

ON THE SIGNIFICANCE OF BACTERIAL RESISTANCE TO OXYTETRACYCLINE IN SEDIMENTS UNDER NORWEGIAN FISH FARMS

BY P. SMITH, L. PURSELL, F. MCCORMACK,
A. O'REILLY AND M. HINEY

Husevåg and Lunestad (1995) have recently published, in this Bulletin, a paper which presented data on the analysis of the microbial flora of sediments under Norwegian fish farms. Amongst other parameters reported they presented data on the frequency of bacteria resistant to oxytetracycline. The definition of resistance implicit in this paper was the ability to form colonies on tryptone soya citrate agar (TSC) which contained $25\mu\text{g ml}^{-1}$ oxytetracycline. Smith *et al.*, (1994) have recently reviewed the many difficulties encountered in interpreting the meaning of such data. These issues will not be discussed further here. However, any attempt to assess the impact of fish farms on the frequency of resistance to antimicrobial agents requires a knowledge of the background frequency that would be detected in areas free of such impact. Such a background frequency is obviously dependent on the methods used. Any attempt to understand the significance of the data presented by Husevåg and Lunestad (1995) is frustrated by their failure to provide this background data.

In an attempt to provide such data we have analysed eight sediment samples taken from sites in Galway Bay, Ireland. The sites were at least 5 km distant from any fish farm or human sewage outfall and were at least 1 km distant from each

other. As far as possible the samples were collected and analysed by the methods of Husevåg and Lunestad (1995). The only difference in methods, of which we are aware, was our use of 32‰ Instant Ocean (Aquarium Systems, Sarrenbourg, France) rather than Rila Marine synthetic seawater as a diluent. Husevåg and Lunestad (1995) did not provide information as to the incubation conditions they used. In this work we therefore used the conditions reported by Samuelsen *et al.* (1992) and the plates were incubated at 18°C for seven days. Results obtained from the analysis of the samples we collected are shown in Table I together with the relevant data from Husevåg and Lu-

Table I Comparison of the total colony forming units detected on TSC and the frequency of colonies obtained on TSC containing $25\mu\text{g ml}^{-1}$ in samples collected under fish farms in Norway (data taken from Husevåg and Lunestad (1995)) and at sites distant from fish farms in Ireland.

	Total bacterial counts on TSC (10^4 cfu ml^{-1})		% Resistant cfu	
	Norway	Ireland (a)	Norway	Ireland (a)
Samples	10	8	11	8
Min	1.3	2.8	0.02	0.43
Max	50	90	2.5	1.4
Median	7.7	15	0.50	0.71
Mean	13	22	0.79	0.76
Standard deviation	15	28	0.79	0.30

(a) Duplicate aliquots of sediment were prepared and diluted. Each set of dilutions were plated in duplicate on both TSC and TSC with $25\mu\text{g ml}^{-1}$ oxytetracycline. The frequency of oxytetracycline resistant bacteria in a sample was calculated by averaging the data from these four results.

nestad (1995). The frequencies of bacteria resistant to oxytetracycline detected in samples collected from sites free of fish farm effluent in Ireland are remarkably similar to those reported under abandoned and operative fish farms in Norway. Samuelsen *et al.* (1992), who also used TSC medium and concentrations of $25\mu\text{g ml}^{-1}$ oxytetracycline, reported background frequencies of $<1\%$ in marine sediments. In this study, however, they provided no information on the number of samples used to generate this figure. The most detailed study of the background frequency of resistance to oxytetracycline in marine sediments has been provided by Kerry *et al.* (1994). They analysed 153 samples from sediments that were not exposed to fish farm effluent to determine the background frequency to oxytetracycline in Galway Bay. Their use of Zobell's media means that the frequency of $1.2 + 1.8\%$ they reported cannot be compared directly to frequencies determined in studies where TSC media was used.

It is recognised that eight samples are inadequate to provide an accurate estimation of the background frequency of resistance that will be detected by any particular method. Equally it is possible to argue that Galway Bay cannot provide suitable samples for the estimation of the background expected in studies of Norwegian sediments. Despite these serious limitations, the data presented in this note does provide some indication of the probable significance of the frequencies of resistance to oxytetracy-

cline reported by Husevåg and Lunestad (1995). The most plausible interpretation of their data is that they present evidence that the operation of fish farms has had a negligible long term impact on this parameter of the microflora present in the under cage sediments.

Authors' address

Fish Disease Group, Department of Microbiology, University College Galway, Ireland. Fax: +353 91 750514
E-mail: peter.smith@ucg.ie

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