Transferable resistance factors in *Aeromonas salmonicida*

by M. Popoff and Yvette Davaine

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Culure medium
Transferable Resistance Factors in Aeromonas salmonicida(*)

by

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INTRODUCTION

The existence of transferable resistance factors in the Enterobacteria was shown first in Japan by Ochiai (9) and Akiba (1): the multiple antibiotic resistance of Escherichia coli isolated from the intestine of patients could be transferred to sensitive Shigella strains. This infectious multiple resistance was then recognized by many authors: Datta (6) and Anderson (2) in England, Maten (8) in Holland, Lebek (7) in Germany, Chabbert and Le Minor (5) in France.

While studying the fish pathogen Aeromonas salmonicida, we thought that it would be worthwhile to check the antibiotic sensitivity of a collection of strains, and eventually, to demonstrate the presence of transferable resistance factors in this species. Our first results are presented here.

MATERIALS AND METHODS

1) Culture Media

Broth cultures used a nutritive broth, pH 7.2 (Pasteur Institute, Paris).

Trypticase-soy agar (Difco) was chosen as solid medium and used for culture, antibiotic and sulfonamide sensitivity testing.

2) Antibiotic and Sulfonamide Sensitivity

The disc method recommended by Chabbert (4) was used. The results were read after 18 hours of incubation at 26°C because A. salmonicida is thermosensitive.

3) Strains

a) A. salmonicida Strains Studied

The 104 A. salmonicida strains tested belong to the collection of the Laboratoire d'Ichtyopathologie de l'Institut National de la Recherche Agronomique. Details of their origin were published earlier (10).
3) Strains (cont'd)

b) Donor Strains

These strains were selected from the results of the antibiotic and sulfonamide sensitivity study.

c) Receiver Strains, *A. salmonicida* 163-68

It is sensitive to all the antibiotic but resistant to sulfonamides. In order to recognize the successful transfers after conjugation, this strain was "marked" by Szybalski's gradient technique (11): it was made resistant to a 1000μg/ml streptomycin concentration.

*A. hydrophila* 138-68: Strain isolated from a Cyprinidae affected with infectious dropsy. It was sensitive to antibiotics (including streptomycin) and to sulfonamides. However, differing from *A. salmonicida* in this respect, it used citrate as its sole source of carbon. *E. coli* K-12 AZ 115: this mutant is resistant to sodium azide (115μg/ml) and was sent to us by Dr. Chabbert (Service de Bactériologie Médicale, Institut Pasteur, Paris). It was sensitive to both antibiotics and sulfonamides.

4) Culture Method

The *Aeromonas* strains and *E. coli* K12 AZ115 were incubated for 18 hours in a nutritive broth (26°C) without stirring. They were then diluted to 1/50 with nutritive broth, and incubated 4 hours at 26°C without stirring before conjugation.

5) Conjugation Conditions

They are derived from those used by Watanabe and Fukasawa (12). The donor and receiver cultures having been diluted previously are mixed in a 1/4 ratio during their exponential phase. The mixture is then incubated for 18 hours at 26°C without stirring.
6) Selection of the Transferred Clones

Various selecting agents were used for selecting the transferred clones, depending on the characteristics of the donor and receiver strains. Table I shows the combinations which were used.

### TABLE I

<table>
<thead>
<tr>
<th>Donor</th>
<th>Strains Used</th>
<th>Receiver</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. salmonicida</td>
<td>A. salmonicida 163-68</td>
<td></td>
</tr>
<tr>
<td>A. salmonicida</td>
<td>A. hydrophila 138-68</td>
<td></td>
</tr>
<tr>
<td>A. salmonicida</td>
<td>E. coli K12 AZ 113</td>
<td></td>
</tr>
</tbody>
</table>

The selective agents were incorporated into trypticase soy agar and used at the following concentrations: Sodium azide 115 μg/ml, chloramphenicol 20 μg/ml, streptomycin 1,000 μg/ml, tetracycline 20 μg/ml.

The citrate medium used is the synthetic citrate medium of Simmons (Institut Pasteur, Paris).

The resistance to antibiotics will be indicated by the first letter of the name of the antibiotic concerned and sulfonamide resistance by Su.
RESULTS

I) Resistance spectrum of the *A. salmonicida* strains

The study of our 104 strains gave the following results: all strains are sulfonamide resistant; 92 (or 88.5%) remained sensitive to all antibiotics used; among the antibiotic resistant strains, 4 were resistant to tetracyclines, 5 to chloramphenicol, and 3 to both tetracyclines and chloramphenicol.

II) Resistance Transmission

The study of the results of the antibiotic and sulfonamide sensitivity tests has led us to search for transferable resistance factors in the 12 *A. salmonicida* which are resistant to antibiotics. Using the 12 resistant strains as donors and the sensitive strains as receivers, we were able to do the following transfers:

1) Transfer to *A. salmonicida* 163-68

This trial showed the resistance markers T, C, and the series T-C to be present in *A. salmonicida*.

2) Transfer to *A. hydrophila* 138-68

The series T-Su, C-Su and C-T-Su were transferred from *A. salmonicida* to *A. hydrophila* 138-68.

3) Transfer to *E. coli* K12 AZ115

The series T-Su, C-Su and C-T-Su were transferred to that strain.

Our results appear in Table II.
TABLE II

<table>
<thead>
<tr>
<th>Number of Resistant Strains</th>
<th>Resistance Spectrum</th>
<th>Characteristics Transferred to E. coli K12</th>
<th>Number of Strains Capable of Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>- - - - - -</td>
<td>T Su</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>- - - - - -</td>
<td>T</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>- - - - - -</td>
<td>C Su</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>- - - - - -</td>
<td>T C Su</td>
<td>3</td>
</tr>
</tbody>
</table>

* Number of strains studied: 104

III Study of the Reversibility of the Transfers.

E. coli K12 AZ115 and A. hydrophila strains made resistant to tetracyclines, chloramphenicol and sulfonamides were used as donors, while A. salmonicida 163-63 was the receiver. The following transfers were successful:

1) Transfer from E. coli K12 AZ115 to A. salmonicida 163-68 (selection characteristics: resistance to streptomycin, resistance to tetracyclines or chloramphenicol).

   The resistance markers T and C, as well as the series T-C were transmitted from E. coli K12 AZ115 to A. salmonicida.

2) Transfer from A. hydrophila to A. salmonicida 163-68 (selection characteristics: resistance to streptomycin, resistance to tetracyclines or chloramphenicol).

   Results were identical to those reported above.

DISCUSSION AND CONCLUSIONS

It is usual to treat trout ponds infected with A. salmonicida (agent of the furunculosis of Salmonidae) with sulfonamides or antibiotics. The antibiotic and sulfonamide resistance spectrum of our 104 strains shows
that a sulfonamide treatment is useless because all the strains are resistant to these drugs. The use of antibiotics is more justified as 88.5% of the strains are still sensitive to them. We should note, however, the apparition of strains (11.5%) which are resistant to the tetracyclines and to chloramphenicol.

It is more interesting to know that this tetracycline and chloramphenicol resistance is due to transferable resistance factors among which we have demonstrated the series T-Su, C-Su and C-T-Su. It is also interesting to note that these factors are easily transferred among strains of the same genus (*A. salmonicida* and *A. hydrophila*) but also that they are easily transmitted to strains of a different family (in this work *E. coli* K12 AZ115).

Many articles have shown the extension of the resistance factors among the Enterobacteria: series such as A. S. K. T. C. Su are not uncommon (Baudens and Chabbert (3)). Such series may well be transferred to *Aeromonas*, thus creating new problems in the treatment of furunculosis in Salmonidae.

It would appear to us that the number of efficient therapeutics against *A. salmonicida* infections decreases, and the evolution of the resistance of the strains should be followed over the next few years.

**SUMMARY**

The study of the antibiotic and sulfonamide resistance spectrum of 104 strains of *A. salmonicida* show that they are all resistant to sulfonamides while 88.5% of them remain sensitive to antibiotics.

The antibiotic resistance is due to transmissible resistance factors which can be transferred to strains belonging to the same genus but also to the enterobacteria. The series C-Su, T-Su, C-T-Su have been demonstrated.

Acknowledgment

Our sincere thanks go to Dr. Chabbert, Chef, Service de Bacteriologie Médicale, Institut Pasteur, Paris, and to Professor Le Minor, Chef, Service des Enterobactéries, Institut Pasteur, Paris, for their advice during this project.