Relation between densities of indicator organisms and *Staphylococcus aureus* in sea water

Mladen SOLIC and Nada KRSTULOVIC

Institute of Oceanography and Fisheries, Split (Yugoslavia)

The distribution of Staphylococcus aureus and its relation to the indicators of faecal pollution were studied at 17 stations in the coastal area of Split (Central Adriatic) during the summer period in 1989. Areas with different degrees of pollution (from very low to high) were chosen for the study.

The average values of S.aureus concentration ranged from 16 to 1000/100 ml, total coliforms from 20 to 100000/100 ml, faecal coliforms from 4 to 25100/100 ml and faecal streptococci from 2 to 7900/100 ml sea water (Fig.1).

The relation of S.aureus with each of the studied groups of indicators was analysed. In the total number of samples no correlation was established with total coliform and faecal coliform, and scarcely a significant one with faecal streptococci. However the analysis of S.aureus and indicators of faecal pollution in samples with different degrees of pollution showed different results (Tab.1). Faecal coliforms were used as the criterion to evaluate the degree of pollution of the studied area which was accordingly divided into less polluted area up to 100 FC/100 ml) and more polluted one (over 100 FC/100 ml).

Table 1. Correlations established between concentrations of Staphylococcus aureus and indicator organisms.

	<100 FC/100 ml			>100 FC/100 ml			Total samples		
	r	n	P	r	n	P	r	n	P
TC	-0.11	43	-	0.49	20	0.05	0.07	63	-
FC	0.10	50	-	0.41	23	0.05	0.17	73	-
FS	-0.05	50	-	0.61	23	0.01	0.24	73	0.05

In cases of low polluted sea water no correlation between S.aureus and indicators of faecal pollution was established whereas in more polluted sea water there was a correlation between them.

The highest correlation coefficient was established between S.aureus and faecal streptococci, the indicator of faecal pollution with the longest period of survival (1,2). It could be explained by the fact that S.aureus is more chlorine resistant in sea water (3), which probably causes its longer period of survival in relation to indicator organisms.

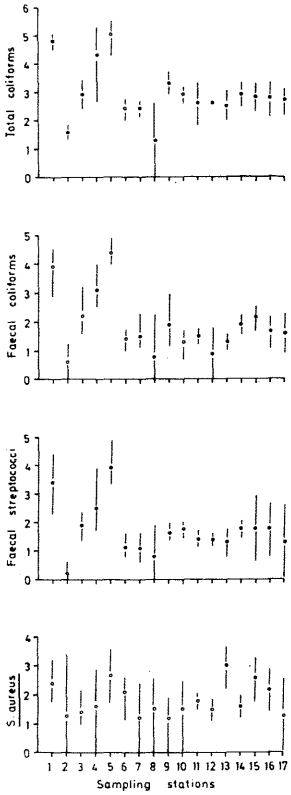


Fig.1. Ranges and mean values of indicator organisms and Staphylococcus aureus (log n/100 ml)

References

1. Fujioka,R.S.,H.H.Hahimoto, E.B.Siwak and R.H.F.Young. 1981. Effect of sunlight on survival of indicator bacteria in sea water. Appl.Environ. Microbiol., 41: 690-696.

2. Tudor,M.,M.Šolić and N. Krstulović. 1989. T_{90} of total coliforms, faecal coliforms and faecal streptococci in the Kaštela Bay. Acta Adriat., 30 (in press).

3. Bergey's Manual of Determinative Bacteriology (8th edit.). 1975. R.E.Buchanan and N.E. Gibbons. Ed. Williams and Wilkins Co. Baltimore.

Recovery of stressed Coliforms from seawater samples

J.-J. BOREGO, M.-A. MORINIGO, R. CORNAX, M.-A. MUNOZ, E. MARTINEZ-MANZANARES and P. ROMERO

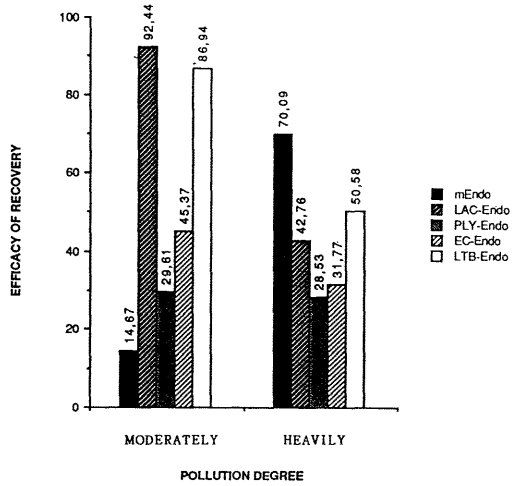
Department of Microbiology, Faculty of Sciences, University of Malaga, 29071 - Malaga (Spain)

The 16th edition of Standard Methods for the Examination of Water and Wastewater [1] and the World Health Organization guidelines [8] specified mEndo agar as the choice medium for coliform enumeration. Several authors have pointed out that this medium has several shortcomings, such as: (i) low recoveries of injured and stressed coliforms [4-6]; and (ii) poor differentiation among coliforms and non coliforms [2-3]. For this reason, a comparative study of the standard method for the enumeration of coliforms (MF and mEndo agar) and several resuscitation methods to recover the stressed coliforms from seawater has been the main objective of the present work.

Seawater samples were collected from ten beaches on the coast of Malaga (Spain). The membrane filtration technique was carried out as described by Standard Methods [1]. The filtrations were five-fold replicated for each one of the methods tested with 0.45µm membrane filters (Millipore Ibérica, Madrid, Spain). Phosphate-buffered saline [1] was used as diluent solution. Four resuscitation membrane filtration methods were conducted according to established procedures [4, 6,7] using Millipore HC-type filters and the following resuscitation media: LAC broth [3]; PLY agar (Difco); EC broth (Difco); and LTB broth (Difco). When the media were liquids, filters were placed onto sterile pads (Millipore) saturated with sterile broth (LAC, EC or LTB), and incubated at 36°C for 2 h. The filters were then transferred to mEndo agar plates and incubated for an additional 22 h. The recovery efficacy of each medium is calculated with respect to the maximal count obtained for one medium and sample, applying the following equation: Relative Percentage of Recovery (Medium A) = (Count on medium A)/ (Maximum count on any medium) x 100.

The comparison of the quantitative recovery of coliforms on the different media tested was carried out using 60 seawater samples with different faecal pollution degree(30 moderately polluted and 30 heavily polluted). The efficacy of recovery of each method is represented in Fig. 1. All the methods detected high percentages of coliforms from the samples analysed, except LAC-Endo resuscitation method, which obtained 1% of the coliform recovery from MIS sampling station in comparison with the best method . Statistically significant differences of the efficacy of recovery were obtained for each sample groups, moderately and heavily polluted seawater, and for the media mEndo, LAC-Endo and LTB-Endo. The best methods for the recovery of coliforms from moderately polluted seawater were LAC-Endo and LTB-Endo, with figures of 92.4% and 86.9% of recovery, respectively, in comparison with 14.7% obtained for the standard method (mEndo). On the other hand, for samples with high pollution degree, the best efficacy of recovery was obtained for m Endo agar (70.1%) in comparison with PLY-Endo and EC-Endo methods which achieved only percentages of 28.5 and 31.8%, respectively.

In short, it seems to be that the pollution degree of the samples affect significantly the recovery of stressed and non-stressed coliforms, being advised the use of the resuscitation methods for samples with a low or moderate pollution degree.



REFERENCES

1. APHA, AWWA & WPCF (1985). Standard Methods for the Examination of Water and Wastewater, APHA, Washington, D.C.
2. Avila, M.J.; M.A. Moriñigo; R. Cornax; P. Romero & J.J. Borrego (1989). J. Microbiol. Methods **9**: 175-193.
3. Evans, T.M.; R.J. Seidler & M.W. Lechevallier (1981). Appl. Environ. Microbiol.**41**:1144-1151.
4. Lechevallier, M.W.; S.C. Cameron & G.A. McFeters (1983). Appl. Environ. Microbiol. **45**: 484-492.
5. McFeters, G.A.; G.K. Bissonnette; J.J. Jezeski; G.A. Thompson & D.G. Stuart (1974). Appl. Microbiol. **27**: 823-829.
6. McFeters, G.A.; S.C. Cameron & M.W. Lechevallier (1982) Appl. Environ. Microbiol. **43**: 97-103.
7. McFeters, G.A.; M.W. Lechevallier & M.J. Domeck (1984) US Envrion. Prot. Agency.
8. WHO/UNEP (1982) Coastal water quality control in the Mediterranean Sea. WHO, Copenhagen.