Bacterial Study of Axolotls

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We recently researched the presence of microbial pathogens in axolotls. The Indiana University Axolotl Colony uses injections of the antibiotic amikacin approximately twice a year on symptomatic adults to control disease, but still suffers a one to three percent death rate. The purpose of our experiment was to attempt to isolate and identify a common bacterial water pathogen, in particular *Aeromonas hydrophila*, possibly responsible for the infection of axolotls at the Indiana University Axolotl Colony and to test any isolates for antibiotic sensitivity.

The procedure was as follows:

1) Sterile dissection of two symptomatic axolotls—one which had not received amikacin injections and one which had received these injections.
(2) Inoculation of selected organ and blood samples onto Sheep's Blood Agar (Kay et al. 1985).
(3) Incubation at 37° Celsius.
(4) Isolation and purification of bacterial colonies.
(5) Identification of colonies using Tween 80 test, Glucose fermentation, indole test, catalase test, and Oxi-Ferm tubes.
(6) Drug sensitivity disk testing with amikacin and various other antibiotics.

The animals were anesthetized and sterilized externally with Betadine. Upon dissection, we found enlarged livers. The liver from the amikacin positive animal had a fairly large (5 mm diameter) yellowish growth. Sheep's Blood Agar was inoculated with the liver growth as well as with various samples from the blood and other organs. Individual plates were used for each inoculation.

Several types of bacterial colonies appeared as a result of the initial inoculum. These colonies were purified on Sheep's Blood Agar containing ampicillin in order to select for gram negative bacteria. The following table presents the origin of the organisms recovered. (Note: Several other colonies were present but were disregarded on the basis of the oxidase test, since we limited our search to *Aeromonas hydrophila*).

Table 1
Origin of Each Inoculum

Organism Number	Origin of Inoculum	Amikacin + or - Animal
1	liver	–
2	blood	–
3	liver	–
4	blood	–
5	blood	+
6	liver growth	+
7	liver	–

Organisms 2, 3, and 4 developed large, opaque, flat, greenish colonies with a yellowish center.

Organisms 1, 5, 6, and 7 developed very small, creamy, glistening, round, convex colonies.

Characteristics of *Aeromonas hydrophila* are as follows (Starr et al. 1981):

(1) Oxidase positive (tested for on Nutrient Agar).
(2) Presence of Tween 80 esterase
(3) Indole production in 1% peptone water
(4) Gas production in glucose
(5) Catalase positive

Test Results

Table 2
Tween 80

Organism	Halo Formation
1	–
2	+
3	+
4	+
5	+
6	–
7	slight

Table 3
Glucose Broth Fermentation Tubes

Organism	Growth	Acid	Gas Production
1	–	–	–
2	+	+	+
3	+	++	++
4	+	+	+
5	+	–	–
6	+	–	–
7	+	–	–

Table 4
Indole

Organism	Positive or Negative
1	–
2	+
3	+
4	+
5	– (no growth)
6	–
7	– (no growth)

Table 5
Catalase

Organism	Positive or Negative
1	–
2	+
3	+
4	+
5	–
6	+
7	–

According to Starr et al. (1981), organisms 2, 3, and 4 were the only isolates that fit the criteria for *Aeromonas hydrophila*. Oxi-Ferm Identification kits were used to identify four of the seven organisms. Organisms 2, 3, and 4 were identified because they were catalase positive and resembled *Aeromonas hydrophila* when grown on Blood Agar. Organism 6 was also identified using the Oxi-Ferm kits because it was also catalase positive. The results of the Oxi-Ferm tests confirmed that organisms 2, 3, and 4 were *Aeromonas hydrophila* and that organism 6 was a type of Flavobacterium. From these results, we concluded that Sheep's Blood Agar with ampicillin is an effective medium for the isolation of *Aeromonas hydrophila*.

We also decided to test the isolates with various amounts of amikacin and standard amounts of other antibiotics. The results are as follows:

Amikacin Negative Animal

Table 6
Organism Number 2

Amikacin Disks		Multi-Disks	
Amount of Drug (µg)	Diameter of Clearing (mm)	Drug	Diameter of Clearing (mm)
1	9	Te	9
3	11	K	25
10	13	E	17
30	15	P	0
100	19	Am	0
		B	0

Table 7
Organism Number 3

Amikacin Disks		Multi-Disks	
Amount of Drug (µg)	Diameter of Clearing (mm)	Drug	Diameter of Clearing (mm)
1	13	Te	15
3	15	K	27
10	17	E	25
30	21	P	0
100	25	Am	9
		B	0

Table 8
Organism Number 4

Amikacin Disks		Multi-Disks	
Amount of Drug (µg)	Diameter of Clearing (mm)	Drug	Diameter of Clearing (mm)
1	11	Te	11
3	13	K	27
10	17	E	25
30	19	P	0
100	23	Am	0
		B	0

Organisms #1 and #7 did not grow on either plate.

Amikacin Positive Animal

Table 9
Organism Number 6

Amikacin Disks		Multi-Disks	
Amount of Drug (µg)	Diameter of Clearing (mm)	Drug	Diameter of Clearing (mm)
1	9	Te	27
3	13	K	19
10	17	E	29
30	19	P	29
100	21	Am	27
		B	12

Organism #5 did not grow on either plate.

Te = Tetracycline
B = Bacitracin
Am = Ampicillin
E = Erythromycin
K = Kanamycin
P = Penicillin

In accordance with the Kirby-Bauer Disk Diffusion Test and values for clearing zones as given in the Tenth Edition of the DIFCO Manual, we conclude that amikacin appears to be still effective. Erythromycin and kanamycin could also be effective against these organisms.

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