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Isolation and counting of S. aureus from sea water samples

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Attempts to isolate and count staphylococci by direct plating on selective media come up against a number of difficulties as a higher number of other cocci (planococci, micrococci) grow and mask or inhibit the growth of S.aureus colonies.As a result small quantities of sea water (0.2-0.5 ml) can be plated in order to obtain growth which permits the recognition and isolation of S.aureus.To analyse a greater quantity of sea water and to get better results with greater volumes of sea water we used the membrane filtration technique and the membranes were incubated on 4 selective media:1)Vogel & Johnson agar(VJ) (DIFCO),2)110 staphylococcus agar (OXOID), 3)Chapman medium (OXOID), 4)4-S agar modified by Mintzel-Morgenstern & Katzenelson (Yospe-Purer & Golderman,1987).The referencemethod by direct plating 0.2-0.5 ml of sample on Baird-Parker medium was applied in parallel.Plates were incubated at 37°C for 24 - 48 hours. A number of typical for staphylococci and atypical colonies were identified according to Morello & Randall 1981 (TABLE 1).The VJ agar was proved to be the most selective for S.aureus colonies. A great number of typical colonies on VJ agar were shown to be coagulase positive.The 4-S agar gave the greatest number of atypical colonies, then the Chapman and the Baird-Parker media.Again on VJ agar the highest number of other staphylococci species grew, followed by Baird-Parker medium and the smallest number of other Gram-positive cocci (mocrococci,planococci).

However, even with the more selective VJ agar we could not get satisfactory results when volumes such as 100 ml of sea water were filtered We turned to a MPN technique proposed by Standard Methods 1981 for drinking and surface water samples.The m-staphylococcus broth formulated for drinking and surface water samples produced appreciable amount of precipitation with sea water.Appropriate modifications were applied and finaly the medium was formulated as follows:

Single strength:Tryptone 10gr,yeast-extract 2,5gr, lactose 2,0gr, mannitol 10,0gr,sodium chloride 75gr,sodium azide 0,049gr, dist. water 11, final pH 7,0.

Double strength: Tryptone 20,0gr,yeast extract 5,0gr, lactose 4,0gr, mannitol 20,0 gr,sodium chloride 70,0 gr,sodium azide 0,098gr, Dist.water 11, final pH 7,0.

The modified medium was found to perform properly both in supporting the growth of staphylococci and not to produce precipitation. Positive tubes were streaked on Vogel - Johnson agar (VJ) (DIFCO) and on Lipovitellin - Salt - Mannitol agar (LSM), proposed by the Standard Methods, 1981.Typical staphylococci colonies were typed according to Morello & Randall, 1981.Of the 68 samples examined 27 (39,7%) were positive when the VJ agar was used while 11 (16,2%) were positive when the LSM agar was used.Nineteen samples positive on VJ plates were negative on LSM ones but only 3 positive on LSM plates were negative on VJ ones(TABLE 2).On samples positive with both media the MPN was generally higher on the VJ agar.False positive colonies appeared on VJ plates of 7 samples and on LSM plates of 28 samples ($P < 0,001$).

In conclusion MPN technique using modified m-staphylococcus broth and plating on VJ agar seems to be a suitable method for the detection and enumeration of S.aureus in sea water samples.Further research should include the growth of other staphylococci sp., as S.epidermitis, on VJ agar and the evaluation of other media proposed in the bibliography.

TABLE 1

Identification of 179 strains recovered on staphylococcus selective media.

MEDIA	TYPICAL COLONIES S. aureus	planococcus	micrococcus	staphylococcus sp	atypical colonies
Vogel and Johnson agar	8	0	1	15	3
110 staphylococcus agar	2	7	4	4	13
Chapman medium	2	10	1	7	18
4 - S agar (modified)	3	6	1	6	25
Baird - Parker medium	1	2	3	10	17

TABLE 2

Results of the isolation of S.aureus from sea water samples using the MPN technique.

	No of samples	positive	negative	P
Vogel - Johnson agar	68	27	41	
Lipovitellin - salt Mannitol agar	68	11	57	<0,01

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Serologic characterization of Salmonellas isolated from polluted seawaters

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Introduction.

More then two thousands serotypes of *Salmonella* are known considering to this microorganism potentially pathogen to human and animal populations (1,2). Salmonellosis are transmitted usually by fecal contamination of the water and food, and some serotypes, such as *S. typhimurium* and *S. enteritidis*, cause a large number of infections.

In the present study were characterized serologically 172 isolates belong *Salmonella* genus. All the microorganisms were isolated from two marine zones subject to polluted discharges.

Sampling Areas.

Two zones were selected for the sampling. One of them was sited in one marine area influenced by the discharges of Guadalhorce river (Málaga-Spain). This area is polluted by fecal and industrial discharges. The second area of study was established in the zone influenced by the upwelling of a submarine outfall in Fuengirola (Málaga-Spain). The pollution of this zone is produced by domestic sewage discharges.

Results and Discussion.

In Tables 1 and 2 are described the *Salmonella* serotypes isolated and their frequency percentages. As can be seen in the marine area affected by river discharges, there is a lower number of serotypes, due probably to the nature of discharge (mixed pollution). In both zones, the same serogroup of *Salmonella* were isolated, and C_1 serogroup was preponderant in both zones. Others serogroups that were significantly detected were C_2 in marine zone affected by river discharges and B in the area of submarine outfall influence.

The percentages of detection of *Salmonella* serotypes were lower than 10 % except in the cases of *S. typhimurium* and *S. ohio* in the zone affected by the river (25 and 23.36 %, respectively), and *S. thompson*, *S. bloctley* and *S. typhimurium* in the zone affected by the outfall discharges, although the values of these threes serotypes never were equal or higher than 20 %. *S. bloctley* was only isolated from source that posses a domestic fecal influence and for this reason their detection may be associated to exclusivelly fecal origin.

In Tables 1 and 2 are exposed the relationship between isolation percentage and drift time in the sea. As can be seen a close relation may not be established between these parameters, results that are in agreement with those obtained by other authors (3,4) in *Salmonella* survival studies.

Table 1: Detection percentage of different serotypes isolated from marine zone affected by discharges of the river.

Serotypes	Serogroup	Drift Time in the sea 0 min (n=33)	0-10 min (n=18)	>10 min (n=25)	Total (n=76)
Self-agglutinable	-	21.21	5.55	24.00	18.42
Inmobile	-	3.03	-	-	1.31
S. typhimurium	B	12.12	27.77	40.00	25.00
S. infantis	C_1	9.09	9.09	-	5.26
S. ohio	C_1	18.18	33.33	-	7.89
S. paratyphi	C_1	-	5.55	-	1.31
S. postdam	C_1	3.03	-	-	1.31
S. thompson	C_1	3.03	-	4.00	2.62
S. bloctley	C_2	3.03	-	-	1.31
S. bovis-morbificans	C_2	-	-	4.00	1.31
S. muenchen	C_2	6.06	-	-	2.62
S. enteritidis	D	12.12	-	4.00	6.57
S. london	E_1	6.06	22.22	-	7.89
S. senftemberg	E_4	-	5.55	-	1.31
S. tatsony	E_4	3.03	-	-	1.31

Table 2: Detection percentage of different serotypes isolated from marine area affected by discharges of the submarine outfall.

Serotypes	Serogroup	Drift time in the sea 0 min (n=60)	0-10 min (n=22)	>10 min (n=14)	Total (n=96)
Self-agglutinable	-	-	-	-	-
Inmobile	-	-	-	-	-
S. typhimurium	B	13.33	-	9.09	10.41
S. braenderup	C_1	1.66	-	-	1.04
S. infantis	C_1	6.66	-	7.14	5.20
S. menden	C_1	-	4.54	-	1.04
S. montevideo	C_1	-	4.54	-	1.04
S. ohio	C_1	8.33	13.63	7.14	9.37
S. orunaiemburg	C_1	1.66	4.54	-	2.08
S. postdam	C_1	-	13.63	-	3.14
S. thompson	C_1	15.00	22.70	9.09	16.66
S. virchow	C_1	10.00	-	7.14	7.29
S. bloctley	C_2	8.33	22.70	7.14	11.45
S. bovis-morbificans	C_2	-	-	7.14	1.04
S. muenchen	C_2	-	-	7.14	1.04
S. enteritidis	D	3.33	4.54	-	3.12
S. london	E_1	10.00	-	7.14	7.29

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