.

The observed increase of the frequencies of heavy metals resistant strains and pyocin types 13B and 12A in seawater suggests that these strains may have better characteristics of survival in the marine environment. It is probably due to a higher resistance to environmental factors, such as sunlight or biotic agents, which usually affect negatively to *P. aeruginosa* cells and another allochtonous microorganisms in seawater (6).

It is not possible assume a direct selective pressure by heavy metals on these bacteria (3,4) as explanation to the antibiotics and metals resistant selection in seawater, because the heavy metal concentrations in the studied area are clearly lower than the MIC of these metals for the studied *P. aeruginosa* strains. The results of this study suggest an association between some characteristics of these strains (heavy metals resistance and pyocin types) and a higher survival ability in the marine environment.

TABLE 1. Antibiotic and heavy metal resistance of *Pseudomonas aeruginosa* isolated from polluted waters. Frequency of resistant strains (%).

RESISTANCE TO:	ISOLATION SITE: Pollution source (n=83)	Mixing area (n=45)	Seawater (n=60)	Overall (n=188)
GM (10 µg)[a]	21.69	22.22	28.33	24.47
SD (1000 µg)[a]	36.14	35.56	38.33	37.23
Hg^{2+} (7.5 µg/ml)[b]	16.87	26.67	36.67	25.53
AsO_2^{3-} (5702 µg/ml)[b]	7.23	4.44	15.00	9.04
CrO_4^{2-} (956 µg/ml)[b]	6.02	6.67	5.00	5.85

[a]:Disk potency
[b]:Concentration
GM:Gentamicin
SD:Sulfadiazine

TABLE 2. Pyocin types of *P. aeruginosa* strains isolated from polluted waters. Percentage of strains of each type.

PYOCIN TYPE	ISOLATION SITE: Pollution source (n=83)	Mixing area (n=45)	Seawater (n=60)	Overall (n=188)
88D	22.89	20.00	15.00	19.68
13B	6.02	8.89	20.00	11.17
16A	7.23	8.89	5.00	6.91
12A	2.41	6.67	11.67	6.38
16B	8.43	4.44	3.33	5.85
Others	53.00	51.11	45.00	50.00

TABLE 3. Relationship between pyocin types and antibiotic and heavy metal resistance of *P. aeruginosa* strains.

PYOCIN TYPE (%)	RESISTANCE TO: Gm	SD	Hg^{2+}	AsO_2^{3-}
88D (19.68)	29.73	51.35	18.92	13.51
13B (11.17)	0.00	4.76	9.52	23.81
16A (6.91)	23.08	38.46	30.77	7.70
12A (6.38)	50.00	66.67	100.00	0.00
16B 5.85)	0.00	63.64	0.00	0.00
Others 50.00)	27.66	31.91	24.47	6.38
OVERALL	24.47	37.23	25.53	9.04

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