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MULTIPLE ANTIBIOTIC SENSITIVITY PATTERNS OF *AEROMONAS SALMONICIDA* WITHIN DIAGNOSTIC SPECIMENS FROM OUTBREAKS OF FURUNCULOSIS

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Introduction

Antimicrobial therapy remains a critical adjunct to vaccination (Hastings, 1988) and good management practices (Roberts & Shepherd, 1986) in the control of furunculosis in Atlantic salmon *(Salmo salar* L.). At present four classes of antimicrobial agents are licensed for use in aquaculture in the UK; oxytetracycline, oxolinic acid, trimethoprim-sulphadiazine and amoxycillin. Isolates of *Aeromonas salmonicida* have been found, increasingly, to be resistant to the first three of these, and frequently the resistance is multiple (Hastings and McKay, 1987; O'Grady *et al,* 1987, DAFS, 1989 and Inglis *et al,* 1991a) but as yet resistance to amoxycillin is rare (Inglis *et al,* 1991a). Treatment, which is usually supported by laboratory diagnosis of the antimicrobial sensitivity pattern of the causative isolate, however is not always successful. This may be due to a failure to deliver an adequate dose to the fish for a sufficient length of time, as has been shown in some amoxycillin treatments (Inglis *et al,* 1991b). It may be associated with the timing of the intervention, general environmental factors or with the presence of more than one *A. salmonicida* variant, not all of which are sensitive to the antibacterial being used (Inglis *et al,* 1991a). This study was carried out to investigate the occurrence of *A. salmonicida* isolates with different antibiotic sensitivity patterns both within individual fish and on different fish within the same outbreak of furunculosis.

Materials and Methods

Eleven outbreaks of furunculosis in Atlantic salmon at six fish farms were investigated. There were three farms with one outbreak, one farm had two episodes, and two farms had three outbreaks. On one of these latter farms, a visit was made during one of the outbreaks, and fresh mortalities were sampled from the kidney. Kidney swabs plated out on tryptose soya ajar were sent as diagnostic specimens to the laboratory from all outbreaks providing 68 cultures of *A. salmonicida.* From each specimen, ten well separated colonies were selected and tested by the disc diffusion test (Bayer et al, 1966) for sensitivity to oxytetracycline (OT), Oxolinic acid (OA),trimethoprim-sulphadi-

Table 1 Different antibiotic patterns within specimens obtained from individual fish.

Site and Outbreak	Specimens in Outbreak	Number of specimens with variation
1.1	10	3[1]
1.2	17	1[2]
1 farm visit	9	-
2.1	1	-
2.2	1	-
3.1	7	1[3]
3.2	5	-
3.3	6	-
4.1	10	1[4]
5.1	1	1
6.1	1	1

Details of variation

1) two specimens each had 2 colonies which differed on oxytetracycline, oxolinic acid and potentiated sulphonamide and one specimen had one colony which differed on potentiated sulphonamide
2) one specimen had one colony which differed on amoxycillin
3) one specimen had one colony which differed on oxytetracycline and oxolinic acid
4) one specimen had two colonies which differed on oxolinic acid

azine (ST), furazolidine (FE), nitrofurantoin (F) and amoxycillin (AMY). Details of sensitivity and scoring were given in Inglis *et al* (1991a).

Results

The origins of the specimens and the antibiotic sensitivity patterns found in them are shown in Table 1. Within each of the 68 specimens of which 10 individual colonies were tested, a single antibiotic sensitivity pattern predominated. Six specimens each contained a sub-group which was different. This occurrence was not frequent and, on average, over 70 colonies had to be tested to reveal a single variant (see Table 1).

Thirty six isolates were obtained from one site; arising separate outbreaks of furunculosis including the farm visit. Table 2 lists ten different antibiotic sensitivity patterns. In the first outbreak, four isolates could be differentiated; in the second there were six, and from the third (farm visit) there were four. One antibiotic sensitivity pattern recurred in each of the three collections.

The differences detected were reproducible and sometimes marked, as between patterns 1 and 3 in Table 2. Others were slight, as between 1 and 2. Pattern 2 was only detected in one colony in the first outbreak, although it came to dominate the subsequent episodes. Three specimens contained pattern 3, on one, it was the only pattern; on the other two, it appeared in a minority of the colonies. Pattern 5 was only found in one colony of one specimen.

Discussion

A. salmonicida isolates with different antibiotic sensitivity patterns were detected within specimens from individual fish. This was not a frequent occurrence and problems arising from small numbers of resistant variants could be overcome by performing antibiotic sensitivity tests on broad inocula of mixed growth from the wells of the isolation plates. Of more significance was the occurrence of *A. salmonicida* variants of different antibiotic sensitivity taken from different fish on the same site. This problem can be reduced by testing several fish in a population of each sampling point. The importance of re-testing each time there is a need to use antibacterial therapy was emphasised by the variation found between isolates taken from the same location at different times.

Table 2 Various antibiotic sensitivity pattern found in 36 specimens taken from one site during two outbreaks of furunculosis and one farm visit

Sensitivity pattern in terms of antibacterial sensitivity zone diameter (in mm)							Number of specimens with sensitivity pattern shown in the		
Antibiotic pattern	OT	OA	ST	F	FE	AMY	1st outbreak (10 specimens)	2nd outbreak (17 specimens)	farm visit (9 specimens)
1	6	12	18	12	16	24	8	0	0
2	6	8	4	12	16	24	1	11	4
3	30	28	26	16	24	26	3	0	0
4	34	12	18	14	16	24	1	0	0
5	6	8	4	14	18	8	0	1	0
6	4	6	26	14	16	26	0	1	1
7	28	4	4	12	16	22	0	3	0
8	6	16	6	18	22	36	0	1	0
9	22	4	20	12	14	20	0	1	3
10	8	26	28	16	20	36	0	0	1

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