transconjugant (AB3 *aer*-5 *rev*-1) was checked for its genetic structure using DNA hybridization and for aerolysin production in hemolysin and cytotoxin assays. As seen in the table, full toxicity was regained with the strain AB3 *aer*-5 *rev*-1 which had regained a LD_{50} of 5×10^7 cells.

All deaths of mice were recorded 30 h post inoculation; the majority of mice succumbed 18 h after i.p. infection. Pure cultures of *A. hydrophila* AB3 could be isolated from individually homogenized livers (5×10^7 cells/gm) and spleens (3×10^7 cells/gm) of dead mice. High concentrations of bacteria were also detected in blood samples of these mice. No loss of antibiotic markers encoded by AB3 (Nal^r) or AB3 *aer*-5 (Nal^r, Kan^r) could be detected upon plating onto selective/non-selective plates. These results clearly demonstrate the association of the toxin with lethality in mice and extends previous results obtained with wild type isolates producing varying amounts of aerolysin[7]. The systemic infection obtained with the mouse model is rapid, reflecting well the rapid course of wound infections and septicemia seen with human infections.

In order to test the ubiquity of the toxin in hemolytic *Aeromonas* strains we have used DNA hybridization analysis to probe for the presence of the gene. A 1.2 kb *Sma* I fragment, internal to the aerolysin gene, was labeled by nick-translation and used for hybridization to chromosomal DNA isolated from various hemolytic strains. All strains were hemolytic on both sheep and human blood agar plates, with the exception of strains AH 160 and AH 29607 which were hemolytic only on sheep blood agar plates. The results are shown in figure 2. All strains with the exception of AH 160 and AH 29607 hybridize to the aerolysin DNA probe (the hybridization to strain AH 9 is poorly seen). Clearly, there are hemolytic factor(s), other than aerolysin, present in *Aeromonas* species; it is also likely that a single strain may harbor more than one hemolytic activity. The other conclusion that can be drawn from the data is that the gene is associated with a different *Hind* III restriction fragment in independent isolates. This pattern has also been observed when the chromosomal DNA is restricted with the enzymes *Sma* I, *Bam* HI, *Eco* RI or *Kpn* I (data not shown). These results suggest a variable location of the aerolysin gene on the chromosome of independent isolates of *A. hydrophila*.

In summary, we have used the cloned aerolysin gene to introduce specific deletions into the chromosome of a clinical isolate of *A. hydrophila* AH 2. Mouse lethality studies with the parental strain and its isogenic mutant point to an important role of the toxin in systemic infections. DNA hybridization analysis indicates that the gene is unique and is present in variable locations on the chromosome of independent isolates. The presence of hemolytic factors other than aerolysin in *Aeromonas* species is suggested by the fact that not all hemolytic strains hybridize with the aerolysin gene probe.

Effect of the strains AB3, AB3 *aer*-5, and AB3 *aer*-5 *rev*-1 on mouse lethality

Strain	No. of cells injected	No. of mice dead/ No. tested
AB3	8×10^8	5/5
	6×10^8	9/9
	4×10^8	5/5
	2×10^8	5/5
	1×10^8	3/5
	5×10^7	1/5
AB3 *aer*-5	8×10^8	0/5
	6×10^8	1/9
	4×10^8	0/5
	2×10^8	0/5
	1×10^8	0/5
	5×10^7	0/5
AB3 *aer*-5 *rev*-1	8×10^8	5/5
	6×10^8	5/5
	4×10^8	5/5
	2×10^8	5/5
	1×10^8	3/5
	5×10^7	2/5

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Surface structure of pathogenic *Aeromonas*

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Key words. Aeromonas; pathogens; lipopolysaccharide; S-layers; proteins; surfaces; fish disease.

Macromolecular arrays of protein subunits known as surface protein arrays or S-layers are found on the outermost surfaces of a wide range of bacteria. In the case of pathogenic bacteria they interface with the host, and so are ideally placed to play important roles in pathogenesis. Virulent strains of *Aeromonas salmonicida* produce a protein array known as A-layer. The A-layer is composed of tetragonally arranged subunits of molecular weight (MW) 50,000[5]. Computer image processing of electron micrographs of negatively stained A-layer has shown that it contains two square patterns having p4 symmetry. Both square arrays are composed of two different morphological units arranged alternatively to give a face-centered lattice in which the four nearest neighbours of each unit are the other type of unit[7]. The lattice constant is slightly but significantly different in the two patterns,

and the orientation of one of the morphological units is changed by about 20° between patterns. It is possible that the two patterns may reflect a structural transformation of the layer, and may be related to a change in permeability of the A-layer. The structural gene for the subunit protein has been cloned using the vector λgt11. The protein expressed by this gene has the same subunit MW as the native mature exported A-proteins. Initial molecular genetic evidence indicates that the A-protein gene is highly conserved in the species *A. salmonicida*.

Transposon mutagenesis has provided a range of single-insertion mutants with altered ability to produce the A-layer. One Tn5 mutant accumulated A-protein in the periplasm, and did not produce an A-layer[1]. Virulence assays showed that this mutant had lost its ability to kill fish confirming the requirement of an assembled A-layer for virulence. The layer contributes to serum resistance[6], and appears to have an important role in the interaction of *A. salmonicida* with macrophages[8]. Interestingly, the Tn5 mutant which accumulated A-protein in the periplasm appeared to be unaltered in its ability to produce and export proteins such as hemolysin and protease, suggesting that the export pathway for A-protein may be unique.

Strains of *Aeromonas hydrophila* which exhibit high virulence for fish also produce a tetragonally arrayed surface layer composed of a protein of subunit MW 52,000[4]. Similar layers also occur on a number of strains of *A. hydrophila* isolated from clinical disease in humans and other mammals. The array protein from one strain of *A. hydrophila* has been purified and characterized. The *A. hydrophila* protein is similar in overall amino acid composition to the *A. salmonicida* A-protein, but differs with respect to the N-terminal amino acid sequence. Immunochemical analysis shows that the *A. salmonicida* and *A. hydrophila* proteins differ antigenically. The *A. salmonicida* protein appears to be antigenically conserved while the *A. hydrophila* array proteins display antigenic diversity.

Surface array-producing strains of both species of *Aeromonas* also produce a lipopolysaccharide (LPS) which is characterized by the presence of O-polysaccharides of unusually homogeneous chain length[2,3]. This morphological form of LPS appears to be important for the assembly of the surface protein array. This is well illustrated by a Tn5-insertion mutant which is unable to produce a smooth-type LPS with its characteristic O-polysaccharides of homogeneous chain length, but still retains the ability to produce and export A-protein. When grown in liquid media these cells are unable to assemble A-layer on their surface resulting in the excretion of large amounts of A-protein into the culture media. The chain length of the O-polysaccharide of *A. salmonicida* contains 16 to 18 repeat units while that of *A. hydrophila* contains approximately 10 repeat units. The O-polysaccharide chains protrude through the surface arrays and contribute to the serum resistance of both species. The O-polysaccharides are also major surface antigens. The O-chains of *A. salmonicida* are antigenically conserved, as are the homologous length O-chains of the *A. hydrophila* LPS. Although the chemical composition of the *A. salmonicida* and *A. hydrophila* homologous length O-polysaccharides are different, both species of O-chains carry an antigenically cross-reactive epitope, as well as a species serospecific epitope. Other strains of *A. hydrophila* produce an LPS with O-polysaccharides of heterologous length. These O-polysaccharides are not antigenically cross-reactive with the homologous chain length O-polysaccharides. Such strains of *A. hydrophila* appear not to produce S-layers.

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Aeromonas adhesin antigens

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Key words. Adhesion-adhesins; *Aeromonas* antigens; haemagglutinins.

Many strains of *Aeromonas* species (aeromonads) produce adhesins[4] which are readily detected using simple haemagglutination assays, and although there is yet little convincing evidence that adhesins are virulence factors for aeromonads, it seems probable that they do play a role in aeromonad disease. For example, diarrhoeal isolates of motile aeromonads co-produce Vero cell cytotoxin and HAG significantly more frequently than do non-diarrhoeal faecal isolates (table).

Both the motile and non-motile aeromonads produce haemagglutinins (HAG)[4,10]. There is a large diversity of HAG types, as demonstrated by what species of erythrocytes are agglutinated, co-aggregation with yeast, and carbohydrate inhibition assays. On this basis alone approximately 40 different types have been recognised[2]. The numbers of HAG types may be extended further by using co-aggregation studies with *Salmonella* strains which have known lipopolysaccharide (LPS) structure[3].

Of the *Aeromonas* HAG's studied to date, all apear to be protein[1,4]. At first it was thought that the HAGs might be associated with fimbriae because some of the early work on *Aeromonas* HAGs revealed the presence of fimbriae on haemagglutinating strains[4]. Subsequent work with the fimbriated strain (A6)

Relationship between diarrhoea-association and co-production of cytotoxin and haemagglutinin by 135 *Aeromonas* strains isolated from human faeces. $p > 0.005$ (Fisher's exact test)

	Diarrhoea-associated	Not diarrhoea-associated	
Co-production positive	87	4	91
Co-production negative	26	18	44
	113	22	135